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## How diatom-, invertebrate- and fish-based diagnostic tools can support the ecological assessment of rivers in a multi-pressure context: Temporal trends over the past two decades in France

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1 **TITLE PAGE**

2 **How diatom-, invertebrate- and fish-based diagnostic tools can support the ecological**  
3 **assessment of rivers in a multi-pressure context: temporal trends over the past two**  
4 **decades in France**

5

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8

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## 1 **ABSTRACT**

2 The degradation of aquatic ecosystems, induced by worldwide intensification in the use of  
3 both land and aquatic resources, has highlighted the importance of managing water quality  
4 and the critical role of innovative diagnostic tools for defining robust management responses  
5 to anthropogenic pressures. In this study, we simultaneously analyzed the outputs of three  
6 diagnostic tools. Each diagnostic tool estimated the impairment probabilities by any given  
7 anthropogenic pressures in French shallow streams using taxonomy- and/or trait-based  
8 metrics of three biological compartments: benthic diatoms, macroinvertebrates and fishes.  
9 Our objective was to explore how the simultaneous analysis of the outputs of the three tools  
10 could be used to (i) disentangle the effects of multiple pressures and (ii) provide policy-  
11 relevant information for stream managers and decision makers. A large proportion of the  
12 impairment probabilities exhibited no significant temporal patterns over the two decades  
13 (61.5% to 87.8 %, depending on the used tests). Among time series exhibiting significant  
14 change, positive shifts in mean and slope as well as in general trends (i.e. degradation)  
15 occurred respectively in 4.6 %, 2.1 % and 6.9 % of the time series and were less frequent than  
16 negative ones (7.6 %, 4.7 % and 15.6 %, respectively), indicating a modest improvement in  
17 water quality over the study period. Biological compartments presented contrasted responses  
18 over time; none, one, two or three compartments exhibiting a significant temporal trend for  
19 the same site and pressure category. Convergent temporal responses over the three biological  
20 compartments were detected according to the pressure category (hydromorphology vs. water  
21 chemistry) and regional context (e.g., lowland plains vs. mountains, pristine vs. agricultural  
22 regions). Altogether, this study advocates that enhancing the integration of a variety of  
23 information on natural and anthropized environments should be a priority prior to implement  
24 evidence-based sustainable conservation and restoration actions.

25

26 **KEYWORDS:** ecological risk assessment; bioindicator; functional trait; hydromorphology;

27 machine learning; water chemistry

28

## 29 **1. Introduction**

30 Worldwide intensification of the use of both land and aquatic resources has induced an  
31 increasing degradation of aquatic ecosystems from local to global scales (Carpenter et al.  
32 1992; Dudgeon 2000; Vaughan & Ormerod 2012; Castello & Macedo 2016). Expanding  
33 urbanization (Kail et al. 2012; Luo et al. 2018), agricultural intensification (Lange et al.  
34 2014), deforestation (Leitão et al. 2018), water abstraction (Benejam et al. 2010; Matthaei et  
35 al. 2010), habitat degradation, homogenization or fragmentation (Kruk 2007; Hamstead et al.  
36 2019; Merg et al. 2020), overharvesting of (biological) resources (Embke et al. 2019),  
37 invasive species (Hermoso et al. 2011), have led to a drastic increase in the intensity and the  
38 diversity of anthropogenic pressures simultaneously driving changes in local biotic  
39 communities (Statzner & Bêche 2010; Mondy & Usseglio-Polatera 2013; Radinger & Wolter  
40 2015; Haase et al. 2019; Dézerald et al. 2020).

41

42 These various co-occurring, and often interacting, pressures elicit a complex range of  
43 deleterious effects on aquatic organisms (Gandar et al. 2016; 2017), taxonomic and trait  
44 diversity of communities (Dolédec & Statzner 2008; Archambault et al. 2010; Lainé et al.  
45 2014; Mondy et al. 2016; Flourey et al. 2017; Smeti et al. 2019; van Klink et al. 2020),  
46 ecosystem functioning (Smeti et al. 2019) and ecosystem services (Cheng & Li 2020) over  
47 multiple spatial and temporal scales (Feld & Hering 2007; Friberg 2014; Villeneuve et al.  
48 2018; Tóth et al. 2019). The persistence of populations in changing environments indeed  
49 greatly depends on their ability to cope with the interactive effects of different stressors  
50 occurring simultaneously or sequentially over various time scales (Christensen et al. 2006;  
51 Downes 2010; Bouraï et al. 2020). The effects on communities are often difficult to predict  
52 due to the combination of direct lethal or sublethal effects on organisms and indirect  
53 functional effects that percolate in various ways through food webs (Haase et al. 2019). Some

54 of them need several days, months or years for inducing detectable shifts in the taxonomic  
55 and functional composition of biotic assemblages, both in a degradation or restoration context  
56 (Arce et al. 2014; Friberg et al. 2014; Funnell et al. 2020).

57

58 This critical context has highlighted the importance of managing ecosystem quality and the  
59 urgent need for developing innovative diagnostic tools supporting the selection of robust  
60 management responses to anthropogenic pressures. Most of recent methods of ecosystem  
61 ecological assessment have been based on multimetric indices that are far more consistent in  
62 the detection of ecosystem impairment in a multi-pressure context than single metrics (Mondy  
63 et al. 2012; Verdonschot et al. 2012; Friberg 2014). Nevertheless, these ones poorly feed the  
64 ecological diagnostic in precise information on the exact nature of the pressures really  
65 impairing local communities within the cocktail of potential pressures (Mondy et al. 2012),  
66 especially at large spatial scale (Lemm et al. 2019). The major goal of such community-based  
67 multi-metric indices is indeed to provide an efficient evaluation of the main impact of  
68 anthropogenic pressures impairing the monitored ecosystems, but not to precisely disentangle  
69 the effects of co-occurring multiple stressors on ecosystem structure and functions. As a  
70 result, many authors have recently underlined the need to focus research efforts on developing  
71 conceptual frameworks and diagnostic tools, capable of disentangling such complex effects of  
72 multi-pressures (Feld et al. 2016; Pistocchi et al. 2017; Baattrup-Pedersen et al. 2019). These  
73 diagnostic tools, complementary to traditional biotic indices, recently developed for more  
74 precisely identifying the nature of the anthropogenic pressures significantly impairing  
75 ecosystems, have already proved their efficiency, robustness and flexibility in assessing  
76 specific risks of river degradation under multi-pressure scenarios using a given biological  
77 compartment (e.g., benthic diatoms, macroinvertebrates, fishes), over various spatial and  
78 temporal scales (Mondy et al. 2016; Baattrup-Pedersen et al. 2017; Waite et al. 2019).

79

80 Most of these diagnostic tools are trait-based, firmly grounded upon the hypothesis that the  
81 habitat is a flexible templet for the selection of ecological strategies (Southwood 1977, 1988;  
82 Townsend & Hildrew 1994). As a result, the multiple combinations of species functional traits  
83 (i.e., the morphological, physiological and behavioral characteristics of organisms) in natural  
84 communities arises from evolutionary “trade-offs” of costs versus benefits in the process of  
85 population/community adaptations to the spatial and temporal variability of their environment  
86 (Southwood 1977). Two major processes drive this functional trait selection in local species  
87 assemblages: competition (e.g., with non-native species) and habitat filtering  
88 (HilleRisLambers et al. 2012). The latter results from various natural environmental filters  
89 acting at different nested scales (from reach to ecoregion) and anthropogenic pressures (e.g.,  
90 nutrient pollution, toxic contamination, hydromorphological alteration, ecological continuity  
91 disruption) that are able to modify natural environmental filtering and dispersal-based  
92 processes of community assembly. Both selective processes are leading to combinations of  
93 traits that are often specific to a given pressure category (Dolédec et al. 2011; Colas et al.  
94 2013; Mondy et al. 2016) and could be used in turn to inform on the major causes of  
95 community impairment.

96

97 Responses to natural and anthropogenic pressure gradients can differ between biological  
98 compartments, water categories, pressure types and levels (Carlisle et al. 2008; Heino 2010;  
99 Hering et al. 2013; Waite et al. 2020). For example, if benthic diatoms, macrophytes,  
100 macroinvertebrates and fishes all exhibited clear responses to eutrophication or organic  
101 pollution (Hering et al. 2006; Marzin et al. 2012), they respond diversely to micropollutant  
102 contamination, hydrological regime alteration, reach scale hydromorphological degradation or  
103 land use changes (Hering et al. 2006; Marzin et al. 2012, 2013; Mondy & Usseglio-Polatera

104 2013; Larras et al. 2017; Dézerald et al. 2020). Moreover, due to lower migratory capacities  
105 and shorter life-cycles, benthic diatom and macroinvertebrate assemblages seem to react to  
106 lower levels of pressure (especially if related to water quality) than fish assemblages, giving  
107 them a higher capacity to be used as early warning indicators of local degradation (Hering et  
108 al. 2006; Marzin et al. 2012; Mangadze et al. 2016), even if contrasted results have been  
109 obtained (Johnson et al. 2006). In contrast, fish assemblages, exhibiting longer generation  
110 times, might be better late warning indicators for monitoring long-term environmental  
111 changes (Hering et al. 2006). This variety of response patterns justifies the selection of a  
112 multi-organism group approach to optimally diagnose stream impairment. However, to our  
113 actual knowledge, no studies have simultaneously analyzed temporal trends in both  
114 taxonomic and bio/ecological responses of several biological compartments to a wide range of  
115 anthropogenic pressures from reach to watershed scale over both a large biogeographic area  
116 and a long period (10+ years).

117

118 In this context, we have developed a series of models individually able to estimate the risk of  
119 stream impairment by a given category of anthropogenic pressure from functional and/or  
120 taxonomy-based characteristics of different biological compartments: benthic diatoms (Larras  
121 et al. 2017), macroinvertebrates (Mondy & Usseglio-Polatera 2013) and fishes (Dézerald et al.  
122 2020). These models have provided good impairment risk assessment for various pressures  
123 related to water quality (e.g., organic matter, nitrates, other nitrogen compounds, phosphorous  
124 components, pesticides) or hydromorphology and land-use alterations (e.g., riverine  
125 vegetation alteration, urbanization at the reach scale, clogging risk or hydrological instability),  
126 even at moderate risk level and in a multi-pressure context, from headwaters (Mondy &  
127 Usseglio-Polatera 2013; Larras et al. 2017) to very large rivers (Desrosiers et al. 2019;  
128 Dézerald et al. 2020).



129

130 In this study, we have illustrated how these cost-effective diagnostic tools are capable of (i)  
131 disentangling the effects of multiple pressures and (ii) providing policy-relevant information  
132 for stream managers and decision makers. Based on benthic diatom, macroinvertebrate and  
133 fish assemblages simultaneously surveyed on several hundred of sites over the 2004-2017  
134 period with standardized protocols, we have (i) examined spatio-temporal variation of  
135 impairment risk for ten categories of chemical and four categories of hydromorphological  
136 pressures in a multi-pressure context, from hydroecoregional to national scale and small to  
137 large rivers, (ii) highlighted commonalities and differences between biological compartments'  
138 response patterns according to stressor combination, hydroecological region and stream order,  
139 (iii) analyzed the ecological rationale underlying the main observed patterns and (iv) tried to  
140 identify if the observed patterns allow to define priorities in actions that would enable  
141 ecological preservation or restoration of ecosystems within the global change context.

142

## 143 **2. Material and methods**

### 144 *2.1. Benthic diatom, macroinvertebrate and fish data*

145 In France, the Water Framework Directive (WFD) implementation has initiated since 2004  
146 the spatial and temporal survey of diatom, macroinvertebrate and fish communities through  
147 monitoring programs and coordinated national networks. Standardized protocols, following  
148 the recommendations of national and international standards, were used to sample those  
149 biological compartments yearly, between May and October. These protocols were the  
150 normalized Biological Diatom Index sampling protocol (AFNOR 2000), the multi-habitat  
151 sampling protocol for macroinvertebrates (AFNOR 2009) and the standardized electrofishing  
152 protocol (CEN 2003). The diatom, macroinvertebrate and fish communities were sampled at  
153 least once in 4653, 4405 and 2169 sites in mainland France, respectively, between 2004 and

154 2017. The record dataset was thus composed of the abundances of 1816 diatom, 371  
155 macroinvertebrate and 72 fish taxa distributed in 21770, 25374 and 10466 sampling events  
156 (site x sampling date), respectively.

157

158 Literature searches were conducted to build a biological dataset gathering any descriptive  
159 information related to the physiology, morphology, behavior and life history strategy of  
160 organisms (Tachet et al. 2010; Mondy et al. 2012; Larras et al. 2017; Dézerald et al. 2020),  
161 hereafter referred to as “traits”. Traits were resolved into a number of modalities. Some traits  
162 were coded using a fuzzy-coding technique (i.e., scores ranging from 0, indicating no affinity  
163 of the taxon for a given trait modality, to 5, indicating high affinity of the taxon for this trait  
164 modality; Chevenet et al. 1994), whereas others were coded using a full disjunctive system  
165 (i.e., presence/absence of trait modalities). This biological dataset was then combined with the  
166 record dataset (i.e., diatom, macroinvertebrate and fish abundances) to calculate biological  
167 metrics at the community level for each sampling event. These metrics included, for instance,  
168 the relative utilization of each trait modality by taxa within a given assemblage (e.g., the  
169 proportion of taxa within a given size class) or Rao functional diversity indices calculated at  
170 the scale of each characteristic (e.g., the Rao diversity index calculated on the distribution of  
171 organisms in functional feeding guilds). The record dataset was also used to calculate  
172 abundance-based and richness-based metrics describing the taxonomic structure of biotic  
173 assemblages for each sampling event, and taxonomy-based diversity indices calculated at the  
174 community level (e.g., the Shannon diversity index). Although diatoms, macroinvertebrates  
175 and fishes share some biological traits, many of those traits are specific to or described in a  
176 specific way for (e.g., trait modality boundaries for body-size) a given biological  
177 compartment. We therefore calculated the biological and taxonomic metrics for each  
178 community independently, for a total of 409, 216 and 1576 metrics, respectively for diatoms,

179 macroinvertebrates and fishes (see Larras et al. 2017, Mondy & Usseglio-Polatera 2013 and  
180 Dézerald et al. 2020 for more details about the biological characteristics and metrics used for  
181 diatoms, macroinvertebrates and fishes, respectively).

182

## 183 *2.2. Environmental data*

184 Environmental data were obtained for each sampling event by defining 20 pressure categories  
185 that describe impairments of water quality at the site scale (WQ: chemistry; 13 categories;  
186 217 individual parameters taken into account; Table S1) and habitat degradation at various  
187 spatial scales, from site to watershed scale (HD: hydromorphology; 7 categories; 10  
188 individual parameters; Table S2). These pressure categories were selected and defined  
189 according to available information, which dictated our ability to reliably describe pressure  
190 gradients, and their potential relevance for the three biological compartments (diatoms,  
191 macroinvertebrates, fishes; Larras et al. 2017, Mondy & Usseglio-Polatera 2013 and Dézerald  
192 et al. 2020). Each pressure category (WQ or HD) was defined by one (e.g.,  $[\text{NO}_3^-]$  for the  
193 pressure category nitrates) to several tens (e.g., 22 molecule concentrations for the pressure  
194 category insecticides) of individual parameters, each of which was described according to two  
195 pressure levels (low vs. significant). For parameters related to WQ pressure categories,  
196 pressure levels were assessed by comparing the mean value of individual parameters averaged  
197 over one year with the “good/moderate” quality class boundary of the French water quality  
198 assessment system (i.e., SEQ-Eau V2.0; Oudin & Maupas, 2003), allowing to distinguish  
199 “low” (high/good) from “significant” (moderate/poor/bad) pressure levels. For nitrates, no  
200 good/moderate boundary was proposed by the SEQ-Eau V2.0 and we fixed this one to 10 mg  
201  $\text{NO}_3/\text{L}$ . For parameters related to HD pressure categories, pressure thresholds were defined  
202 according to expert judgements and the literature. The HD pressure levels were evaluated  
203 only once for the whole studied period due to limited information (Mondy & Usseglio-

204 Polatera 2013; Larras et al. 2017; Dézerald et al. 2020). Then, the pressure level allocated to a  
205 given site for a given pressure category was the worst pressure level over all the individual  
206 parameters taken into account for characterizing this pressure category (i.e., following the one  
207 out, all out aggregation rule; Hering et al. 2010). Finally, the environmental data also  
208 included, for each site, information on the stream location (identity of the hydroecoregion  
209 (HER) according to Wasson et al. (2002); n = 22) and size (stream order according to the  
210 Strahler number; n = 8).

211

### 212 *2.3. Random forest models and model performance*

213 Diagnostic tools have been developed to predict the impairment probabilities of diatom  
214 (Larras et al. 2017), macroinvertebrate (Mondy & Usseglio-Polatera 2013) and fish  
215 compartments (Dézerald et al. 2020) by each WQ and HD pressure category to help  
216 identifying specific risks of stream degradation in a multi-pressure context. They consisted in  
217 Random Forest (RF) models, a particular type of machine learning method able to investigate  
218 complex and potentially non-linear relationships (Breiman 2001). One RF model was built  
219 per biological compartment and pressure category taking the pressure level (low vs.  
220 significant) as the response variable and the biological metrics as the predictive variables. The  
221 diagnostic tools have been updated for this study based on the largest available datasets (see  
222 the above description of the record dataset and environmental data) in order to optimize the  
223 comparability of the model settings (i.e., identical pressure integration time, multi-stress  
224 context consideration, similar definition of the boundary between significant vs. non-  
225 significant risk; Tables S3, S4 and S5). We parametrized the RF models following recently  
226 published setting parameters to maximize both robustness and performance of models (e.g.,  
227 1500 classification trees per forest, random selection of 25 biological metrics at each node in  
228 individual trees; Larras et al. 2017, Dézerald et al. 2020). Based on the biological metric

229 values, each biological compartment- and pressure-specific model therefore provided, for  
230 each sampling event, a mean impairment probability (IP) calculated over all the trees defining  
231 the corresponding forest.

232

233 To assess model performances, each RF model was built on a learning dataset and then tested  
234 against a test dataset (Mondy & Usseglio-Polatera 2013; Larras et al. 2017; Dézerald et al.  
235 2020). For each biological compartment, learning and test datasets corresponded to  
236 independent subsamples of the input databases, i.e., the biological metrics and pressure data  
237 measured for all the sampling events. To take into account the temporal correlations among  
238 sampling events within a site, all the sampling events corresponding to a given site were  
239 randomly allocated either to the learning or to the test dataset (75% and 25% of the sampling  
240 events, respectively). Model performance was then evaluated with the Area Under the ROC  
241 Curve (AUC; Fawcett 2006). The AUC values indicate “good” model performances if they  
242 are equal to or higher than 0.70 (i.e., maximizing both sensitivity and specificity; Pearce and  
243 Ferrier 2000). We applied this AUC criterion to assess model performances using the test  
244 datasets. In subsequent analyses, we only used the IPs related to the pressure categories for  
245 which the three biological compartment-specific RF models exhibited an  $AUC > 0.70$   
246 (Supplementary Material, Tables S3-S5). Consequently, 14 pressure categories were retained,  
247 namely acidification, fungicides, insecticides, nitrates, nitrogen compounds (except nitrates),  
248 organic matter, PAH, phosphorus compounds, PCB, suspended particles (WQ), and  
249 catchment anthropization, clogging risk, hydrological instability and transportation facilities  
250 (HD; Tables S1 and S2).

251

252 *2.4. Temporal changes in impairment probabilities*

253 One of our goals was to evaluate long-term changes in IPs. From the initial database, we  
254 selected, for each biological compartment independently, all the time series (i.e., site x  
255 biological compartment x pressure category) that satisfied two conditions: (i) to have at least  
256 eight years between the first and the last sampling dates, and (ii) to have at least five  
257 sampling. The two selection criteria applied represented a trade-off between maximizing the  
258 amount of candidate time series and minimizing the uncertainty in the information contained  
259 in the database. The mean of IP values over each year (in case of multiple surveys during a  
260 given year) was calculated. Overall, this restricted dataset was composed of 47040 time series  
261 (i.e., 10556 for diatoms, 21560 for macroinvertebrates, and 14924 for fishes).

262

263 In the restricted dataset, the temporal changes in IPs within each time series were  
264 characterized according to ten temporal metrics. All these temporal metrics have been  
265 discretized into categorical variables (i.e., from three to 13 ordinal or nominal modalities).  
266 More details on the modalities, the definition and the interpretation of temporal metrics can be  
267 found in Table 1. These temporal metrics have been distributed in three groups: the  
268 descriptive statistics, the shifts and the trends. The descriptive statistics included the mean and  
269 standard deviation of IPs over time. The shifts were estimated using two parametric tests that  
270 evaluate one probable abrupt change in the mean (Buishand test; Buishand 1982) and slope  
271 (Davies test; Davies 2002) of IP values over time. We assessed the general trend over the  
272 study period by a linear regression on the IP values against time. For a finer understanding of  
273 temporal changes in IPs, we further distinguished the direction from the magnitude of  
274 potential changes, estimated by the last two groups of metrics (i.e., shifts and trends). Finally,  
275 to reduce the potential biases induced by an unbalanced design (i.e., numbers of sampling  
276 sites different among biological compartments), we performed all the statistical analyses on a  
277 final dataset ( $n = 13104$  time series), which represented all the combinations between the

278 three biological compartments (i.e., diatoms, macroinvertebrates and fishes), 14 pressure  
279 categories, and 312 sampling sites (common to the three compartments) distributed across  
280 mainland France (Fig. 1). Since most time-series analyses require data with complete  
281 observations, linear interpolations were applied to estimate missing IP values between two  
282 sampling dates with available IP values. In this final dataset, the number of missing IP values  
283 varied from zero (n = 5446 time series) to seven (n = 14 time series) with a mean ( $\pm$  SE) of  
284  $1.97 \pm 0.02$  missing IP values per time series. Overall, 16.5 % of IP values were interpolated.

285

## 286 *2.5. Statistical analyses*

287 A permutational multivariate analysis of variance allowed us to investigate the drivers of  
288 temporal changes in IPs (PERMANOVA; Anderson 2001). Given that the values of the  
289 quantitative temporal metrics had been discretized (Table 1), PERMANOVA was based on  
290 the pairwise dissimilarities between time series using the Gower's distance. In this analysis,  
291 the biological compartment, the pressure category, the HER, the stream order and all the two-  
292 way interactions were used as predictors. Note that since our goal was to investigate general  
293 temporal patterns, more idiosyncratic responses, which are represented by higher-ordered  
294 interactions, were not evaluated. PERMANOVA is a particularly well suited method to  
295 evaluate the differences in multivariate locations among groups (e.g., biological  
296 compartments, pressure categories), which can be assimilated to the comparison of the  
297 average composition of groups in terms of time series characteristics. However,  
298 PERMANOVA fails to identify potential differences in intra-group variability. To this end,  
299 we used the analysis of homogeneity of multivariate group dispersions (PERMDISP;  
300 Anderson 2006) with the same model configurations as in PERMANOVA. In order to  
301 visualize the most significant patterns in the data, we used a Nonmetric Multidimensional  
302 Scaling (NMDS) with stable solution from random starts.

303  
304 Spatial variations of temporal metrics of interest were investigated, focusing on the mean IPs  
305 and the directions of both linear regressions and Davies tests (shift in slope; Table 1). For  
306 each HER, and all the biological compartments being pooled, the relative frequencies of time  
307 series over the different modalities of each temporal metric were calculated. Based on these  
308 frequencies, the HERs were hierarchically clustered using Euclidean distances and the Ward  
309 linkage method (Ward1; Murtagh & Legendre 2014). Relative frequencies were then  
310 visualized through heatmaps, with dendrograms adding information on the clusters of HERs.  
311 All the pressure categories were simultaneously considered for the mean IPs, whereas five  
312 pressure categories (clogging risk, nitrates, phosphorous compounds, fungicides and PCB,  
313 chosen based on the NMDS results) were independently considered for the directions.

314  
315 All the statistical analyses were performed within the R software (R Core Team 2018) using  
316 the *ecodiag* package (for RF-based diagnostic tools; currently hosted on GitHub;  
317 <https://github.com/CedricMondy/ecodiag>), the *cluster* package (for dissimilarity matrices;  
318 Maechler et al. 2019), the *vegan* package (for PERMANOVA and NMDS; Oksanen et al.  
319 2019), the *RVAideMemoire* package (for PERMDISP; Hervé 2020) and the *gplots* package  
320 (for heatmaps; Warnes et al. 2020).

321

### 322 **3. Results**

#### 323 *3.1. Temporal changes in impairment probabilities*

324 The time series (n = 13104) constituting our final dataset were unevenly distributed across 21  
325 HERs (min. = 42 [HER13], max. = 2268 [HER5], mean  $\pm$  SE = 624  $\pm$  129) and the eight  
326 stream orders (min. = 84 [stream order 8], max. = 4116 [stream order 3], mean  $\pm$  SE = 1638  $\pm$   
327 508). Due to sampling design, none of the sites, and therefore time series, from the HER19  
328 had information on all three biological compartments (Fig. 1).



329

330 Overall mean IP of individual time series varied from 0.0001 to 0.9887 (mean  $\pm$  SE = 0.2961  
331  $\pm$  0.0020) while standard deviations varied from 0.0000 to 0.4092 (mean  $\pm$  SE = 0.0751  $\pm$   
332 0.0004). A small fraction of the time series showed a significant temporal trend according to  
333 the linear regressions of IPs as a function of time, with positive regressions (meaning  
334 degradation of the ecological quality; 6.9 %) being less frequent than negative ones (meaning  
335 improvement of the ecological quality; 15.6 %; Table 2). The shift detection tests also  
336 indicated that only a small fraction of time series displayed at least one significant shift in  
337 either the mean (Buishand test) or slope (Davies test) of IPs over time (Table 2). Significant  
338 positive shifts in means and in slopes of IPs (meaning degradation) occurred respectively in  
339 4.6 % and 2.1 % of the time series and were less frequent than negative ones (meaning  
340 improvement), respectively 7.6 % and 4.7 % (Table 2). The significant shifts in mean IPs  
341 occurred most often in 2010 (Buishand test), whereas the significant shifts in slopes occurred  
342 mainly in 2009 (Davies test; Table 3).

343

344 Shifts in means and slopes, and general trends (from the linear regressions) of IPs were  
345 further described in terms of magnitude. Overall, among the time series exhibiting significant  
346 response patterns, a small majority displayed a high magnitude of change. Precisely, 7.0 %,  
347 21.1 %, and 12.9 % of the time series displayed low magnitude of shift in mean and slope and  
348 of general trend, respectively, while 5.2 %, 17.4 %, and 9.6 % exhibited high magnitude  
349 (Table 2).

350

### 351 *3.2. Variance partitioning of temporal changes in impairment probabilities*

352 Most of the explained variance (EV) was generated by differences (i.e., distances between  
353 group centroids) among biological compartments (3.55 %), pressure categories (3.59 %), and

354 by the interaction between pressure categories and HERs (4.42 %; PERMANOVA; Table 4).  
355 Residual variance represented 78.20 % of the total variance. It must be noted that since the  
356 number of time series was unevenly distributed among HERs and stream orders, the results of  
357 the PERMANOVA including those factors should be interpreted with caution. The  
358 PERMDISP results indicated that among the main sources of variation, the main effects of the  
359 biological compartment (5.69 %), and its interactions with pressure category (7.15 %), HER  
360 (8.16 %), and stream order (6.54 %) explained the largest fraction of the total variance in  
361 community dispersion (average distance to group centroids; Table 4).

362

363 The directions of (i) shifts in slope (Davies test; Fig. 2A), (ii) general trends according to  
364 linear regression (Fig. 2B), and (iii) shifts in mean IPs (Buishand test; Fig. 2C) within time  
365 series were shown on the first factorial plane of the NMDS. Besides, Fig. 2D highlights areas  
366 of this factorial plane with a high number of superimposed time series. The time series were  
367 mainly discriminated along the first axis (NMDS1) according to the results of the Davies test.  
368 The more negative NMDS1 values were associated to non-significant shifts in slope (*none*  
369 modality) whereas positive NMDS1 values were associated to other modalities (either  
370 *pos.pos*, *neg.neg*, *pos.neg* or *neg.pos* modalities; Fig. 2A). In contrast, the time series were  
371 discriminated according to the results of the linear regressions along the second axis  
372 (NMDS2). The more negative values were associated to either a positive or negative trend  
373 (Fig. 2B), whereas the more positive values were associated to non-significant linear trends.  
374 More contrasted results were observed for the distribution of modalities related to the  
375 direction of shifts in mean IPs (Buishand test; in Fig. 2C). In each of the four main aggregates  
376 of time series highlighted by the NMDS first factorial plane and structured by their patterns of  
377 response to the Davies test and linear regression trend, time series with significant shift  
378 identified by the Buishand test exhibited higher coordinates along NMDS1, but lower

379 coordinates along NMDS2, than time series without significant shift (Fig. 2C). This contrast  
380 (significant vs. non-significant Buishand test) seemed a bit more important for time series  
381 exhibiting a negative (in blue) than positive (in orange) shift in mean IP values.  
382

383 To illustrate differences in the distances among (PERMANOVA) and within (PERMDISP)  
384 groups (Table 4), the centroids and dispersions of the four sources of variation (biological  
385 compartment, pressure category, HER and stream order) have been also provided on the first  
386 NMDS factorial plane (Fig. 3 and Fig. S1). The temporal variation in IPs observed for the fish  
387 compartment was comparatively more distinct (fish centroid was the most distant to other  
388 group centroids) and more variable (larger ellipses) than those observed for the  
389 macroinvertebrate and diatom compartments (Fig. 3A). A comparison of Fig. 2A and Fig. 3A  
390 indicated that fish communities may exhibit more significant shifts in slope (Davies test) than  
391 macroinvertebrate and diatom communities. The centroids of the hydromorphological  
392 pressure categories were negatively located along NMDS1 and positively located along  
393 NMDS2 (Fig. 3B) and thus seemed to be often associated to the *none* modality of direction  
394 metrics (i.e., to non-significant tests; Fig. 2). Regarding the chemical pressure categories, the  
395 centroids were also discriminated along NMDS1 and NMDS2 (Fig. 3B) but they seemed to be  
396 more often associated to modalities of significant changes in direction metrics (Fig. 2). The  
397 centroids of the pressure categories insecticides, PAH and suspended particles were  
398 negatively located along NMDS1 and NMDS2, whereas those of the pressure categories  
399 acidification, organic matter and PCB were negatively located along NMDS2, but positively  
400 located along NMDS1. The centroids of the other four chemical pressures (i.e., fungicides,  
401 phosphorous compounds, nitrates, nitrogen compounds) were positively located along both  
402 NMDS1 and NMDS2. The lowest and highest dispersions in temporal responses were related  
403 to hydrological instability and PCB, respectively (Fig. 3B). The centroids of the temporal

404 responses corresponding to the different stream orders were clearly discriminated along  
405 NMDS1 (Fig. 3C). For example, small streams (stream orders 1 and 2) seemed to be more  
406 prone to significant shifts in slope (centroids were more positively distributed along NMDS1)  
407 but their temporal responses were also more variable (larger ellipses) than those of larger  
408 streams (stream orders 7 and 8; Fig. 2A and 3C). No clear between-HER pattern was observed  
409 along NMDS1 and NMDS2. Between-HER differences were observed, but it was difficult to  
410 relate them to the characteristics of the HERs (Fig. S1). Finally, other temporal metrics  
411 (direction of shifts in mean IPs according to the Buishand test, quantiles of the mean and  
412 standard deviation of impairment probabilities), as well as the group centroids and dispersions  
413 of the biological compartments, pressure categories and stream orders are shown along the  
414 NMDS3 and NMDS4 in Fig. S2 and S3.

415

### 416 *3.3. Spatial distribution of temporal changes in impairment probabilities*

417 Hierarchical cluster analysis revealed four groups of HERs based on their mean IP values  
418 across all the time series, corresponding to the different combinations of biological  
419 compartment x pressure category (Fig. 4; see also Fig. 1A for the spatial distribution of the  
420 HERs). This underpins the previously observed spatial effect on the response patterns of  
421 metrics (EV = 3.16% for HER; Table 4). The first group of HERs, located at the top of the  
422 dendrogram (in blue on Fig. 4; HER1, 2, 4, 7 and 8), was composed by HERs globally  
423 exhibiting low IPs. The second group of HERs (in green on Fig. 4; HER3, 5, 6, 16 and 21)  
424 exhibited intermediate mean IPs. Conversely, the remaining two other groups, with six (in  
425 orange on Fig. 4; HER9, 10, 12, 13, 20 and 22) and five HERs (in red on Fig. 4; HER11, 14,  
426 15, 17 and 18) globally exhibited the highest mean IPs whatever the pressure category. The  
427 fourth group displayed higher mean IP values than the third group for transportation facilities,

428 hydrological instability, phosphorous compounds and insecticides, but lower mean IP values  
429 for nitrates and acidification.

430

431 Such a spatial effect was also obvious when focusing on the temporal trends (direction of the  
432 linear regressions and Davies tests) of pressure categories such as clogging risk, nitrates,  
433 phosphorous compounds, fungicides and PCB (Fig. 5). The contrasted locations of their  
434 centroids on the NMDS1-NMDS2 plane (Fig. 3B) suggested indeed the differences among the  
435 most important temporal changes in metric values. Linear regressions of the IP values as a  
436 function of time for phosphorous compounds and PCB exhibited negative directions in almost  
437 all the HERs (*neg* modality on Fig. 5A), suggesting a decrease in IP values (i.e., improvement  
438 in ecological quality) over time for these pressure categories. The pattern was more complex  
439 for clogging risk, nitrates and fungicides, with the time series of sites located in the HER9, 12,  
440 17 and 18 more often exhibiting an IP increase than an IP decrease over the study period in  
441 contrast to most of the other HERs. Whatever the pressure category, HERs of the “red” and  
442 “orange” groups on Fig. 4 are those exhibiting an IP increase over the study period (*pos*  
443 modality on Fig. 5A; i.e., degradation in site conditions).

444

445 The variations in direction of change identified by the Davies tests (shifts in slope; Fig. 5B)  
446 have shown that the temporal pattern of change in IPs may have been more complex than  
447 what was suggested by the results of the linear regressions, at least for some pressure  
448 categories. For instance, for the phosphorous compounds and PCB, both the *neg.pos* and  
449 *pos.neg* patterns of response were relatively frequent in all these HERs, suggesting that either  
450 the overall decrease (highlighted by the linear regressions) possibly occurred quite early in the  
451 time series (*neg.pos* modality), or later in the time series (*pos.neg* modality), at least for time  
452 series for which both regressions and Davies tests were significant (Table S6). Compared to

453 the other pressure categories, the *neg.neg* modality was also more frequently observed for  
454 phosphorous compounds and PCB, confirming the possibility of a two-step decrease in IP.

455

#### 456 **4. Discussion**

457 We have sought to estimate specific risk of stream impairment by various anthropogenic  
458 pressures from the trait- and taxonomy-based characteristics of benthic diatoms,  
459 macroinvertebrates and fishes. These risks, reflected in the form of IPs, have been estimated  
460 with RF models for each sampling event and pressure category (i.e., one biological  
461 compartment x one site x one sampling date) in a context of multiple co-occurring pressures.  
462 Once calibrated, those models have the advantage of not requiring information on the  
463 chemical or hydromorphological conditions of the tested sites at the local (reach) or regional  
464 (watershed) scale. IPs are thus original, integrated and robust descriptors of local community  
465 responses (both functional and taxonomic) that can help environmental managers in  
466 prioritizing and designing conservation or restoration programs. In this study, we have  
467 diagnosed the overall state of rivers at both local and large spatial scales and over a rather  
468 long period (10+ years) from the simultaneous use of the IPs provided by three biological  
469 compartments.

470

##### 471 *4.1. A typology of temporal changes in impairment probabilities*

472 A large proportion of the time series exhibited no significant temporal patterns (61.5%, 77.5%  
473 and 87.8% of all the time series, according to Davies tests, linear regressions and Buishand  
474 tests, respectively), while significant patterns were mostly negative and of low magnitude,  
475 over the study period (Tables 2 and 3; Fig. 2). Such results suggest the existence of a global  
476 and continuous, but modest, improvement in environmental conditions in many French  
477 shallow streams. These local improvements in site conditions are in line with recent scientific

478 findings in France (Marzin et al. 2012; Dézerald et al. 2020), in Europe (Grizzetti et al. 2017;  
479 EEA 2018) and more generally with the relatively recent and increasing societal concerns  
480 towards environmental issues through the implementation of novel legislation and  
481 management actions (European Commission 2000).

482

483 Nevertheless, the widespread absence of significant patterns may not necessarily reflect the  
484 absence of temporal responses to environmental changes. Interpreting these results requires  
485 recasting the temporal window of the times series that covers the last two decades within the  
486 larger context of human impacts on lotic ecosystems. Major human modifications of running  
487 waters (hydrology, morphology, water chemistry) have indeed started long before the onset of  
488 the studied time series (Allan 1995; Malmqvist & Rundle 2002). In this sense, most of the  
489 local communities may have already adapted to past pressure events before the study period  
490 (Lytle & Poff 2004; Nodine & Gaiser 2015 for diatoms).

491

492 When a significant shift has been identified in a time series, our approach offers also the  
493 opportunity to specifically pinpoint this shift. A larger proportion of time series displayed a  
494 significant shift in mean IP values in 2010 (3.5%; Buishand tests) or in slope values in 2009  
495 (5.4%; Davies tests; Table 3). These general patterns in shifts were, however, difficult to  
496 interpret and potential cause-effects relationships may remain unclear. For instance, a  
497 discernable biological response occurring in a given year 'N', resulting from a major  
498 disturbance that occurred in the year 'N-2' (time-lag effect), may be wrongly attributed to a  
499 smaller disturbance that occurred in the year 'N-1'. Similarly, a general pattern could hide  
500 multiple causes (Coffey et al. 2013; Gessner & Tlili 2016; Villeneuve et al. 2018) that may  
501 only be visible after a detailed analysis of the temporal changes in local conditions (Metzger  
502 et al. 2009); a task however beyond the scope of this study considering the high number of

503 studied sites and time series. Nevertheless, possible (spatial) explanations for such shifts have  
504 been explored in the section 4.3 of this discussion.

505

#### 506 *4.2. Differences in temporal patterns between biological compartments*

507 Overall, the patterns in fish temporal responses to any given pressure were more distinct (i.e.,  
508 larger distance or between-group variance) and more variable (i.e., higher dispersion or  
509 within-group variance) than those of macroinvertebrates and diatoms. Time series involving  
510 fish communities also displayed a higher proportion of significant responses to the Davies test  
511 (i.e., single shift; positive coordinates along NMDS1; 54.6%; n = 4368) than those involving  
512 diatoms (31.3%; n = 4368) or macroinvertebrates (29.6%; n = 4368; Fig. 3A). Such  
513 differences were further highlighted by the interactions between biological compartments and  
514 the other main sources of variation (i.e., pressure category, HER and stream order; Table 4;  
515 see also Fig. S4-S6). For instance, fishes are overall less taxonomically diversified, exhibit  
516 better dispersion capacities, and more efficient escape behaviors once subjected to  
517 disturbances and/or unsuitable conditions than the other two biological compartments. In  
518 contrast, the benthic diatoms evolve mostly on the surface of substrata (e.g., rocks), embedded  
519 in the biofilm matrix (Morin et al. 2016). They have to adapt (genetically and taxonomically)  
520 to the environmental changes. The biofilm matrix has been also known to interact with the  
521 components of the water column such as the organic compounds (Lawrence et al. 2001;  
522 Flemming & Wingender 2010), which may interfere in the exposure of the organisms. These  
523 taxonomic and bio-ecological characteristics would thus make the fish assemblages more  
524 responsive than the diatom assemblages to environmental changes but also potentially more  
525 resilient after the removal of disturbances.

526



527 Further differences in temporal patterns between biological compartments were highlighted  
528 through their interactions with other main sources of variation (Table 4; Fig. S4-S6). For  
529 instance, fish responses to individual pressure categories seemed more homogenous than  
530 those observed for macroinvertebrates, as indicated by the lower dispersion of centroids of the  
531 fishes:pressure interaction in the NMDS1-NMDS2 biplot (Fig. S4). The dispersion of  
532 centroids of the diatoms:pressure interaction was intermediate. These observations suggest  
533 that, although fishes may be more responsive than macroinvertebrates and diatoms, they may  
534 also be less efficient to discriminate the temporal responses to individual pressure categories  
535 compared to the other two compartments. Altogether, these results have highlighted the  
536 complementarity of the temporal responses of individual compartments to the various  
537 pressures categories.

538

#### 539 *4.3. Spatial distribution of temporal responses*

540 Regardless of the identity of the biological compartment, there were some clear differences in  
541 patterns of temporal responses across HERs and pressure categories. Temporal responses  
542 could be readily explained by the land-use characteristics of the HER. For instance, sites  
543 located in plains known for intensive agricultural activities in North-western (e.g., in HER9  
544 and 12; Fig. 1), North (HER20) or North-eastern France (HER10 and 18), exhibited higher  
545 mean IPs and more frequent significant increases in IPs (i.e., an increasing degradation) over  
546 the study period for the pressure categories more directly linked to agricultural activities (such  
547 as clogging risk, nitrates and fungicides; Fig. 4 and 5). Conversely, the sites mainly located in  
548 mountain areas (Pyrénées = HER1; Alps = HER2 and 7 or Vosges = HER4) and/or in the less  
549 anthropized areas (Cévennes = HER8) exhibited lower mean IPs and more frequently either a  
550 relative temporal stability or a significant decrease of IPs over the study period for the same  
551 set of pressure categories.

552

553 The results of the Davies tests (shifts in slope) also suggested that temporal changes in IPs  
554 could correspond to different HER patterns. For instance, the majority of the HERs seemed to  
555 exhibit an overall decrease of IPs by phosphorous compounds, based on linear regressions.  
556 Despite this trend, shift analyses of the same pressure category showed that this overall  
557 decrease may mask a higher diversity of temporal patterns at the HER scale. Some sites  
558 exhibited a response in two steps, with either a quite early improvement (sometimes followed  
559 by a new degradation phase during the most recent years; e.g., see the modality *neg.pos* in  
560 Fig. 5B for HER20 and 22) or a later improvement (after an initial period of degradation; e.g.,  
561 see the modality *pos.neg* for HER7, 11 and 18) during the time series. The reverse is also true,  
562 with increases in IPs (i.e., degradation) frequently linked to the modality *pos.neg* (e.g., sites  
563 located in North-western France in Fig. 6 and 7), highlighting rather recent improvement of  
564 water and habitat quality. Overall, such temporal trends in IPs may underline contrasted  
565 management strategies of these pressure categories: the various entities that manage rivers at  
566 the national, regional and local scales have not necessarily exhibited the same timelines  
567 and/or prioritizations for implementing new national or international directives for water  
568 preservation.

569

570 Our results have also suggested that sites on small streams (stream orders 1 and 2) could  
571 overall exhibit more often significant temporal changes in their IPs than sites on large streams  
572 (stream orders 7 and 8). Such differences between time series of sites located on small vs.  
573 large streams may be explained by different dynamics, sensitivity to pressure changes and  
574 recovering capacities (Allan & Castillo 2007), even more so if the changes are local (e.g., a  
575 sewage discharge) and/or one-time events. We can argue that pressure-driven changes, either  
576 linked to the deterioration or the restoration of water quality and/or habitats, would first

577 impact streams at the local scale, especially if they exhibit a small catchment area and/or a  
578 low discharge, whereas large streams would only exhibit significant changes of their IPs if  
579 pressure changes occurred at the regional scale, over long period and/or if they are extreme  
580 (e.g., the 1986 Sandoz accident which greatly impacted the Rhine river; Plum & Schulte-  
581 Wülmer-Leidig 2014). Our results seemed to empirically confirm that pressure-driven  
582 changes may take more time to occur at the regional scale rather than at the local scale, even  
583 if the success of reach-scale restoration depends highly on the availability, ecological quality  
584 and connectivity of habitats as well as the diversity of potential colonizers at the regional  
585 scale (Nakamura et al. 2005; Verdonschot et al. 2013; Stoll et al. 2016).

586

#### 587 *4.4. Implications for environmental management initiatives and future challenges*

588 The selection of adequate mitigation measures is crucial for the sustainable management and  
589 conservation of ecosystems (Hering et al. 2015) and requires identifying individual pressures  
590 as specific causes of deterioration of communities. Such an approach can help inform decision  
591 makers and water managers about the hierarchy of pressure impacts and the more appropriate  
592 management options (Murphy et al. 2013; Matthaei & Lange 2016), but its application can be  
593 extremely difficult in multi-pressure environments. A widely used conservation and  
594 restoration practice is to consider biotic indices, based on one or more metrics, to assess the  
595 response of either one or multiple biological compartments simultaneously to a limited  
596 number of pressure categories within a single site (Chen et al. 2017; Silva et al. 2017;  
597 Segurado et al. 2018). However, even when several metrics are considered, the number of  
598 metrics remains limited. Based on 297 biotic indices used in Europe, Birk et al. (2012)  
599 estimated that on average two, four and seven metrics had been used to construct diatom,  
600 macroinvertebrate and fish indices, respectively. A too small combination of metrics cannot  
601 precisely capture the variety and complexity of biological responses to multiple co-occurring

602 pressures. The integration of a very large number of metrics in diagnostic tools has improved  
603 the robustness of the estimation of ecosystem impairment in a multi-pressure context  
604 (Verdonschot et al. 2012; Mondy & Usseglio-Polatera 2013; Larras et al. 2017; Dézerald et al.  
605 2020). Moreover, aggregating the differential responses of several biological compartments to  
606 a given pressure category provides complementary information likely to improve  
607 management and conservation programs (Kelly et al. 2016).

608

609 A current trend is thus to propose multi-pressure and multi-compartment approaches for the  
610 evaluation of the ecological status of aquatic ecosystems (Caroni et al. 2013; Herrero et al.  
611 2018). Our study has provided concrete implications for such an approach, because (i) the  
612 time series data have allowed tracking community changes and gaining insights into their  
613 vulnerability to environmental changes and (ii) the diagnosis tools have flagged the presence  
614 of a particular pressure category acting on a given biological compartment, irrespective to  
615 other pressure categories being observed or not. The comparability of models (i) between  
616 biological compartments for a given pressure category and (ii) between pressure categories  
617 for a given compartment, has allowed (i) to compare the spatial and temporal responses to  
618 pressures within and between biological compartments and (ii) to identify the rivers  
619 exhibiting an improvement or a deterioration of the taxonomic and functional structures of  
620 their biological compartments facing a given pressure category, at the regional (i.e., HER) and  
621 local (i.e., sites) scale. For instance, regardless of the biological compartment, linear  
622 regressions have shown a regionalization in the obvious consequences of nitrate management  
623 measures at the biological compartment scale (Fig. 6). In Eastern France, a higher proportion  
624 of sites exhibited an improvement in the taxonomic and functional structures of at least one  
625 biological compartment facing nitrate contamination (i.e., *neg* modality) whereas a trend to  
626 degradation (i.e., *pos* modality) was more frequently observed in sites located in Western

627 France (Fig. 6). Further identification of the temporal trends obtained in particular by the  
628 Davies test has shown which sites, among those with a deterioration in the taxonomic and  
629 functional structures of biological compartments over the study period, should be considered  
630 as priorities because the deterioration was greater (higher magnitude) and/or happened in  
631 more recent years (i.e., after the date of change identified by the Davies test; Fig. 7). Such  
632 river reaches are of special interest to water managers as they exhibit high ecological risk and  
633 should therefore be prioritized in the management initiatives. In a conservation context or  
634 when the first signs of degradation occur, these results could be combined with the  
635 computation of indices assessing the degree of uniqueness of sites in terms of taxonomic and  
636 functional composition (e.g. LCBD index; Legendre & De Cáceres 2013), as well as  
637 information about the location of sites into the hydrographic network at the catchment scale.  
638 Such an approach could facilitate the prioritization of sites and management measures to be  
639 applied (Erős et al. 2011). For example, if a degraded site is located in a poorly connected  
640 area of the watershed (e.g., a headwater stream), the management plan should focus on the  
641 local pressures acting on the reach. Conversely, if a degraded site is located in a highly  
642 connected area of the hydrographic network (e.g., nearby the successive confluences of  
643 several watercourses), it may be necessary to consider management measures at both local  
644 and watershed scales. These results could thus provide information on sites where ongoing  
645 management actions (e.g., related to the implementation of the Directive Nitrates; European  
646 Commission 1991, Directive 91/676/EEC) may have had a positive effect (e.g., *neg* modality  
647 in linear regression) on their ecological status (i.e., improvement) compared to sites where an  
648 effort is still needed to improve their ecological status for a given pressure.

649

650 Biological compartments displayed contrasted responses to pressure categories; none, one,  
651 two or three compartments of a same site exhibiting a significant temporal trend (e.g., for

652 nitrates in Fig. 6 and 7). We may suppose that a management measure for reducing a given  
653 pressure category can cause different effects on different biological compartments. For  
654 instance, the removal of human-made barriers (e.g., weirs, dams) can have a direct positive  
655 effect on the taxonomic and functional structure of fish communities both upstream and  
656 downstream of the former dam, more likely because of the rehabilitation of stream  
657 longitudinal connectivity and the development of more suitable habitats, such as riffles that  
658 are favorable habitats for many riverine fishes (Stanley & Doyle 2003; Cook & Sullivan  
659 2018). At the same time, dam removal can facilitate the spread of undesirable, nonnative  
660 and/or highly invasive species (Doyle et al. 2005; Lejon et al. 2009; Kornis et al. 2015), and  
661 modify the flux and the composition of sediments (Bellmore et al. 2019), which have indirect  
662 negative effects on communities (e.g., diatoms, Thomson et al. 2005; macroinvertebrates,  
663 Carlson et al. 2018). These results can be taken into account by water managers when  
664 adjusting management actions.

665

666 Finally, this study has encountered and identified two main challenges when trying to  
667 integrate the information from multiple biological compartments and pressure categories  
668 across various spatio-temporal scales. The first challenge is related to the quality and quantity  
669 of the information itself. As in any other ecological dataset, the proportion of missing values  
670 represents a major limitation to the use of particular statistical analyses, especially those  
671 involving time-series data. To bypass this limitation, we applied a set of selection criteria of  
672 time series and performed, at last, punctual interpolations of missing data to increase the  
673 completeness of the dataset. Another approach to handle missing data could be an ordination  
674 method (based on the Principal Component Analysis) allowing to distinguish different  
675 mechanisms generating missing data and thus to have an estimation closer to the real values  
676 (Dray & Josse 2015). The second challenge involves the way this information was

677 synthesized. In this study, we used an *a posteriori* approach. We established a simple  
678 typology from the outputs of models built independently for the different biological  
679 compartments. Another approach could consist in building models that directly combine the  
680 taxonomic and functional information brought by all the biological compartments of interest  
681 in order to model IPs based on their simultaneous and asynchronous responses. Although  
682 conceptually more challenging, this latter approach may provide new insights in future  
683 attempts to achieve a more integrative understanding of stream functioning.

684

## 685 **5. Conclusion**

686 Integrating the information about multiple biological compartments and anthropogenic  
687 pressure categories across various spatio-temporal scales into a single comprehensive  
688 framework is an essential task in an era of increasing management of natural resources under  
689 rapid environmental changes. To this end, we have leveraged the power of National databases  
690 (i.e., faunal, floral, hydromorphological and chemical surveys documented over more than ten  
691 years across metropolitan France) by adding taxonomy- and trait-based information to  
692 estimate stream impairment probabilities by multiple pressure categories. The temporal  
693 changes in IPs were further broken down into a simple typology of temporal patterns  
694 according to ten metrics and evaluated in light of the identity of the biological compartments,  
695 pressure categories and environmental conditions (HER and stream order). Our results have  
696 highlighted that combining biological compartment responses provides a real added value to  
697 the ecological diagnostic of streams: (i) fishes displayed more distinct but also more variable  
698 temporal responses to any pressure compared to those of macroinvertebrates and diatoms; (ii)  
699 macroinvertebrates and diatoms exhibited more distinguishable temporal responses than  
700 fishes to the studied pressure categories. In contrast, similar temporal responses among the  
701 three biological compartments were detected according to the pressure category (e.g.,

702 hydromorphology vs. water chemistry) and environmental context (mountain vs. lowland  
703 plain). Finally, this study advocates that enhancing the integration of a variety of informations  
704 on natural and anthropized systems should be a priority when implementing sustainable  
705 conservation and restoration actions.

706

### 707 **Supplementary Online Material**

708 Table S1. Water quality pressure categories and individual chemical parameters

709 Table S2. Habitat degradation pressure categories and individual hydromorphological and  
710 land use-related parameters

711 Table S3. Performances of the original and updated diatom diagnostic models

712 Table S4. Performances of the original and updated macroinvertebrate diagnostic models

713 Table S5. Performances of the original and updated fish diagnostic models

714 Table S6. Number of time series for each combination of modalities from the Buishand test,  
715 Davies test and linear models

716 Figure S1 Non-metric multidimensional scaling displaying the distribution of the centroids  
717 and the dispersions around centroids of hydroecoregions among time series

718 Figure S2 Non-metric multidimensional scaling displaying the distribution of the modalities  
719 of temporal metrics along NMDS3 and NMDS4

720 Figure S3 Non-metric multidimensional scaling displaying the distribution of the centroids  
721 and the dispersions around centroids of the biological compartments, pressure categories and  
722 stream orders along NMDS3 and NMDS4

723 Figure S4 Non-metric multidimensional scaling displaying the distribution of the centroids of  
724 the interaction between the biological compartments and the pressure categories

725 Figure S5 Non-metric multidimensional scaling displaying the distribution of the centroids of  
726 the interaction between the biological compartments and the hydroecoregions



727 Figure S6 Non-metric multidimensional scaling displaying the distribution of the centroids of  
728 the interaction between the biological compartments and the stream orders

729 **TABLES & FIGURES**

730 **Table 1.** Modalities, definitions, and elements of interpretation of temporal metrics describing  
 731 the temporal patterns in time series. Note: a time series (N = 47040) is composed of the  
 732 impairment probabilities (IP) calculated for all the sampling dates of a given site x biological  
 733 compartment x pressure category. The *none* modality was assigned when a given test was not  
 734 significant.

<b>Metrics: modalities; Definition: interpretation</b>	
<b>Descriptive statistics:</b>	<p><b>Mean IP</b> (5 modalities): <i>Q0-Q0.2; Q0.2-Q0.4; Q0.4-Q0.6; Q0.6-Q0.8; Q0.8-Q1</i>            Limits of associated quantiles across all the time series: <i>indicate the overall mean of temporal variations in IP values.</i></p> <p><b>IP Standard Deviation</b> (5 modalities): <i>Q0-Q0.2; Q0.2-Q0.4; Q0.4-Q0.6; Q0.6-Q0.8; Q0.8-Q1</i>            Limits of associated quantiles across all the time series: <i>indicate the overall variability of temporal variations in IP values.</i></p>
<b>Shifts:</b>	
Buishand test	<p><b>Shift point</b> (11 modalities): <i>2005, 2006, 2007, 2008, 2009, 2010, 2011, 2012, 2013, 2014; none</i>            Estimated year: <i>indicates the point in time of a probable change in mean IP</i></p> <p><b>Direction</b> (3 modalities): <i>pos; neg; none;</i>            Sign of the difference in mean IP between the second (after the shift point) and first (before the shift point) period: <i>indicates a probable positive or negative change in mean IP values</i></p> <p><b>Magnitude</b> (3 modalities): <i>high; low; none;</i>            For the significant tests, we took the absolute values of the differences in means on either side of the shift point estimate, we estimated the median of those values and considered as ‘high’ or ‘low’ the values which were above or below the median, respectively: <i>indicates to what extent the mean IP values change after the shift point estimate relative to other times series with significant temporal patterns.</i></p>
Davies test	<p><b>Shift point</b> (13 modalities): <i>2005, 2006, 2007, 2008, 2009, 2010, 2011, 2012, 2013, 2014, 2015, 2016; none</i>            Estimated year: <i>indicates the point in time of a probable change in slope</i></p> <p><b>Direction</b> (5 modalities): <i>pos-pos; neg-neg; pos-neg; neg-pos; none</i>            Signs of the slopes in the periods before and after the shift point: <i>indicates whether the signs of the slopes differ or remain similar before and after the shift point estimate</i></p> <p><b>Magnitude</b> (3 modalities): <i>high; low; none</i>            For the significant tests, we took the mean value of the two absolute slopes on either side of the shift point estimate, we estimated the median of those values and considered as ‘high’ or ‘low’ the values which were above or below the median, respectively: <i>indicates to what extent the slopes of IP values over time change after the shift point estimate relative to other times series with significant temporal patterns.</i></p>
<b>Trends:</b>	
Linear regression	<p><b>Direction</b> (3 modalities): <i>pos; neg; none</i>            Sign of the slope: <i>indicates the sign of the overall trend in IP values over time</i></p> <p><b>Magnitude</b> (3 modalities): <i>high; low; none</i>            For the significant tests, we took the absolute values of the slopes, we estimated the median of those values and considered as ‘high’ or ‘low’ the values which were above or below the median, respectively: <i>indicates to what extent the overall trend in IP values changes relative to other time series with significant temporal patterns.</i></p>

735

736 **Table 2.** Distribution of the time series among the different modalities of the temporal metrics  
737 described in terms of direction and magnitude of change. Within each metric, modalities were  
738 arranged following increasing percentages. The *none* modality was assigned when a given test  
739 was not significant.

	<b>Metrics</b>	<b>Modalities</b>	<b>Percent of time series</b>
<b>Shift in mean:</b> Buishand test	Direction	Pos	4.6
		Neg	7.6
		None	87.8
	Magnitude	High	5.2
		Low	7.0
		None	87.8
<b>Shift in slope:</b> Davies test	Direction	Pos-pos	2.1
		Neg-neg	4.7
		Pos-neg	14.4
		Neg-pos	17.3
		None	61.5
	Magnitude	High	17.4
<b>Trend:</b> Linear regression	Direction	Low	21.1
		None	61.5
		Pos	6.9
	Magnitude	Neg	15.6
		None	77.5
		High	9.6
		Low	12.9
		None	77.5

740

741

742 **Table 3.** Distribution of the time series among the different modalities of the shift points  
 743 detected by the Buishand test (shift in mean) and the Davies test (shift in slope). The *none*  
 744 modality was assigned when a given test was not significant.

745

<b>Modalities:</b>	<b>Percent of time series</b>	
	Buishand test	Davies test
None	87.8	61.5
2006	0.4	4.6
2007	0.4	3.8
2008	1.6	3.4
2009	2.3	5.4
2010	3.5	2.9
2011	1.7	4.7
2012	1.4	3.1
2013	0.7	5.0
2014	0.2	3.3
2015	-	2.2
2016	-	0.1

746

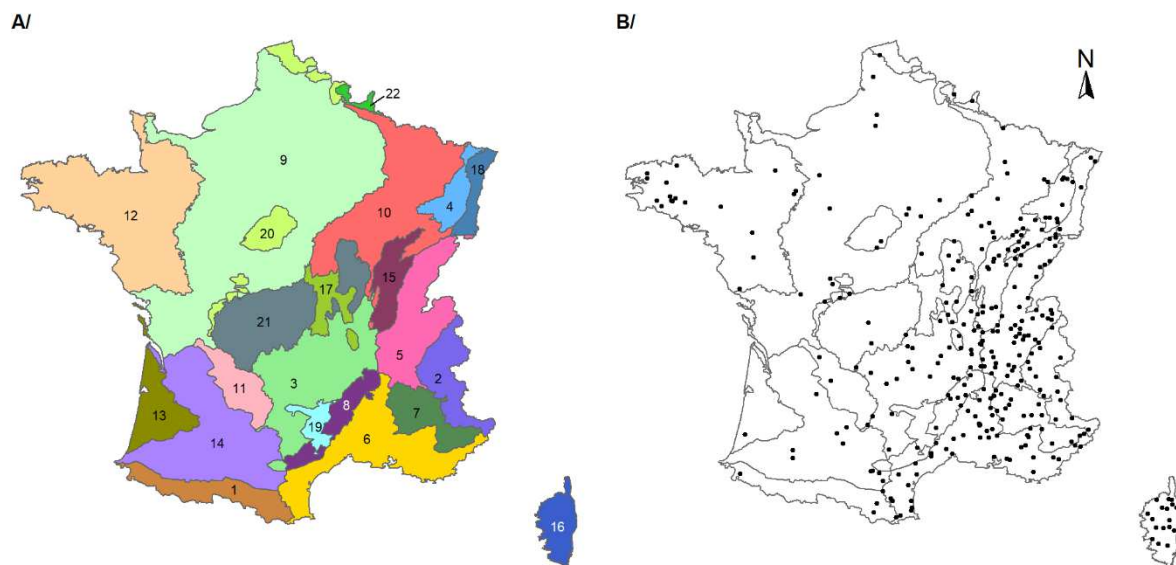
747

748 **Table 4.** PERMANOVA and PERMDISP analyses of the effects of biological compartments,  
749 pressure categories (Pressures), hydroecoregions (HER), stream orders and two-way  
750 interactions on the metrics describing the temporal variations in impairment probabilities of  
751 time series. Notes: SS (MS), Sum of Squares (Mean Squares); Df, degrees of freedom; F,  
752 Pseudo F-statistic by permutation; EV (P), Explained Variance in % (p-value by permutation);  
753 PERMANOVA and PERMDISP were based on 499 and 999 permutations, respectively;  
754 PERMDISP analyses were applied independently on each of the main effects and two-way  
755 interactions; therefore residual variances are not shown but are included in the calculation of  
756 the explained variance (EV).

Sources of variation :	PERMANOVA				PERMDISP			
	SS (MS)	Df	F	EV (P)	SS (MS)	Df	F	EV (P)
Biological compartment	31.79 (15.89)	2	285.43	3.55 (0.002)	19.47 (9.74)	2	395.32	5.69 (0.001)
Pressure	32.18 (2.48)	13	44.45	3.59 (0.002)	1.67 (0.13)	13	4.60	0.46 (0.001)
HER	28.31 (1.42)	20	25.42	3.16 (0.002)	4.33 (0.22)	20	7.86	1.19 (0.001)
Stream order	5.50 (0.79)	7	14.11	0.61 (0.002)	2.04 (0.29)	7	10.68	0.57 (0.001)
Biological compartment:Pressure	5.48 (0.21)	26	3.78	0.61 (0.002)	24.78 (0.60)	41	24.55	7.15 (0.001)
Biological compartments:HER	12.81 (0.32)	40	5.75	1.43 (0.002)	27.81 (0.45)	62	18.70	8.16 (0.001)
Biological compartments:Stream order	4.24 (0.30)	14	5.44	0.47 (0.002)	22.29 (0.97)	23	39.79	6.54 (0.001)
Pressure:HER	39.58 (0.15)	260	2.73	4.42 (0.002)	13.78 (0.05)	293	1.69	3.72 (0.001)
Pressure:Stream order	8.52 (0.09)	91	1.68	0.95 (0.002)	7.30 (0.07)	111	2.37	1.98 (0.001)
HER:Stream order	20.15 (0.37)	55	6.58	2.25 (0.002)	13.85 (0.17)	82	6.47	3.91 (0.001)
Residuals	700.25 (0.06)	12575		78.2 (-)				
Total	895.44	13103						

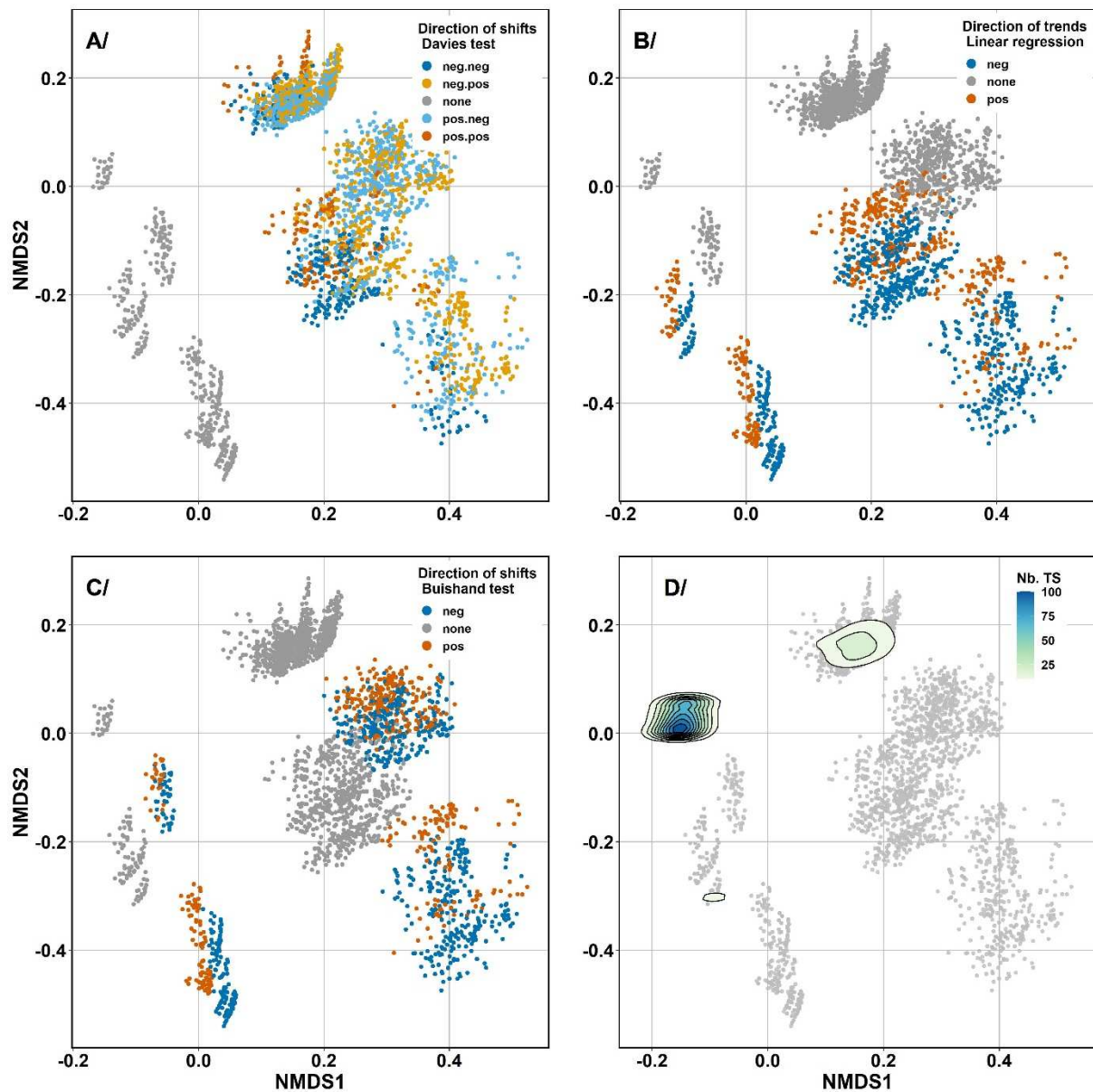
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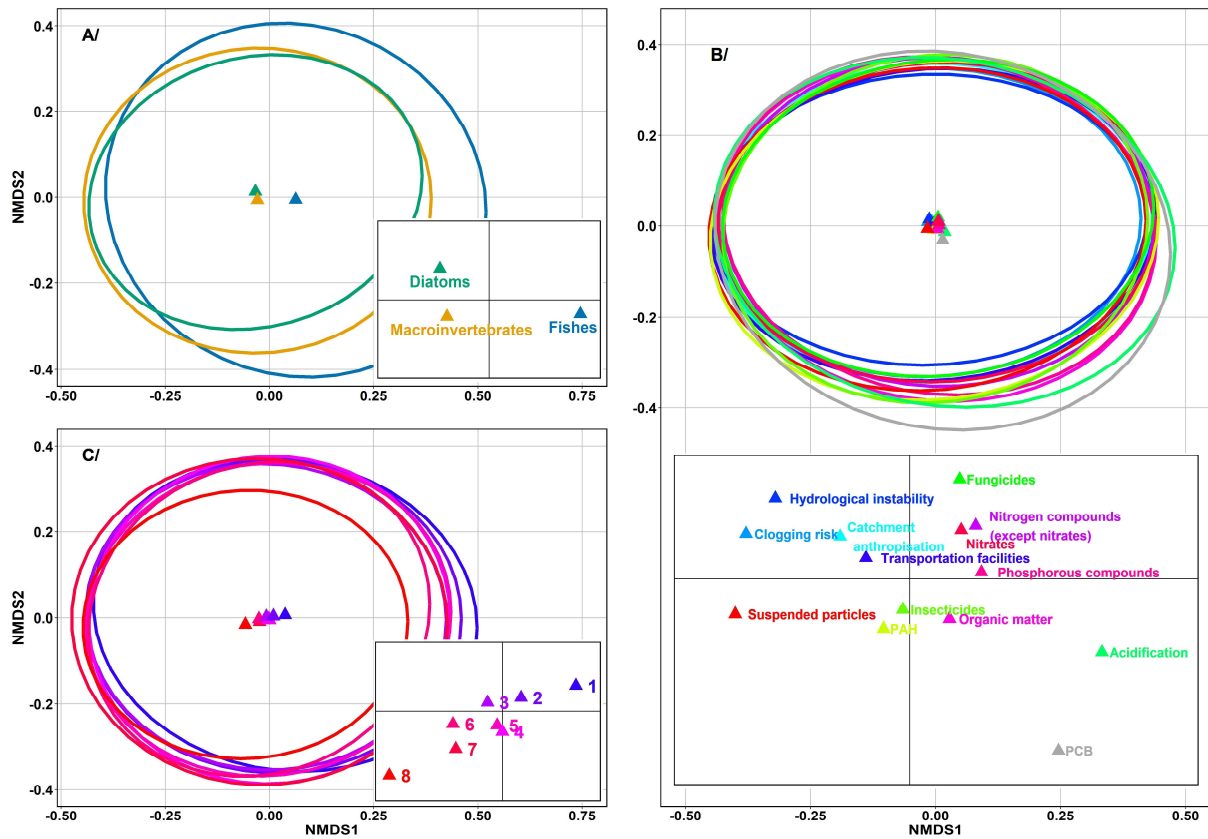
759

760 **Figure 1.** Locations of (a) the 22 French hydroecoregions and (b) the 312 shallow river sites  
 761 (black points) for which data about the three biological compartments were simultaneously  
 762 available. 1: Pyrénées (high mountain), 2: Alpes Internes (high mountain), 3: Massif Central  
 763 Sud (mountain), 4: Vosges (mountain), 5: Jura - Préalpes du Nord (mountain), 6:  
 764 Méditerranéen (small relief), 7: Préalpes du Sud (mountain), 8: Cévennes (mountain), 9:  
 765 Tables Calcaires (plain), 10, Côtes Calcaires Est (small relief), 11: Causses Aquitains (small  
 766 relief), 12: Armoricain (plain), 13: Landes (plain), 14: Côteaux Aquitains (small relief), 15:  
 767 Plaine Saône (plain), 16: Corse (mountain), 17: Dépressions Sédimentaires (plain), 18: Alsace  
 768 (plain), 19: Grands Causses (mountain), 20: Dépôts Argilo-Sableux (plain), 21: Massif  
 769 Central Nord (mountain), 22 : Ardennes (small relief).



770

771 **Figure 2.** Non-metric multidimensional scaling (stress = 0.04) displaying the distribution of  
 772 the modalities of temporal metrics in the Gower's dissimilarity space (i.e., between time  
 773 series or temporal changes in impairment probabilities; IP). A/ direction of shifts in slope  
 774 according to the Davies test; B/ direction of general trends according to linear regression; C/  
 775 direction of shifts in mean IP according to the Buishand test; D/ density plot showing the  
 776 main clusters of observations. Nb. TS: number of time series.

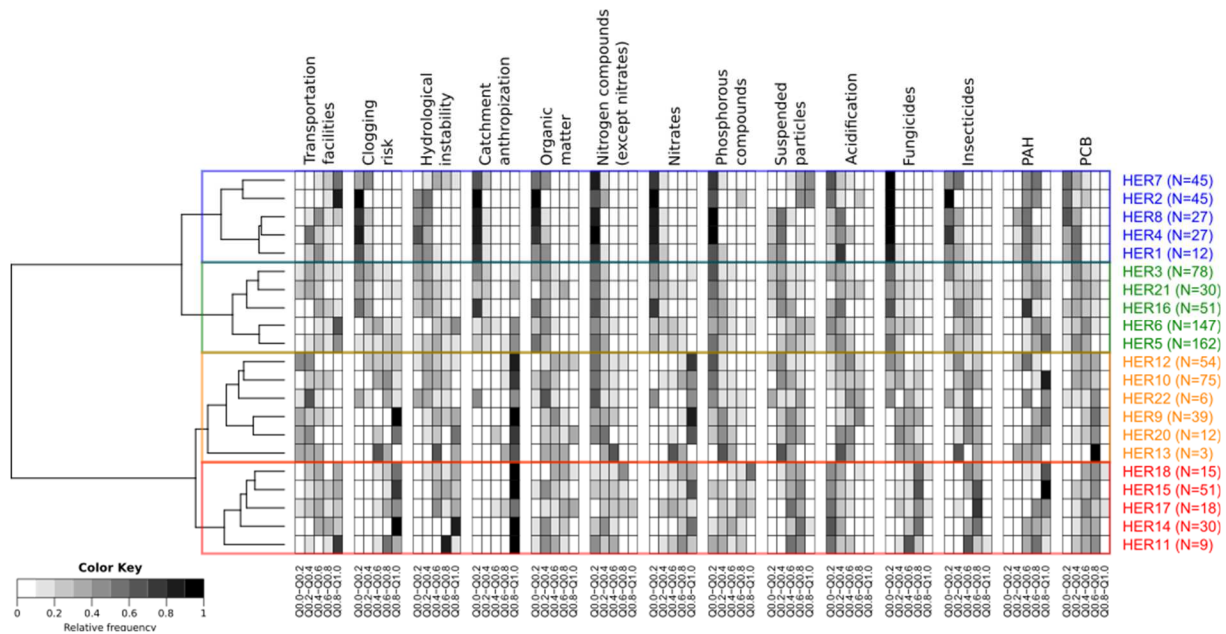


777

778 **Figure 3.** Non-metric multidimensional scaling displaying the distribution of the centroids  
 779 and the dispersions around centroids of three sources of variation in the Gower's dissimilarity  
 780 space (i.e., between time series or temporal changes in impairment probabilities; IP). The  
 781 triangles are used as proxies of group centroids and are located at the average position of each  
 782 group. The ellipses are used as proxies of the average distance of time series to group  
 783 centroids and assume a normal multivariate distribution at a 95% confidence interval. A/ the  
 784 three biological quality elements; B/ the 14 pressure categories; C/ the eight stream orders.  
 785 Inset plots further depict the relative position of group centroids (horizontal and vertical lines  
 786 intersect at NMDS1 = NMDS2 = 0).

787



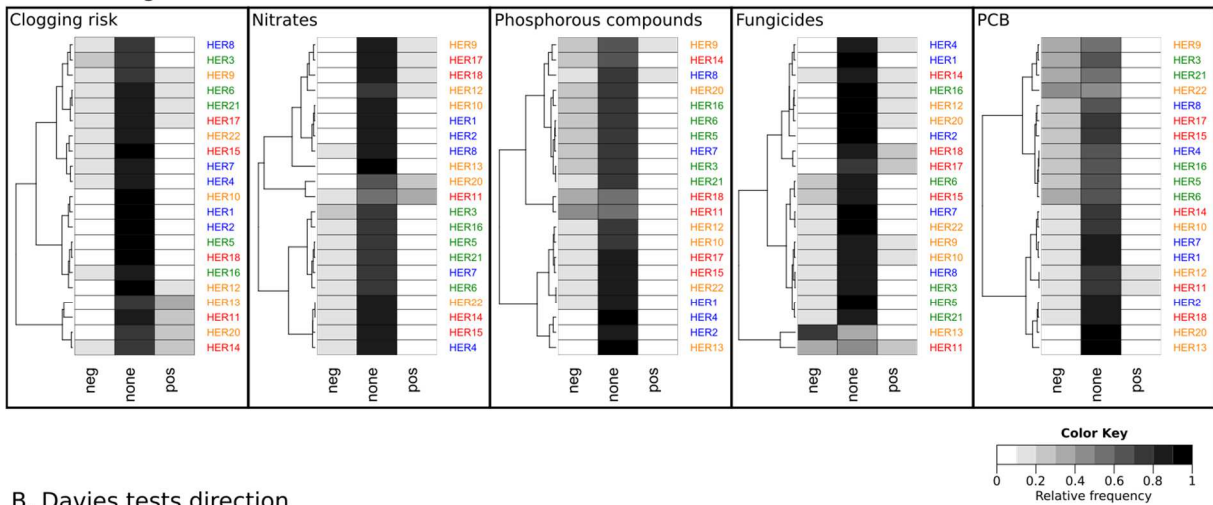


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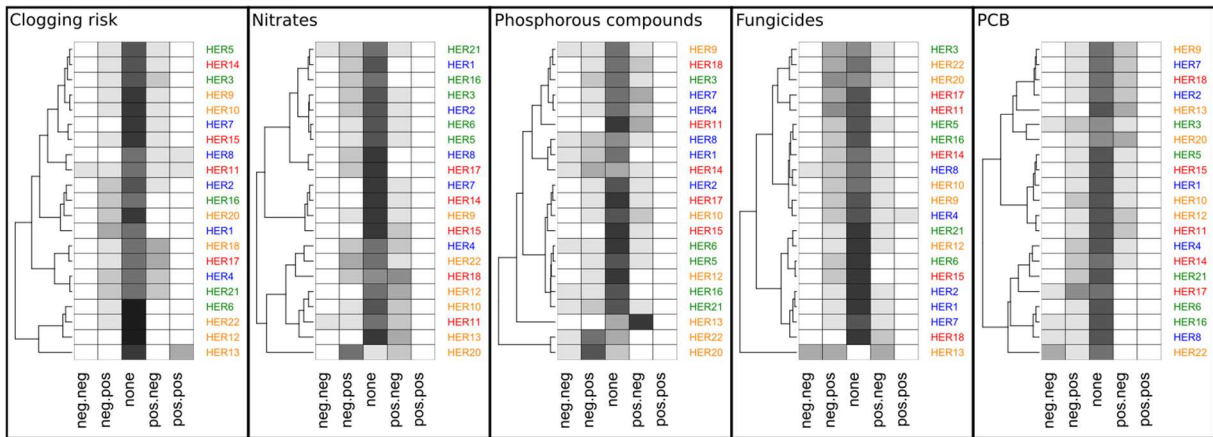
789 **Figure 4.** Heatmap of the relative frequencies of the discrete mean IP for all the pressure  
 790 categories in all the HERs. Numbers of time series per HER are given in brackets. HERs were  
 791 hierarchically clustered using Euclidean distances between rows and the Ward linkage  
 792 algorithm. Range of the values for the quantiles (cf. Table 1): Q0.0-Q0.2 = [0.000125, 0.125[;  
 793 Q0.2-Q0.4 = [0.125, 0.241[; Q0.4-Q0.6 = [0.241, 0.367[; Q0.6-Q0.8 = [0.367, 0.546[; Q0.8-  
 794 Q1.0 = [0.546, 0.996[.

795

A. Linear regressions direction

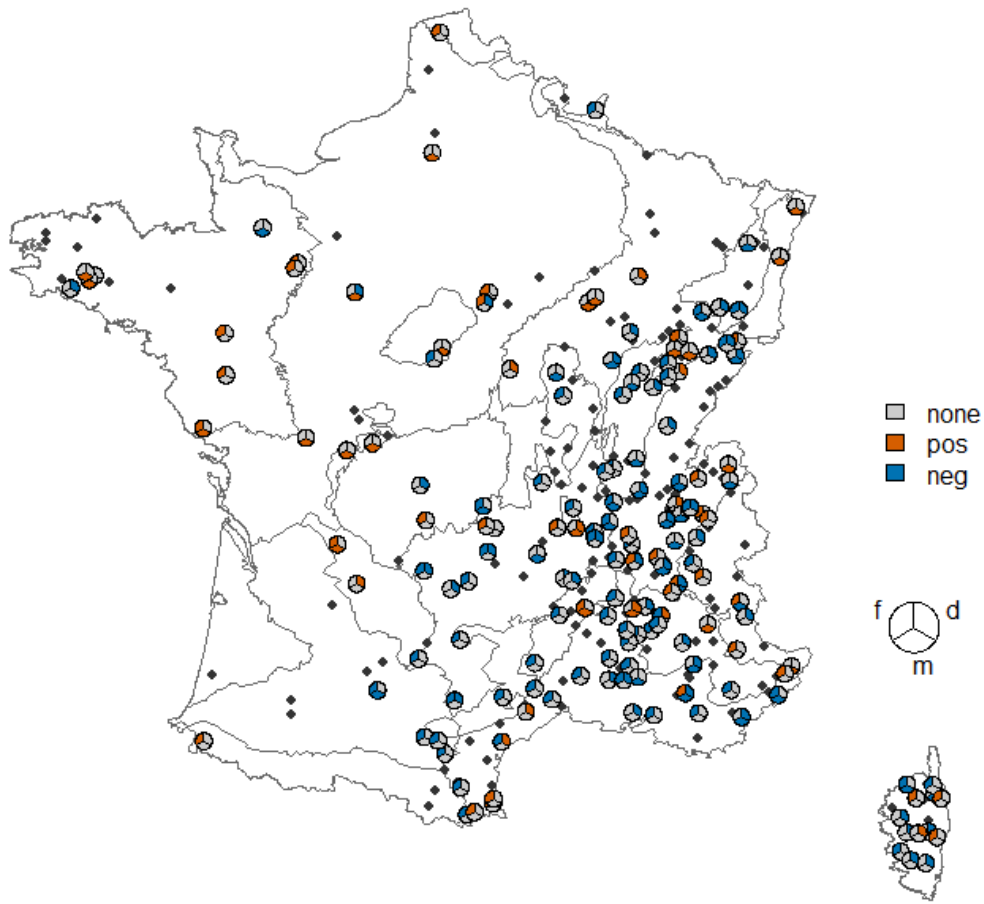


B. Davies tests direction



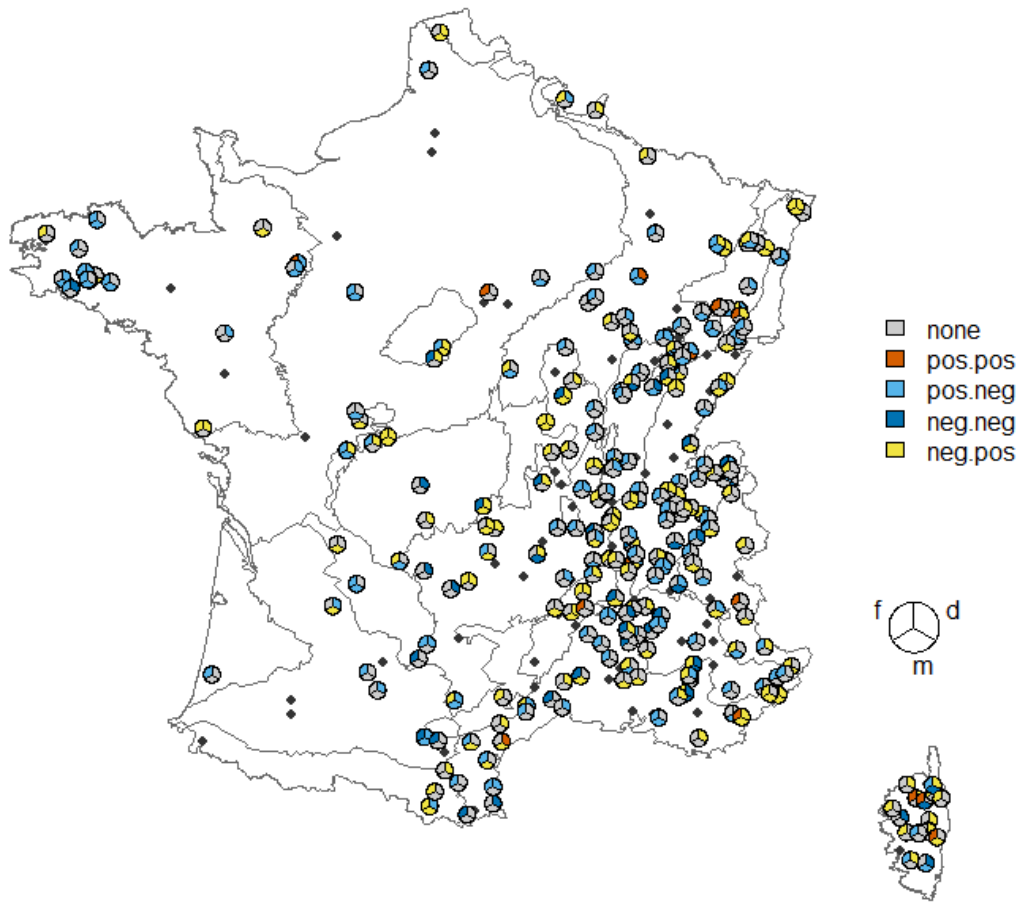
796

797 **Figure 5.** Heatmaps of the relative frequencies of all the modalities of the directions obtained  
 798 with the linear regressions (A/) and the Davies tests (B/). Based on the results of the NMDS  
 799 analysis (Fig. 3B). We chose to only show frequencies for 5 given pressure categories. HERs  
 800 were hierarchically clustered using Euclidean distances between rows and the Ward linkage  
 801 algorithm. The color of the HER labels is based on the groups identified in Fig. 4.



802

803 **Figure 6.** Distribution of the linear trends (according to linear regressions) for the pressure  
 804 nitrates in mainland France. Pie charts represent the significant linear trends observed for each  
 805 biological compartment; d: diatoms, m: macroinvertebrates, and f: fishes. When none of the  
 806 times series corresponding to the three biological compartments displayed a significant linear  
 807 trend, sites were denoted by black dots. The direction of linear trends were resolved into three  
 808 modalities: *none*, *pos*, and *neg* (see Table 1 for the definition of each modality).



809

810 **Figure 7.** Distribution of the shifts in slope (according to Davies test) for the pressure nitrates  
 811 in mainland France. Pie charts represent the significant shifts in slope observed for each  
 812 biological compartment; d: diatoms, m: macroinvertebrates, and f: fishes. When none of the  
 813 times series corresponding to the three biological compartments displayed a significant shift  
 814 in slope, sites were denoted by black dots. The direction of shifts in slope were resolved into  
 815 five modalities: *none*, *pos.pos*, *neg.neg*, *neg.pos* and *pos.neg* (see Table 1 for the definition of  
 816 each modality).

817

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823

#### 824 **Conflicts of interest**

825 The achievement of this manuscript has not been influenced by competing financial, personal,  
826 or professional interests.

827

#### 828 **Data accessibility**

829 Data will be made available upon request.

830

#### 831 **Credit authorship contribution statement**

832 **Benjamin Alric:** Conceptualization, Data curation, Methodology, Formal analysis, Writing -

833 original draft. **Olivier Dézerald:** Conceptualization, Data curation, Methodology, Formal

834 analysis, Writing - original draft. **Albin Meyer:** Data curation, Methodology, Formal analysis,

835 Writing - original draft. **Elise Billoir:** Conceptualization, Methodology, Formal analysis,

836 Writing - original draft. **Romain Coulaud:** Methodology, Writing - original draft. **Floriane**

837 **Larras:** Methodology, Writing - original draft. **Cédric P. Mondy:** Methodology, Writing -

838 original draft. **Philippe Usseglio-Polatera:** Supervision, Conceptualization, Methodology,

839 Formal analysis, Writing - original draft.

840

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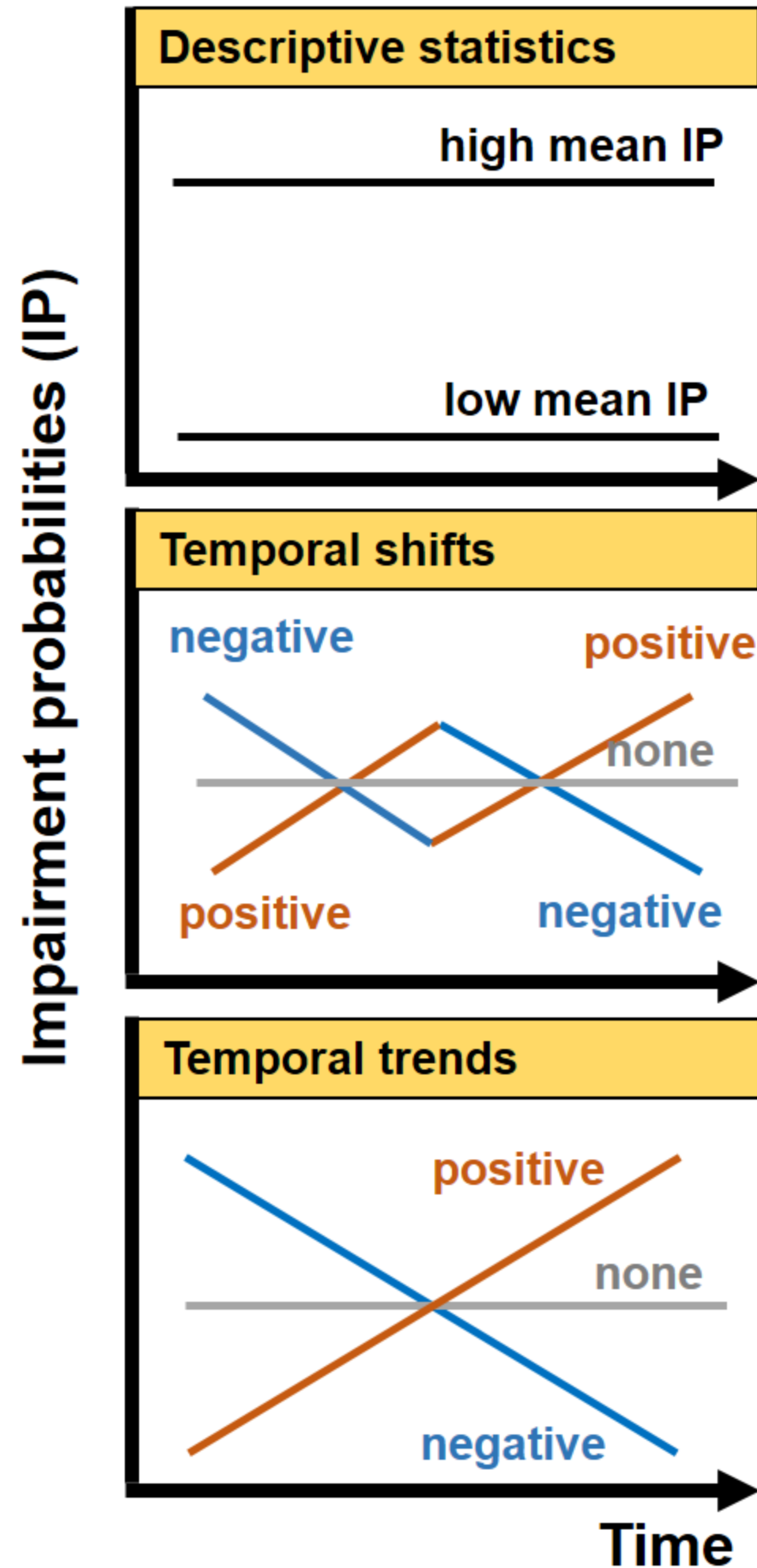
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# Taxonomy and trait-based diagnostic tools



# Typology of temporal responses to anthropogenic pressures



# Integrated assessment of the ecological quality of rivers over two decades

