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

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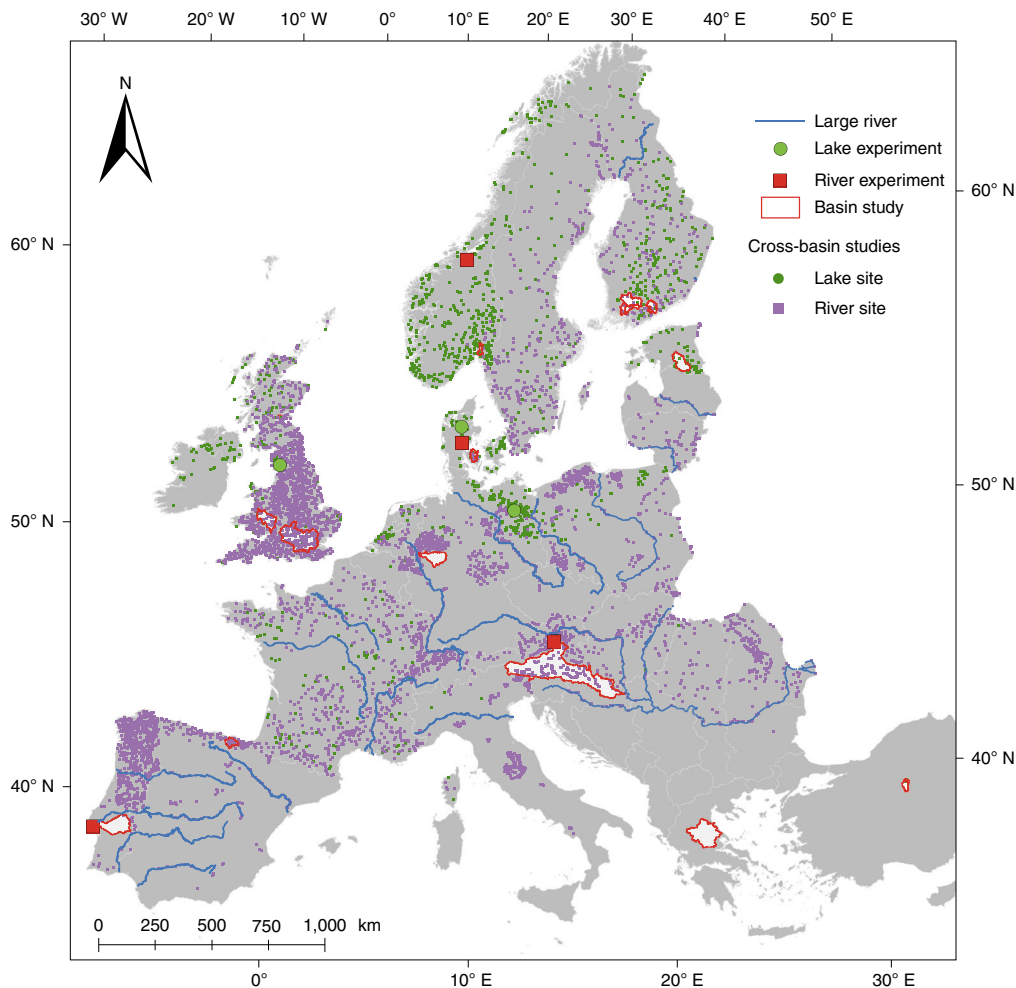
**Climate and land-use change drive a suite of stressors that shape ecosystems and interact to yield complex ecological responses (that is, additive, antagonistic and synergistic effects). We know little about the spatial scales relevant for the outcomes of such interactions and little about effect sizes. These knowledge gaps need to be filled to underpin future land management decisions or climate mitigation interventions for protecting and restoring freshwater ecosystems. This study combines data across scales from 33 mesocosm experiments with those from 14 river basins and 22 cross-basin studies in Europe, producing 174 combinations of paired-stressor effects on a biological response variable. Generalized linear models showed that only one of the two stressors had a significant effect in 39% of the analysed cases, 28% of the paired-stressor combinations resulted in additive effects and 33% resulted in interactive (antagonistic, synergistic, opposing or reversal) effects. For lakes, the frequencies of additive and interactive effects were similar for all spatial scales addressed, while for rivers these frequencies increased with scale. Nutrient enrichment was the overriding stressor for lakes, with effects generally exceeding those of secondary stressors. For rivers, the effects of nutrient enrichment were dependent on the specific stressor combination and biological response variable. These results vindicate the traditional focus of lake restoration and management on nutrient stress, while highlighting that river management requires more bespoke management solutions.**

Multiple stressors are increasingly recognized as a major concern for aquatic ecosystems and for those organizations in charge of their management<sup>1</sup>. 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<sup>2</sup>. Critically, no study has been conducted to systematically confirm the frequency of occurrence of multiple stressor interactions across spatial scales (that is, from water-body to continental scales) and ecosystem types (that is, for lakes and rivers). Using a comprehensive large-scale assessment of multiple stressor interactions, we show that the dominance of a single stressor, namely nutrient enrichment, is still common in

lakes, while for rivers stressor interactions are much more relevant, demanding more complex and informed management decisions.

Single, intense and well-characterized stressors (such as organic and nutrient pollution from point sources) formerly dominated freshwater ecosystem responses<sup>3</sup>. 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<sup>4–6</sup>.

For the simplest case of two stressors acting simultaneously, three main types of effects can be conceptually distinguished<sup>7</sup>: (1) 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); (2) the two stressors act independently



**Fig. 1 | Location of the sampling sites and experimental sites.** Locations of the 7 experimental facilities, 14 basin studies and sampling sites (small dots) for the 22 cross-basin studies of lakes and rivers across Europe (see Supplementary Table 1 for details).

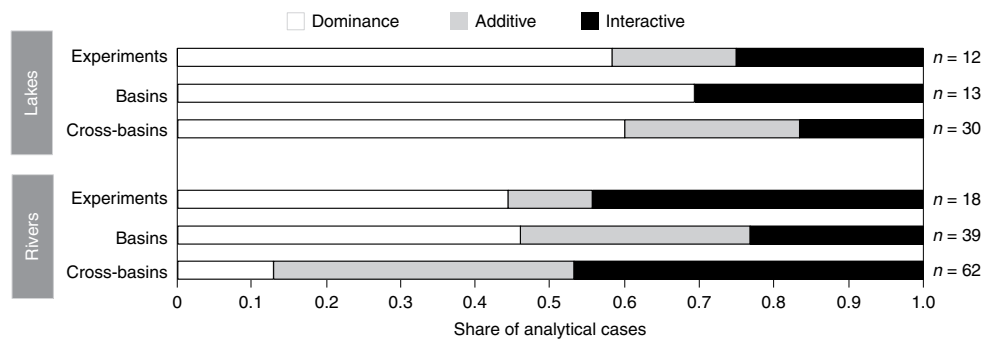
such that their joint effect is the sum of the individual effects (additive effects); and (3) one stressor either strengthens or weakens the effects of the other (interaction). There is a striking lack of information on the frequencies of occurrence of these effect types across spatial scales (that is, from individual water bodies to whole continents) and ecosystem types (rivers versus lakes)<sup>8</sup>.

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 a conceptual understanding of ecological responses to stressor interactions<sup>9–11</sup> to structure an empirical modelling approach, using generalized linear modelling (GLM) and 174 stressor combinations with single biological responses from more than 18,000 observations (Fig. 1). The outputs of the GLMs were interpreted to identify the frequencies of cases with stressor dominance, additive stressor relationships and stressor interactions (synergistic or antagonistic), stratified by ecosystem type (lake or river) and spatial scale (experiments, basin studies and cross-basin studies).

With this approach, we addressed four questions. (1) How frequent are the three different types of stressor effects in lakes and rivers? We expected a high share of additive and interactive relationships in both lakes and rivers, as intense stressors obscuring the effects of secondary stressors rarely occur nowadays<sup>12,13</sup>. (2) To

what extent do ecosystem type (lake versus river) and spatial scale influence the combined effects of two stressors? We expected more frequent stressor interactions in rivers, as their greater heterogeneity increases the likelihood for two stressors to have an impact<sup>14</sup>. We further expected more frequent stressor interactions in small-scale studies (that is, in mesocosms), as these studies are less influenced by confounding factors<sup>15,16</sup>. (3) What is the influence of ecosystem type (lake versus river) and spatial scale on the explanatory power of two stressors and their interaction? We expected the explanatory power to be lower for rivers because of greater heterogeneity and thus potentially confounding factors in comparison with lakes<sup>17</sup>. We also expected a decreasing explanatory power of individual stressors and their interactions with increasing spatial scale, reflecting the increasing importance of confounding factors at large scales<sup>18,19</sup>. (4) Is nutrient enrichment still the most prominent stressor affecting European aquatic ecosystems as suggested by ref. <sup>20</sup>, despite the progress in wastewater cleaning, and does the importance of costressors differ between lakes and rivers? We expected a dominating effect of nutrient stress in lakes (due to the dominance of primary producers) and a greater relevance of hydrological and morphological changes in rivers<sup>21,22</sup>.

Our study pursues a phenomenological approach (sensu ref. <sup>23</sup>) and seeks to disclose stressor interrelations under real-world conditions, contributing to solving some of the pertinent issues in ecosystem management<sup>2</sup>.



**Fig. 2 | Stressor effect types in lakes and rivers.** 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.

## Results and discussion

**Impact of ecosystem type on stressor effect types.** Stressor interactions are regularly reported in synthesis papers on multiple stressors in freshwater ecosystems<sup>8,10</sup>. We therefore hypothesized that high proportions of both lake and river case studies would indicate additive or interactive paired-stressor relationships. However, this was not supported. Among the 174 cases, 39% of models indicated single-stressor dominance, 28% indicated additive paired-stressor effects and 33% indicated paired stressors interacting significantly (Fig. 2; see also ‘Data availability’).

We expected a higher proportion of river cases to exhibit stressor interactions, compared with lakes, as a result of greater habitat heterogeneity in rivers<sup>14</sup>. This was supported. The proportions of effect types differed between lakes (62% dominance, 16% additive and 22% interactive) and rivers (28% dominance, 33% additive and 39% interactive; Fig. 2) (chi-squared test,  $P < 0.001$ ).

We assumed that the different frequencies of effect types between lakes and rivers might have been rooted in different frequencies of the stressor types investigated<sup>8</sup>: nutrient enrichment was one of the two stressors in 95% of the lake cases but in only 76% of the river cases. However, these differences between lakes and rivers in the share of stressor dominance remain if only cases with nutrient enrichment are considered: 60% (lakes) versus 27% (rivers), compared with 62% (lakes) versus 29% (rivers) considering all cases.

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

An alternative explanation is the different exposures of organisms inhabiting rivers and lakes to stressor effects. While freshwater ecosystems in general are sinks that collect anthropogenic stressors, the much higher shoreline length of rivers multiplies the effects of human activities in the catchment, such as land and water uses<sup>24,25</sup>. This results in an increased exposure to hydrological and morphological stressors, the latter also being more relevant in rivers due to their primarily benthic habitats and assemblages<sup>26</sup>. This relationship is also expected for toxic substances that can act more directly in (small) rivers, as much lower compound quantities are needed to reach toxic concentrations<sup>27</sup>. In the 58 cases where models included a significant interaction term, the combinations of nutrients with toxic or morphological stress represented the greatest proportion of

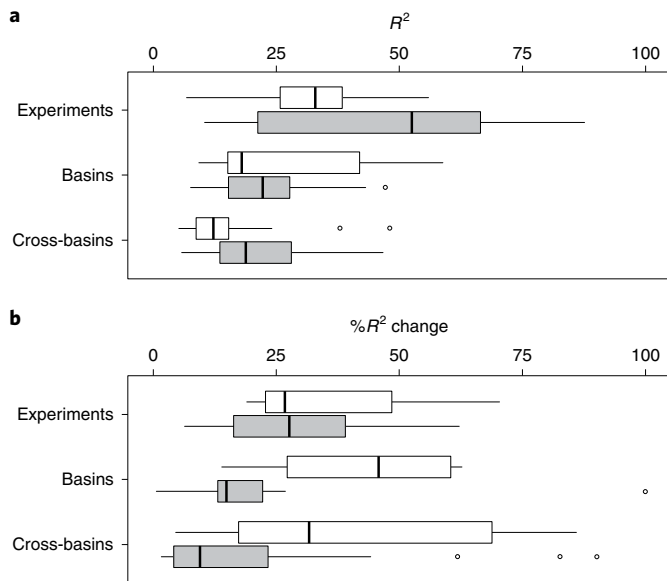
confirmed interaction effects (a ratio of 0.45 or 0.43, respectively; only combinations with the total number of cases  $> 5$ ; no significant correlation between the total number of cases and the share of interactive cases). All but one of the cases with toxic substances as a stressor were rivers.

**Impact of spatial scale on stressor effect types.** We expected that the frequency of interactions would decrease with the 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, an 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 these effects may be masked by other factors not under investigation. On the other hand, an 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 occur only at certain stressor intensities. The latter holds true only if stressors are effective over the whole gradient length—for example, the biological response does not level off at low or intermediate stressor levels (as in case of nutrient saturation<sup>28,29</sup>).

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 as the spatial scale increased.

Though we are not aware of other studies comparing the effects of spatial scale on the explanatory power of stressor interaction models, the observed differences in the frequencies of stressor interactions between experiments and field studies are in line with the synthesis studies of ref. <sup>8</sup> and ref. <sup>10</sup>. While the study of Jackson et al.<sup>10</sup> included only experiments and observed interactive or additive effect types in all cases considered, the study by Nöges et al.<sup>8</sup> focused on field studies, and interactive or additive effect types were given for only 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<sup>20</sup> and are affected by a lower number of stressors<sup>30</sup>. We therefore expected the explanatory power of our models to be lower for rivers because of the greater impacts of stressors that have not been regarded (that is, confounding factors)<sup>8,31</sup>. Contrary to our expectations, however, the river models performed significantly better than the lake models (Mann–Whitney  $U$ -test,  $P < 0.05$ ). This



**Fig. 3 | Explanatory power of models at different spatial scales and in different ecosystems. a**, Percentage of the 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 ( $n=92$ ) in 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 the explained biological variance when the 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 ( $n=34$ ) in lakes (white boxes) and rivers (grey boxes). None of the differences within spatial scales are significant. In each box plot, the centre line indicates the median, the box limits indicate the upper and lower quartiles, the whiskers indicate the  $1.5\times$  interquartile range, and the points indicate the outliers.

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 (such as water quality, hydrology and benthic habitats), and the riverine fauna is sensitive to the impacted oxygen conditions, which may collect the effects of a variety of stressors into a single gradient. Oxygen, however, is rarely measured in a meaningful way in monitoring programs (including the daily maxima and minima) and was thus not considered as a stressor in our analysis. In contrast, lake phytoplankton seems less susceptible to the effects of multiple stressors, as long as nutrients are in the growth-limiting concentration range.

We expected a decreasing explanatory power with an increasing spatial scale, reflecting the increasing importance of confounding factors at large scales<sup>18,19</sup>. This was partly supported. The variance in the biological response explained by the paired-stressor models (expressed as the marginal coefficient of determination ( $R^2$ )) ranged between 0.05 and 0.88, with a median value of 0.19. These ranges differed significantly between experiments (median marginal  $R^2$ , 0.38), basin studies (median marginal  $R^2$ , 0.22) and cross-basin studies (median marginal  $R^2$ , 0.16) (Bonferroni-corrected Mann-Whitney  $U$ -test,  $P<0.05$ ; Fig. 3a). The marginal  $R^2$  differed significantly between lakes and rivers, with river cases showing on average slightly higher explanatory power (lakes,  $R^2=0.15$ ; rivers,  $R^2=0.22$ ; Mann-Whitney  $U$ -test,  $P<0.05$ ). The importance of the interaction term (expressed as % $R^2$  change) was significantly higher for lakes than for rivers (Mann-Whitney  $U$ -test,  $P<0.01$ ). For rivers, this importance tended to decrease with an increasing spatial scale

**Table 1 | Numbers of paired-stressor cases analysed across lakes and rivers**

| Paired stressors                           | Lakes | Rivers |
|--|-------|--------|
| Nutrient   hydrological                    | 11    | 24     |
| Nutrient   morphological                   | 0     | 46     |
| Nutrient   thermal                         | 34    | 9      |
| Nutrient   toxic                           | 1     | 10     |
| Nutrient   chemical                        | 6     | 1      |
| Hydrological   morphological               | 0     | 6      |
| Hydrological   thermal                     | 3     | 8      |
| Hydrological   chemical                    | 0     | 5      |
| Morphological   morphological <sup>a</sup> | 0     | 1      |
| Morphological   toxic                      | 0     | 5      |
| Morphological   chemical                   | 0     | 2      |
| Toxic   chemical                           | 0     | 2      |

<sup>a</sup>Connectivity disruption and morphological river alteration.

of investigation, but the differences between investigation scales were generally not significant (Fig. 3b).

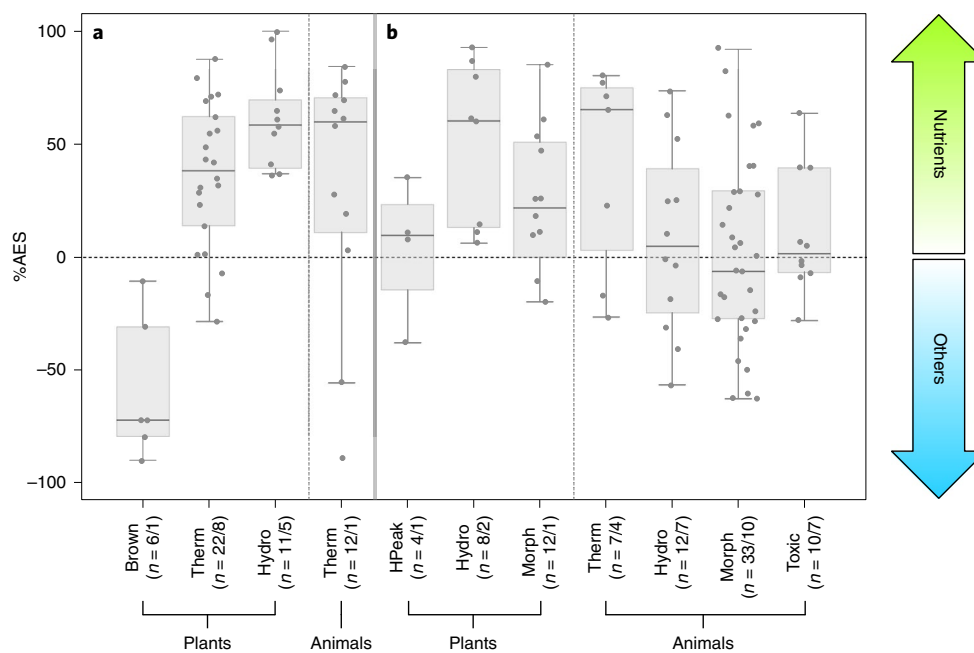
For the experiments addressed in our study, the high level of control on potentially confounding factors can account for the greater explanatory power (on average) when compared with field studies. Furthermore, the experimental studies had lower numbers of observations and less-complex biological communities, whereas factors such as temperature variation are already temporally pronounced at the basin scale, and the spatial variation across basins is considerable.

**Role of nutrient stress in lakes versus rivers.** The recent surveys by refs.<sup>8,20</sup> suggest that eutrophication is still the most prominent stressor affecting the biota of Europe's water, particularly lakes, while rivers are also strongly affected by hydrological and morphological stressors. We therefore expected that responses to nutrient stress are retarded by the presence of secondary stressors in rivers more than in lakes, where responses to nutrient enrichment are the strongest<sup>21,22</sup>. This was supported.

We identified 11 combinations of nutrient stress paired with another stressor, covering morphological, hydrological (including hydropeaking), thermal, toxic and chemical stress (brownification) (Table 1). The numbers of analytical cases in each stressor combination ranged from 4 to 33, with the combinations including hydropeaking and brownification stress exclusively comprising data collected at the experimental scale. All other combinations comprised data from up to ten different studies, most of which originated from two or more spatial scales. Best represented were the combinations of nutrient stress paired with thermal stress affecting autotrophs in lakes, and nutrient stress paired with morphological stress affecting heterotrophs in rivers (Fig. 4).

Nutrient stress often had the stronger effect in the paired-stressor models. Hence, 9 of the 11 combinations in lakes and rivers showed a positive median absolute effect size difference (%AES), implying stronger effects of nutrients on average compared with the other stressor. Five combinations even showed a positive 25th-percentile %AES, indicating that in three-quarters of the cases in these combinations, nutrient effects outweighed the other stressors. This was evident for all lake stressor combinations except nutrients and brownification, represented by a single case study. The few additional lake cases for which the non-nutrient stressor was stronger included warming affecting cyanobacterial biomass in European lakes, and lithophilous or piscivorous fish abundance in French lakes.





**Fig. 4 | %AES for stressors across case studies. a, b.** Range of %AES for nutrient stress and selected other stressors across case studies from lakes (a) and rivers (b). Positive %AES values indicate stronger effects of nutrient stress; negative %AES values indicate stronger effects of 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. In each box plot, the centre line indicates the median, the box limits indicate the upper and lower quartiles, the whiskers indicate the 1.5 $\times$  interquartile range, and the points indicate the individual analytical cases. The vertical dotted lines in a and b separate plants from animals in both ecosystem types.

The dominance of nutrients over secondary stressors in lakes also applies to temperature stress, which is often considered to interact in a synergistic way with eutrophication in rivers and lakes<sup>32</sup>. One mesocosm experiment even demonstrated an antagonistic relationship at high nutrient stress<sup>33</sup>. Water temperature may affect lake communities by modifying the food-web structure (by supporting planktivorous fish, for example)<sup>34</sup>; the two temperature-driven functional fish-trait responses mentioned above perhaps indicate the emergence of such modification.

Brownification is a remarkable exception from this general pattern but is observed here only in a single case study. It strongly superimposes the effects of nutrient stress, in particular by decreasing light transmission in the pelagic zone, which inhibits productivity despite excess nutrient concentrations (an opposing interaction) and favours mixotrophic phytoplankton species. Brownification is triggered by global warming and a wetter climate, and it becomes increasingly relevant in boreal regions, as it originates from dissolved organic carbon in leachates of bogs and permafrost soils mineralizing due to increasing temperatures and flushing and to the recovery from acidification<sup>35,36</sup>.

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

The pattern of nutrient stress outweighing the effects of hydrological or morphological stress for river autotrophs is similar to that in lakes. Here, “the response variable matters”<sup>37</sup>—while river autotrophs have been shown to be responsive to hydrological or morphological stress elsewhere (for example, refs. <sup>38,39</sup>), their effect

size was overruled by the nutrient signal in our study. In one case, however, hydropeaking outweighed the nutrient signal on river autotrophs. The immediate mechanical effect of flush flows is very pervasive, but is presumably limited to short river stretches downstream of hydropower dams.

By contrast, river heterotrophs were equally affected by paired stressors when nutrient enrichment was paired with hydrological, morphological or (to a lesser degree) thermal stress. This indicates that these paired stressors act together on oxygen content or habitat availability. In our study, we found small but consistent antagonistic interactions, in particular for channelized rivers, probably due to increased current velocities facilitating the oxygen availability. In the case of toxic stress, our conjectures on mechanistic pathways remain speculative. The diversity of compound-specific modes of action across xenobiotics in each mixture renders toxic stress a multistressor issue in itself<sup>40</sup>. Notably, the toxic effects of ambient mixtures were clearly discernible in all respective paired-stressor case studies ( $n = 17$ ), despite the likely different stressor modes of action<sup>41</sup>. Given the lack of adequate monitoring of xenobiotics, our findings support the idea that toxic effects in the multiply stressed freshwater ecosystems of Europe are largely underestimated<sup>42</sup>.

In summary, nutrient enrichment overrules the effects of most other stressors in lakes, but the situation in rivers is more complex. In rivers, plants are more strongly affected by nutrients, while animals are affected equally by nutrient enrichment and by other stressors.

## Conclusions

Our study supports the conjecture that eutrophication is still the most relevant stressor affecting many lakes, irrespective of the spatial scale considered. Other stressors are subordinate but may reveal notable effects if interacting with nutrients. These stressors deserve special attention if antagonistic (such as lake brownification) and synergistic interactions (such as climate warming) can be

expected that control the overall nutrient effect on phytoplankton. Relevant stressors and stressor combinations are more variable in rivers and more strongly affected by spatial scales. While river autotrophs are impacted mainly by nutrients, heterotrophs seem to be influenced mainly by oxygen availability, which is impaired by a range of stressors (pollution, warming, flow reduction and fine sediment entry), on top of nutrient enrichment. While the reduction of nutrient stress is the most relevant for lakes, particularly under the conditions of climate warming, rivers require mitigation measures addressing several stressors simultaneously. Options include the establishment of woody riparian buffer strips that address several stressors (such as eutrophication and 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 multistressor experiments in mesocosms and flumes, river basin studies, and cross-basin studies (Fig. 1 and 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<sup>43</sup>, involving three lake mesocosm facilities in Denmark, Germany and the 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<sup>43</sup>. On the basis of harmonized analytical protocols<sup>38</sup>, the multistressor 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 were collated at the regional, national or international level to investigate the biological effects of various stressors (for example, refs. 44,45). 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.** In this study, we considered a stressor as any external factor modified by human intervention that potentially moves a receptor (that is, a response variable) out of its normal operating range<sup>46</sup>. The analysed stressor variables belonged to six stress categories (see also ref. 39): (1) nutrient stress (142 cases), including the experimental addition or field sampling of phosphorus or nitrogen compounds in the water; (2) hydrological stress (57 cases), including the experimental manipulation or field measurement of high flow (for example, high flow pulse duration), low flow (for example, residual flow), water level change, non-specific flow alteration (for example, the mean summer precipitation as a 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 multisubstance potentially affected fraction<sup>40</sup>, toxic units<sup>47</sup> or runoff potential<sup>48</sup>; and (6) other chemical stress (16 cases), including the experimental application of humic substances and field samples of water quality determinants (for example, dissolved oxygen, chloride and biological oxygen demand).

We always selected the stressor combinations most relevant for the respective broad lake or river type in each river basin or region—that is, stressors that are most likely to affect biota due to their relative strengths compared with other regions and other stressors in the same region<sup>49</sup> (Supplementary Table 1). These included stressors prevalent in European freshwater ecosystems<sup>20</sup> and addressed in previous multistressor studies<sup>8</sup>. In the experimental studies, stressor intensities were applied that emulated real-life conditions in the respective water-body type. For instance, flumes mimicking nutrient-poor calcareous highland rivers were enriched by a tenfold phosphorus increase towards mesotrophic conditions—a realistic scenario in the case of alpine pasture use in the floodplains. Mesocosms mimicking eutrophic shallow lowland lakes were enriched by a fivefold 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 cases, we selected those stressors that were assumed to affect the biota most strongly, on the basis of either their intensity or previous studies on the relevance of the stressors in the region.

Overall, 12 paired-stressor combinations were investigated, including 7 combinations that covered only rivers (Table 1). For rivers, the combination of nutrient and morphological stress was the most frequent, amounting to more than one-third of the cases. For lakes, the combination of nutrient and thermal stress was the most frequent, amounting to more than half of the cases.

**Response variables.** A variety of organism groups was investigated, including phytoplankton (52 cases), benthic flora (that is, macrophytes or phytobenthos; 22 cases), benthic invertebrates (63 cases) and fish (37 cases). Within the 174 cases, four categories of biological response variables were used: (1) biodiversity (76 cases), including indices reflecting the proportion of a taxonomic group in the assemblage (for example, the percentage of Chlorophyta in the benthic algal assemblage), taxon richness, ecological quality ratios (as derived from ecological classification tools for the European Water Framework Directive) and taxon-sensitivity indices (for example, saprobic indices and Average Score Per Taxon); (2) biomass/abundance (51 cases), including biomasses or total abundances of phytoplankton or fish, chlorophyll *a* concentrations or cyanobacterial biomass; (3) functional traits (38 cases), including the absolute or relative abundances of functional groups such as habitat preferences, feeding types or life cycles and trait-based quality indices (for example, SPEAR = species at risk; ref. 49); and (4) behaviour (9 cases), exclusively including drift rates of invertebrates and stranding rates of juvenile fish. While the response category 'biodiversity' covered all organism groups, the category 'biomass/abundance' was limited to phytoplankton (except for two cases each with benthic algae and fish), and both 'functional traits' and 'behaviour' were limited to animals (invertebrates and fish).

**Statistical analysis.** The relationship between the biological response and the paired stressors was investigated for each individual analytical case by GLM on the basis of the general formula

$$E(Y) = g^{-1}(ax_1 + bx_2 + cx_1x_2),$$

where  $E(Y)$  is the expected value of the biological response variable  $Y$ ,  $g$  is the link function that specifies how the response relates to the linear predictors,  $x_1$  is the standardized measurement of Stressor 1,  $x_2$  is the standardized measurement of Stressor 2 and  $x_1 \times x_2$  is the interaction of the standardized measurements of Stressor 1 and Stressor 2. The parameters  $a$ ,  $b$  and  $c$  scale the effects of Stressor 1, Stressor 2 and their interaction, respectively.

**Data processing of stressor and response variables.** For large-scale data (multisite biomonitoring data with no, or a very short, temporal component), long-term average measures of stress were used. For multiyear data (with a single site or multiple sites), each year provided one stress measurement per site. When the data were at a higher temporal resolution, they were preprocessed to an annual level. Categorical stressor variables (for example, experimental flow treatment) had only two levels, representing stressed versus unstressed conditions.

All continuous variables (responses and stressor variables) were standardized by transformation to approach a normal distribution. A version of the Box-Cox transformation was used<sup>50</sup>, including an offset to ensure strict positivity (all values >0). The transformed data were inspected for normality by plotting frequency histograms. If the data exhibited skewness because of extreme outliers, these outliers were excluded from the analysis. After the Box-Cox transformation, each transformed variable was centred and scaled, so they had a mean of zero and a variance of one.

**Choice of regression model.** The type of statistical model used to fit the paired-stressor response data depended on two major considerations. The first was the type of analytical case, which determined whether a GLM was sufficient or whether a generalized linear mixed model (GLMM) with random effects was needed (see Supplementary Table 2 for the criteria). GLMMs were used when the data structure included grouping factors, such as experimental block, site or year (see 'Data availability'). In most cases, the analyses included random effects in the standard way as random intercept terms. However, if considered appropriate (for example, due to a large data volume), models with both random intercepts and random slopes were used. The second consideration was the type of response data, which determined the link function and the error distribution of the model (gaussian errors and an identity link for continuous data, Poisson errors and a logarithmic link for count data). The GLMs were fitted with the base R libraries, and the GLMMs were fitted with the lme4 and lmerTest R packages<sup>51</sup>.

**Testing and correcting for residual autocorrelation.** Where necessary, we tested whether model residuals showed strong evidence of spatial or temporal

autocorrelation, which can cause the statistical significance of model terms to be exaggerated. This was required only when the analysis used GLMs without random effects, since the random effects in the mixed effects models should account for grouping in space and time. Autocorrelation in space or time was identified with Moran's tests on model residuals, and, where substantial autocorrelation was detected, the model was refitted including a trend surface generated using a smoothing spline or polynomial functions<sup>52</sup>. This is a simple and generally effective way of reducing the influence of autocorrelation on the model's stressor effects of interest.

**Model evaluation.** To evaluate our models, we examined the residuals for correlation to the fitted values and deviation from the normal distribution (Shapiro–Wilk test). We excluded 28 models where the residuals were correlated with the fitted values ( $R > 0.35$ ) and non-normally distributed. The model fit was evaluated as the marginal  $R^2$  (that is, the proportion of variance explained by the model fixed effects, ignoring the contribution of any random effects<sup>53</sup>). We excluded models with marginal  $R^2 < 0.05$ . The model fixed effects (the main effects of both stressors and their interactions) were evaluated from the standardized partial regression coefficients and their significance ( $t$ -test), in the following referred to as standardized effect sizes (SES) (see 'Data availability').

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

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

**Interaction classification.** The type of interaction was characterized from the SES and considered only in the case of a significant interaction term. We applied a simple classification scheme to the full model, referring to both stressors' main effects and their interaction. This scheme was based on the direction of the interaction effect, relative to the directions of the main effects of both stressors. A synergistic interaction was assigned when the SES for both stressors and their interaction all had the same sign (that is, all positive or all negative). An antagonistic interaction was assigned when the SES for both stressors had the same sign, but their interaction had the opposite sign. An opposing interaction was assigned when the signs of the SES for both stressors differed, and we distinguished between opposing contributing to either Stressor 1 (that is, Stressor 1 and the interaction with the same sign) or Stressor 2 (that is, Stressor 2 and the interaction with the same sign). A reversal interaction (sensu refs.<sup>9,10</sup>) was assigned when the SES' sum for both stressors had a 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 the interaction term was not. The importances (shares) of these three types of stressor interrelations were compared between ecosystems (from studies of lakes or rivers) and between spatial scales (from experiments, basin studies and cross-basin studies). These comparisons were tested using the chi-squared test. The distribution of marginal  $R^2$  values from the 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 nutrient stress paired with another stressor. The strengths of the stressors' effect sizes were compared, distinguishing between effects on autotrophs and heterotrophs across lakes and rivers. In this analysis, we simply considered the magnitudes 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 the %AES of the nutrient stressor and the %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 %AES of the stressor with which the interaction SES shared the sign (for example, 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.

**Reporting Summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

## Data availability

The data on the regression model outputs and the underlying paired-stressor response data are available at GitHub: <https://github.com/sebastian-birk/MultiStressorImpacts>.

## Code availability

The R script is available at GitHub: <https://github.com/sebastian-birk/MultiStressorImpacts>.

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### Author contributions

D.C., L.C., B.M.S., S.B., L.B., S.J.T. and D.H. conceptualized the study. D.C. and S.B. curated the data. D.H., L.C. and S.B. acquired the funding and administered the project. A.B., A.G., A.S., B.M.S., C.A., C.G.-C., C.P., D.d.Z., D.G., E.B.-K., F.C., G.P., J.J.R., J.R., J.T., J.U.L., K.R., K.S., L.P., L.S., M.C.U., M.J., N.K., N.W., P.B., P.S., P.C.v.d.O., R.B.S., R.-M.C., R.S., S.A., S.B., S.C.S., S.J.M., S.L., S.P., S.J.T., T.B., U.I. and U.M. provided the data and/or conducted the formal analysis. A.B.-P., A.L.S., D.G., E.B.-K., E.J., H.F., J.M.S., J.R., L.C., L.S., M.O.G., P.B., S.A., S.C.S., S.S. and W.G. conducted the experimental investigations. S.B., D.H., B.M.S., M.O.G. and D.C. wrote the manuscript. H.E.A., M.B., A.D.B., A.C.C., C.K.F., M.T.F., M.O.G., L.G., J.H., M.K., P.N., T.N., S.J.O., Y.P., B.S., M.V. and the aforementioned authors reviewed the manuscript and provided the necessary amendments.

### Competing interests

The authors declare no competing interests.

### Additional information

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Data on the regression models, the underlying paired stressor-response data and the R-script are available at GitHub: <https://github.com/sebastian-birk/MultiStressorImpacts>

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|-----------------------------------|--|
| Study description                 | Our study combined data from experimental, river basin and cross-basin studies producing 174 combinations of paired-stressor effects on a biological response variable. The relationship between stressor and response variables was analysed using generalised linear regression modelling (GLM).   |
| Research sample                   | <p>The 174 combinations of paired-stressor effects on a biological response variable were sampled from 33 mesocosm experiments, 14 river basin and 22 cross-river basin studies.</p> <p>The manipulative experiments were conducted within the framework of the European research-project MARS, 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).</p> <p>The MARS project also contributed data on 14 river basin studies selected to cover the main European regions and their representative stressor combinations. Based on harmonised analytical protocols the multi-stressor effects were analysed using comprehensive datasets derived from regional monitoring programmes. 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 2,086 samples (median number of samples per basin: 97, range: 19 to 525).</p> <p>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 scale to investigate biological effects of various stressors. 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).</p> |
| Sampling strategy                 | n/a  |
| Data collection                   | <p>The co-authors provided the data on the regression models based on the internal guidance document of Chapman et al. (2017): Analysing stressor-response relationships and interactions in multi-stressor situations: a WP6 guidance document. <a href="https://tinyurl.com/y4ac5wus">https://tinyurl.com/y4ac5wus</a></p> <p>This process allowed for a harmonised and streamlined data collection.</p>   |
| Timing and spatial scale          | n/a  |
| Data exclusions                   | Regression model residuals were examined for correlation to the fitted values and deviation from the normal distribution (Shapiro-Wilk Test). We excluded 28 models where residuals were correlated with fitted values ( $R > 0.35$ ) and non-normally distributed. Model fit was evaluated as the marginal $R^2$ , i.e. the proportion of variance explained by the models fixed effects, ignoring the contribution of any random effects. We excluded only models with marginal $R^2 < 0.05$ . Model fixed effects (main effects of both stressors and their interactions) were evaluated from the standardized partial regression coefficients and their significance (t Test).   |
| Reproducibility                   | n/a  |
| Randomization                     | n/a  |
| Blinding                          | n/a  |
| Did the study involve field work? | <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No  |

## Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.



## Materials & experimental systems

| n/a                                 | Included in the study                                |
|-------------------------------------|--|
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Antibodies                  |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Eukaryotic cell lines       |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Palaeontology               |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Animals and other organisms |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Human research participants |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Clinical data               |

## Methods

| n/a                                 | Included in the study                           |
|-------------------------------------|---|
| <input checked="" type="checkbox"/> | <input type="checkbox"/> ChIP-seq               |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Flow cytometry         |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> MRI-based neuroimaging |