



ALMA MATER STUDIORUM  
UNIVERSITÀ DI BOLOGNA

## ARCHIVIO ISTITUZIONALE DELLA RICERCA

### Alma Mater Studiorum Università di Bologna Archivio istituzionale della ricerca

Diatom Red Lists: important tools to assess and preserve biodiversity and habitats in the face of direct impacts and environmental change

This is the final peer-reviewed author's accepted manuscript (postprint) of the following publication:

*Published Version:*

Diatom Red Lists: important tools to assess and preserve biodiversity and habitats in the face of direct impacts and environmental change / Cantonati M.; Hofmann G.; Spitale D.; Werum M.; Lange-Bertalot H.. - In: BIODIVERSITY AND CONSERVATION. - ISSN 0960-3115. - ELETTRONICO. - 31:2(2022), pp. 453-477. [10.1007/s10531-021-02339-9]

*Availability:*

This version is available at: <https://hdl.handle.net/11585/904129> since: 2022-11-19

*Published:*

DOI: <http://doi.org/10.1007/s10531-021-02339-9>

*Terms of use:*

Some rights reserved. The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website.

This item was downloaded from IRIS Università di Bologna (<https://cris.unibo.it/>).  
When citing, please refer to the published version.

(Article begins on next page)

This is the final peer-reviewed accepted manuscript of:

Cantonati M.; Hofmann G.; Spitale D.; Werum M.; Lange-Bertalot H.: *Diatom Red Lists: important tools to assess and preserve biodiversity and habitats in the face of direct impacts and environmental change*

BIODIVERSITY AND CONSERVATION

VOL. 31

ISSN 0960-3115

DOI: 10.1007/s10531-021-02339-9

The final published version is available online at:

<https://dx.doi.org/10.1007/s10531-021-02339-9>

Terms of use:

Some rights reserved. The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website.

This item was downloaded from IRIS Università di Bologna (<https://cris.unibo.it/>)

**When citing, please refer to the published version.**

[Click here to view linked References](#)

1 **Diatom Red Lists: important tools to assess and preserve biodiversity and**  
2 **habitats in the face of direct impacts and environmental change**

3

4 **Marco Cantonati** <sup>1,\*</sup>, **Gabriele Hofmann** <sup>2</sup>, **Daniel Spitale** <sup>1</sup>, **Marcus Werum** <sup>3</sup>, **Horst**  
5 **Lange-Bertalot** <sup>4</sup>

6

7

8 \* Corresponding author. E-mail address: marco.cantonati@muse.it (M. Cantonati).

9 <https://orcid.org/0000-0003-0179-3842>

10

11

12 Open Research statement: Currently available underlying data can be accessed at:

13 <https://www.rote-liste-zentrum.de/de/Download-Pflanzen-1871.html>

14

15

16 **Abstract**

17 Freshwater biodiversity is disproportionately endangered and under-prioritized. Algal Red Lists  
18 literature is very limited, and there are only two editions of a single diatom-specific Red List  
19 (developed for Germany), in spite of the importance of diatoms for biodiversity and global ecosystem  
20 functioning. We used and analysed the new diatom Red List, compared to the previous edition, to  
21 show that these diatom microalgae threat status data allow, on one hand, a characterization of the  
22 ecological integrity and of the diatom diversity of the different types of inland-water ecosystems (also  
23 of practical importance to designate the most relevant habitats for conservation purposes), including a  
24 clear assessment of the threat status of the habitat, and, on the other hand, they offer ample  
25 possibilities to track the effects of stressors and of environmental change. Our results revealed, among  
26 other things, that: - threatened taxa concentrate in dystrophic-oligotrophic environments; - 'not  
27 threatened', i.e. tolerant and opportunistic, taxa are most frequent in eutrophic and saline ecosystems;  
28 - most local diatom extinctions happened in carbonate oligotrophic habitats. In this study, nitrates  
29 could be shown to possess a highly significant negative association with the cumulative percentage of  
30 diatom taxa in threat categories of the Red List. Having included heterogeneous studies (diverse  
31 inland-waters, different geographic areas, from close-to-pristine to impacted, neo- and  
32 paleolimnology etc.), we think that this strong negative association is noteworthy and points to the  
33 high potential of diatom-Red-List based approaches, especially if these would be tailored for the  
34 different biogeographic and climatic ecoregions.

35

36 **Keywords** Diatoms · Red Lists · Oligotrophy · Dystrophic environments · Nitrates

37

## 38 **Introduction**

39 International Union for Conservation of Nature (IUCN) Red Lists offer insight into present-day  
40 species declines. Foissner (2008) concludes that it is important to include also protists in conservation  
41 strategies, and Juráň and Kaštovský (2019) underline that there should be Red Lists also of  
42 microalgae because of their role in monitoring and assessment of habitat quality.

43 Current extinction rates are unprecedentedly high in human history (e.g., Davies 2019), and  
44 biodiversity loss in freshwater environments five times higher as compared to terrestrial systems (e.g.,  
45 Ricciardi and Rasmussen 1999). Moreover, inland-water ecosystems are suffering the impact of  
46 multiple stressors acting in combination and the threat multiplier effect of climate change (e.g.,  
47 Downing 2014; Cantonati et al. 2020a). Fresh water is the main strategic, and nevertheless  
48 increasingly scarce, resource but its future, along with that of the species and ecosystems it supports  
49 (almost 7% of global biodiversity in spite of being tiny in relative volume) is uncertain (Cantonati et  
50 al. 2020a). Freshwater biodiversity is disproportionately endangered and under-prioritized when  
51 compared to the marine and terrestrial biota. Yet the Water Framework Directive (WFD; European  
52 Union 2000) lacks adequate consideration of wetlands, freshwater ecosystem services, and smaller  
53 water bodies (van Rees et al. 2020).

54 Species at risk are non-randomly distributed across phylogeny (Davies 2019). Diatoms are the  
55 most species-rich group of algae (Mann 1999). The global diversity of diatoms may be one or more  
56 orders of magnitude higher than the current number of species described (Vanormelingen et al. 2008),  
57 with as many as ca. 100,000 but at least 30,000 extant diatom species (Mann and Vanormelingen  
58 2013). They contribute to global primary production with a proportion as large as 20%, possessing  
59 global significance in the carbon and silicon cycles (Mann 1999). They can be found wherever there  
60 is a little bit of moisture, and in all aquatic habitats. Most of them have very precise preferences for  
61 ecological conditions of paramount relevance in environmental quality assessments, such as pH,  
62 mineralization, nutrients etc. The frustules allow species identification, and are usually preserved in  
63 sediments, thus providing a precious tool for paleo-environmental reconstructions, and diatom mounts  
64 are excellent collection objects, present in many natural history museums. All these characteristics

65 have fostered the use of diatoms in a wide array of disciplines (e.g., Smol and Stoermer 2010). A  
66 combination of mechanisms (“melting pot genome” of ca. 10.000 genes with many diatom specific  
67 genes but also extensive gene transfer from bacteria to diatoms; active urea cycle, probably used to  
68 improve nitrogen storage; fatty acids oxydation in mitochondria; chemical information exchange  
69 among cells etc.) may underlie the rapid diversification rates, and may explain why diatoms have  
70 come to dominate several contemporary systems in a relatively short period of time (Bowler et al.  
71 2008). In oligotrophic habitats, which host diverse and characteristic diatom communities (e.g.,  
72 Lange-Bertalot and Metzeltin 1996), a detailed taxonomy, capable of identifying and characterizing  
73 typical (and, possibly, new) taxa must be applied. Shoe-horning of taxa in aggregates of species  
74 common in meso- and eutrophic waters must be avoided. For diatoms, Mann (1999) advocated a so-  
75 called “Waltonian” species concept, a total-evidence approach combining data from morphology,  
76 genetics, mating systems, physiology, ecology, and crossing behaviour.

77 Vanormelingen et al. (2008) pointed out that detailed taxonomic inventories using fine-grained  
78 morphological characteristics, molecular markers, and crossing experiments revealed that the  
79 geographic distribution of diatoms ranges from global to narrow endemic, and that population  
80 divergence apparently takes place over distances of 100s to 1000s of kilometres. According to Juráň  
81 and Kaštovský (2019) microorganisms should be viewed in Red Lists primarily as poorly-known,  
82 rather than rare.

83 Oligotrophic aquatic habitats have been neglected also by ecologists and taxonomists, including  
84 those working on diatoms (Kociolek and Stoermer 2009), whose attention has been more focussed on  
85 aquatic environments affected by human impacts (eutrophication, acidification etc.). The diatom  
86 microflora of oligotrophic environments has received renewed attention only since the 1990s. Lange-  
87 Bertalot and Metzeltin (1996) found as many as 800 taxa in a small number of samples from three  
88 oligotrophic lakes (from carbonate to dystrophic) at a time when the total diatom flora of Central  
89 Europe was estimated to comprise 1600 taxa. Oligotrophic habitats are threatened by direct  
90 (exploitation for hydropower and drinking water, land-reclamation, nutrient enrichment etc.) and  
91 indirect (diffuse airborne pollution, climate change) impacts. Lange-Bertalot (2007) suggests that

92 there are indeed cosmopolitan diatom species, and that these include, in the first instance, about 250  
93 well-known species adapted to thrive in highly eutrophic to strongly saprobic waters and widely  
94 distributed in cultural landscapes e.g. in Europe. Unluckily, strictly oligotrophic freshwaters are  
95 becoming rarer and rarer because of direct and indirect (diffuse) human impact.

96 Diatom Red Lists provide an account of the diatom flora of a geographic area pointing out the  
97 distribution and threat status of individual species as determined by environmental factors and  
98 impacts. Soininen (2007) identified ion concentration and trophic status as major regional-scale  
99 factors and light, current velocity, and substratum composition as key local-scale factors influencing  
100 diatom distributions. The main environmental variables affecting composition of diatom assemblages  
101 in oligotrophic habitats are pH, conductivity, inorganic N, substrate particle size, and shade (e.g.,  
102 Cantonati 1998). Among the environmental determinants of diatom distribution, nitrate often stands  
103 out as particularly relevant factor (e.g., Bertrand et al. 1999; Tolotti 2001; Gesierich and Kofler 2010;  
104 Cantonati et al. 2012).

105 So far, applications of the diatom Red Lists for Germany (Lange-Bertalot 1996; Hofmann et al.  
106 2018) have been relatively rare but included a fair variety of inland-waters' habitats: springs (e.g.,  
107 Werum and Lange-Bertalot 2004; Cantonati et al. 2012), mountain mires (e.g., Fránková et al. 2009;  
108 Cantonati et al. 2011), glacial streams (e.g., Fell et al. 2018), alpine aquatic habitats (e.g., Falasco and  
109 Bona 2011), high-mountain lakes (e.g., Tolotti 2001, including paleolimnology: Cantonati et al.  
110 2021a), large peri-alpine lakes (Spitale et al. 2011), Mediterranean streams (e.g., Falasco et al. 2016;  
111 Cantonati et al. 2020c).

112 We used and analysed the new diatom Red List (Hofmann et al. 2018), compared to the previous  
113 edition (Lange-Bertalot 1996), to show that these diatom microalgae threat status data allow, on one  
114 hand, a characterization of the ecological integrity and diatom diversity of the different types of  
115 inland-water ecosystems (e.g., Cantonati et al. 2020a), including a clear assessment of the threat status  
116 of the habitat, and, on the other hand, offer ample possibilities to track the effects of stressors and of  
117 environmental change.

118

119

## 120 **Methods**

### 121 **Diatom Red Lists for Germany**

122 The first diatom Red List for Germany was published in 1996 (Lange-Bertalot 1996; hereafter  
123 referred to as RL96), and actualized 22 years later by Hofmann et al. (2018; hereafter referred to as  
124 RL18). The threat analysis for the latter was grounded on a taxonomically harmonized dataset based  
125 on studies from 1985 to 2008, which includes ca. 7600 diatom samples from various German rivers  
126 and 3000 samples from German lakes (Hofmann et al. 2013). These data are not evenly distributed  
127 across the main inland aquatic habitats occurring in Germany (see Supplementary Information Table  
128 1 and Finck et al. 2017) but originate mainly from the stream, river, and lake types considered in the  
129 German monitoring network. These, however, include also a fair amount of data from acidification-  
130 sensitive silicate streams of the low-elevation mountain ranges of Central Germany, which have been  
131 part of the acidification monitoring network since the mid-1980s. Dystrophic aquatic environments,  
132 small water bodies, and dilute (soft-water) lakes are largely underrepresented in the dataset. The  
133 abundance of the species is recorded as per cent (relative abundance) of the assemblage composition,  
134 a quantification method common in diatom studies. Unpublished studies from the years 2008 to 2015  
135 that had not been included in the dataset, several individual papers and books (e.g., Reichardt 1997;  
136 2006; 2012; Werum and Lange-Bertalot 2004), as well as historical samples from various collections  
137 were used to complement habitats not covered by regular/official monitoring.

138 Whilst RL96 was still largely based on empirical assessments by the author based on expertise and  
139 literature, RL18 was mainly generated from the above-described dataset and following the  
140 standardized method of threat analysis (e.g., Gärdenfors et al. 2001; IUCN 2012). In addition to the  
141 current-distribution, the long-term development was the most important criterion to assess population  
142 declines and thus the possible endangerment of the diatom taxa. Long-term development trends were  
143 achieved by comparing the current distribution situation with the literature and historical diatom  
144 samples from the time before the onset of the massive water pollution.



145 Excel files with the data of the RL18 can be downloaded at: [https://www.rote-liste-](https://www.rote-liste-zentrum.de/de/Download-Pflanzen-1871.html)  
146 [zentrum.de/de/Download-Pflanzen-1871.html](https://www.rote-liste-zentrum.de/de/Download-Pflanzen-1871.html).

147 RL18 threat categories are as follows: 0 = extinct or lost, 1 = threatened with extinction, 2 =  
148 strongly threatened, 3 = threatened, G = threat of unknown extent, R = extremely rare, V = declining,  
149 D = data insufficient, \* = not threatened. RL18 current-distribution categories are as follows: ex =  
150 extinct or lost, er = extremely rare, vr = very rare, r = rare, mf = moderately frequent, f = frequent, vf  
151 = very frequent, ? = unknown, na = not assessed.

152 RL18 ecological attributes of the taxa are as follows: ae = aerial, o = oligotraphentic, oc =  
153 oligotraphentic carbonate, od = oligotraphentic dystrophic, eu = eutraphentic to tolerant, hal =  
154 halophilic, ? = unknown.

155

#### 156 **RL18 data processing and statistical analyses**

157 Diatom taxa of RL18 were automatically matched, using code in R (R Core Team 2020), to ecological  
158 attributes in the lists of van Dam et al. (1994; providing information on trophic status, moisture, pH  
159 etc.) and Rimet and Bouchez (2012; providing information on size, growth forms/ecological guilds,  
160 pioneer species etc.). The matches were then checked and expanded manually accounting for  
161 synonyms. Tables 3 and Supplementary Information Table 3 provide details for the most threatened  
162 (cat. 0 + 1) taxa and the putative neophytes, respectively. As concerns the most menaced species' and  
163 neophytes' tables (Table 3, Supplementary Information Table 3), matches with van Dam et al. (1994)  
164 were too few to be used in a meaningful way, and matches with Rimet and Bouchez (2012) were  
165 available in fair amount (50%) for the neophytes only.

166 To test for agreement between RL96 and RL18, using only taxa with congruent taxonomy in the  
167 two Red Lists, we used Cohen's kappa ( $k$ ) (Cohen 1960, 1968), a commonly used measure of  
168 agreement that compares the observed agreement to the congruency expected by chance if two  
169 observers' panels rate independently ( $k = 1$  means perfect agreement). The original (unweighted  $k$ )  
170 only counts strict agreement (the same category is assigned by both panels). A weighted version of  $k$   
171 (Cohen 1968) allows for partial agreement. For example, exact agreements can be given full weight,

172 while a one-category difference might be given a weight of 1/2. This typically makes sense only when  
173 the categories are ordered. Cohen's kappa values were used to construct a contingency table. Based  
174 on this, an Observer Agreement Chart between RL96 and RL18 was plotted (Fig. 1).

175 To measure the association between the RL18 threat categories and the current-distribution  
176 situation of the diatom taxa we used Cramer's V (Friendly and Meyer 2015), varying from 0 (no  
177 association) to 1 (perfect association).

178 To measure the association between the RL18 threat categories and the ecological groups available  
179 in RL18, and between RL18 threat categories and selected ecological attributes reported by van Dam  
180 et al. (1994) and Rimet and Bouchez (2012), we used association plots. In the association plot, each  
181 cell is shown by a rectangle, constructed in a way that each cell is proportional to the respective raw  
182 residual. The rectangles are positioned relative to a baseline representing independence shown by a  
183 dotted line. Cells with observed > expected frequency rise above the line and are coloured blue, cells  
184 with observed < expected frequency fall below it and are shaded red.

185 Associations between RL18 diatom taxa and ecological attributes available in Rimet and Bouchez  
186 (2012) were tested for significance using *Pearson's Chi-squared* ( $\chi^2$ ) tests with Yates' continuity  
187 correction for two-way tables. All numerical analyses were performed using R (R Core Team 2020)  
188 and mainly the packages *vcd* (Meyer et al. 2020) and *vcdExtra* (Friendly 2021).

189

## 190 **Applications of diatom Red Lists**

191 We assessed all studies we were aware of (own, published and unpublished, and those available in  
192 the literature) in which RL96 or RL18 were applied on a variety of current (and past) inland-water  
193 ecosystems from different geographic areas. In particular, we focussed on the relationship between  
194 Red List threat categories and nitrate concentrations, and, therefore, only studies providing these data  
195 were retained.

196 The relationship between the cumulative percentage of diatom taxa in threat categories of the Red  
197 List and mean nitrate nitrogen concentrations was investigated with a Pearson's product-moment  
198 correlation, and applying a linear model on the log-transformed nitrate concentrations.

199

200

## 201 **Results**

### 202 **Diatom Red Lists**

203 An 'Article title, Abstract, Keywords' query ( diatom\* AND "red list\*" ) carried out in Scopus on  
204 May 21<sup>st</sup> 2021 yielded only 29 relevant document results, of which about 50% were authored/co-  
205 authored by one of the authors of the present paper.

206 RL96 contained a total of 1,435 taxa whilst RL18 includes 715 more (2,103). This 50% increase is  
207 mainly the result of intensified investigations and monitoring programs since 1996 which significantly  
208 expanded the database. After 1996, numerous species were detected for the first time from German  
209 waters, e.g. from the previously poorly-investigated waters of the northern German lowlands. Also  
210 newly described species and those which were the result of differentiation/splitting made a significant  
211 contribution: 110 out of the 715 taxa (= 15%) added to the new edition (Hofmann et al. 2018).

212

### 213 **Agreement between RL96 and RL18**

214 The association table (Table 1) shows the extent of overlap in the Red List categories between taxa  
215 with congruent taxonomy in both lists. The highest congruency (0.91) was found for the 'extremely  
216 rare' category, meaning that 91% of the taxa were listed R in RL96 as well as RL18. The lowest  
217 congruency between the same category was related to category 2 with only 36% of taxa in this  
218 category in RL96 persisting in this category in RL18.

219 The excellent consistency between the two Red Lists is confirmed by Cohen's kappa (Fig. 1;  
220 Supplementary Information Table 2).

221 The histogram in Fig. 2 shows the number of taxa (with percentages on each bin) in each of the  
222 threat categories of RL96 and RL18. The 'threat of unknown extent' (G), and 'not threatened' (\*)  
223 categories increased by 16% and 7%, respectively. On the contrary, there was a marked decrease  
224 (22%) of taxa with 'data insufficient' (D).

225

## 226 **The new diatom Red List (RL18)**

227 The association table based on Cramer's V values (Table 1) between the RL18 threat categories  
228 and the current-distribution status of the diatom taxa shows a very strong and consistent association.  
229 The overall Cramer's V value of 0.674 is statistically highly significant ( $P < 0.0001$ ).

230 The histogram in Fig. 3 shows the current distribution status of the diatom taxa of the Red List  
231 (RL18). A striking observation is that rare taxa are about three times more numerous than common  
232 ones.

233 Table 3 shows the 55 most menaced taxa of RL18 (4 taxa in category "0" = extinct or lost, and 51  
234 taxa in cat. "1" = threatened with extinction). The effort of expanding and updating Red List  
235 knowledge allowed to assign three taxa for which Red List status was unknown (D) in 1996 to  
236 category 0, and 28 of these taxa to category 1. Consistently with their threat category, the four taxa in  
237 the 'extinct or lost' category, and the remaining fifty-one considered nearly extinct are 'extremely  
238 rare'. For the 51 non-extinct taxa, the long-term development is predicted to be "decline of unknown  
239 extent" for most taxa, with only two estimated to be affected by "moderate decline". As many as 31 of  
240 the most endangered taxa are restricted to oligo-dystrophic environments, 10 to oligotrophic habitats,  
241 and 8 to oligotrophic calcareous ecosystems (for 6 taxa the preferred environment is unknown).  
242 Consistently, the two most well-represented genera among the 51 taxa 'threatened with extinction' are  
243 *Eunotia* with 18 taxa and *Pinnularia* with 10 taxa.

244 Following Hofmann et al. (2018), Supplementary Information Table 3 lists the ten species that  
245 have been suggested in the literature to be neophytes for central Europe. *Achnantheidium* is the genus  
246 with most species (4) in this group. The most common threat category is 'not threatened' (4) followed  
247 by 'data insufficient' (3). Most (6) of the ten species are classified as eutrapphentic, with two halophilic  
248 species, and two with insufficiently known ecology. As concerns the current distribution, three  
249 species are 'moderately frequent', three are 'rare', one is 'very rare', and three are 'extremely rare'.  
250 All species (3) for which a long-term development indication could be assigned have 'clear increase'.  
251 Life forms/ecological guilds were available for 50% of these species. As concerns size class, three

252 species are large and two are small. The large majority (4) of species for which data were available  
253 are motile.

254

### 255 **Association of RL18 with ecological groups**

256 The association plot between RL18 threat categories and RL18 ecological groups (Fig. 4) was highly  
257 significant ( $\chi^2 = 1385.3$ ,  $df = 48$ ,  $P < 2.2 \times 10^{-16}$ ). It suggests that: - most diatom extinctions happened  
258 in carbonate oligotrophic habitats, - threatened taxa concentrate in dystrophic-oligotrophic  
259 environments, - ‘not threatened’ (\*) taxa are most frequent in eutrophic and saline ecosystems. These  
260 observations are largely confirmed by the highly-significant association plots with ecological  
261 attributes according to van Dam et al. (1994): pH (Fig. 5;  $\chi^2 = 289.9$ ,  $df = 40$ ,  $P < 2.2 \times 10^{-16}$ ); nitrogen  
262 uptake metabolism (Supplementary Information Fig. 1;  $\chi^2 = 204.9$ ,  $df = 24$ ,  $P < 2.2 \times 10^{-16}$ ); saprobity  
263 (Supplementary Information Fig. 2;  $\chi^2 = 249.3$ ,  $df = 32$ ,  $P < 2.2 \times 10^{-16}$ ); trophic status (Fig. 6;  $\chi^2 =$   
264  $377.1$ ,  $df = 48$ ,  $P < 2.2 \times 10^{-16}$ ). The highly-significant association plot with moisture requirement (M)  
265 (Fig. 7;  $\chi^2 = 75.7$ ,  $df = 32$ ,  $P < 2.1 \times 10^{-5}$ ) shows that ‘threatened’ (3) and ‘extremely rare’ (R) taxa are  
266 significantly more frequent than expected in moisture categories 4 and 5, respectively, i.e. the  
267 categories that include pseud- and euaerial species, whilst ‘not threatened’ (\*) taxa are mostly found  
268 in submersed assemblages (M = 1).

269 The only significant association (*Pearson*  $\chi^2 = 11.19$ ,  $df = 1$ ,  $P = 0.0008$ ) between RL18 diatom  
270 taxa and ecological attributes (Rimet and Bouchez 2012) was between threatened and not-threatened  
271 (RL18) and motile and not-motile (Rimet and Bouchez, 2012) taxa (Supplementary Information Table  
272 4).

273

### 274 **The German diatom Red Lists applied to a variety of inland-water ecosystems from different** 275 **geographic areas: strong negative association between threatened taxa and nitrates**

276 In Table 4 we summarize the studies in which RL96 or RL18 were applied to a variety of current (and  
277 some former) inland-water ecosystems from different geographic areas. Even considering all personal  
278 published and unpublished datasets and those available in the literature, only 25 studies provided both

279 Red List data and nitrate concentrations. The habitat types include springs (different types including  
280 limestone precipitating springs), high-mountain lakes, streams, mires, and a large peri-Alpine lake.  
281 The substrata from which diatoms were sampled are benthic, mainly lithic materials but also  
282 bryophytes, surface sediments, etc. The study sites are located mainly in the Alps but also in the  
283 northern Apennines, central Germany, Cyprus, Bosnia and Herzegovina, France, and Belgium. The  
284 main reported impacts are nitrates from agricultural runoff, pastures and cattle watering, alteration of  
285 the morphology and water diversion, airborne pollution.

286 The cumulative percentage of diatom taxa in threat categories (1, 2, 3, G, R, V, D oligotraphentic)  
287 of the Red List is negatively associated to nitrate concentration ( $R = -0.82$ ,  $t = -6.83$ ,  $df = 23$ ,  $P =$   
288  $5.8 \times 10^{-7}$ ) (Supplementary Information Fig. 3). A highly-significant linear model (*Residual standard*  
289 *error* = 8.25,  $df = 23$ , *Multiple R*<sup>2</sup> = 0.67, *Adjusted R*<sup>2</sup> = 0.65,  $F_{[1,23]} = 46.6$ ,  $P = 5.8 \times 10^{-7}$ ) could be  
290 applied on the log-transformed mean nitrate nitrogen concentrations (Supplementary Information  
291 Table 5).

292

293

## 294 **Discussion**

295 There is nowadays specialists' agreement that Red Lists for microorganisms, microalgae, and  
296 heterotrophic protists are important, and that they should be used primarily to guide protection of their  
297 habitats, and only secondarily as a means to achieve protection of particular taxa (Juráň and  
298 Kaštovský 2019). Some authors (Geissler 1988) even claimed that Red Lists of algae point to the  
299 urgent necessity of maintaining high ecological integrity in as many inland waters as possible.

300 Using the two diatom Red Lists and studies in which they were used, we gathered examples and  
301 evidence supporting the high potential of Red Lists for a sound representation and characterization of  
302 the ecological integrity and diversity of the different types of inland-waters' ecosystems. This has also  
303 important applied implications, since IUCN Red Lists of e.g. birds and plants are widely used to  
304 designate the most relevant habitats and conservation areas, and to identify regions where resident

305 species are most vulnerable (e.g., Paine and Mokany 2016), whereas this remains extremely limited in  
306 the case of diatom Red Lists.

307 However, literature on algal Red Lists is scarce and there are basically only two editions of a  
308 single diatom-specific Red List (Lange-Bertalot 1996; Hofmann et al. 2018), in spite of the paramount  
309 importance of this group of protists for biodiversity and global ecosystem functioning. Only in  
310 Estonia (Lilleleht et al. 2008), Hungary (Neméth 2005), Poland (Siemińska et al. 2006), and Slovakia  
311 (Hindák and Hindáková 2001) are diatoms included, more or less extensively, in algal red lists.

312 Here we have shown that this approach is useful, and should be extended to other geographic  
313 areas. It is also an excellent way to preserve expert knowledge, and make it available in an  
314 objectively-ordered way to future scholars (compare Juráň and Kaštovský 2019).

315 There is excellent agreement between the two editions of the Red List for Germany (Table 1, Fig.  
316 3), showing statistical support and validation from a large and taxonomically harmonized database for  
317 an initially expert-based classification. Nevertheless, a marked decrease (22%) of taxa with ‘data  
318 insufficient’ (D) highlights the benefits of a data-driven approach. The accuracy of RL18 is also  
319 underpinned by the very strong, partly-inherent association between the RL18 threat categories and  
320 the current-distribution situation of the diatom taxa.

321 Considering the latter, it appears clearly that rare taxa outnumber common ones. This is consistent  
322 with previous observations, in particular with Lange-Bertalot (1997) who noted that most diatoms are  
323 bound to specific habitats but that there is a relatively small group of cosmopolitan diatom species  
324 that is widely distributed in cultural landscapes, e.g. in Europe.

325 Our focus on the 55 most menaced taxa of RL18 (Table 3) pinpoints clear relationships between  
326 groups of the most endangered taxa and the different types of inland-waters’ ecosystems. This was  
327 reported as being particularly evident in springs and groundwater-dependent ecosystems, in particular  
328 when these are considered from an ecohydrogeological perspective (Cantonati et al. 2020d).

329 Species at risk are non-randomly distributed across space (Davies 2019). The association between  
330 RL18 threat categories and RL18 ecological groups reveals that: - threatened taxa concentrate in  
331 dystrophic-oligotrophic environments, - ‘not threatened’ (= tolerant and opportunistic) taxa are most

332 frequent in eutrophic and saline ecosystems, - most regional extinctions occurred in carbonate  
333 oligotrophic habitats. Carbonate inland waters are common also at low and medium elevations, where  
334 they are often affected by multiple human impacts, such as water abstraction and morphological  
335 alteration, nitrates and other pollutants from intensive agriculture etc. (e.g., Angeli et al. 2010;  
336 Cantonati et al. 2020a). Low-conductivity, naturally acidic (organic acids), oligotrophic (including  
337 nitrogen), dystrophic environments were singled out for their rich and peculiar diatom microflora by  
338 previous works (e.g., Cantonati et al. 2009; 2011; 2020b). Conversely, the fragility of oligotrophic  
339 and dystrophic habitats explains the fact that most oligo- and oligo-mesotraphentic diatom taxa are in  
340 threat categories of the Red List for central Europe (Lange-Bertalot 1997). The high frequency of ‘not  
341 threatened’ (\*) taxa in eutrophic and saline ecosystems reflects two of the current main impacts on  
342 freshwaters: eutrophication (pollution) and salinization (e.g., Downing 2014; Kaushal et al. 2018).

343 The significantly more frequent than expected occurrence of ‘threatened’ (3) and ‘extremely rare’  
344 (R) taxa among aerial species probably reflects the fact that studies on terrestrial diatoms are still  
345 relatively few (e.g., Foets et al. 2021) compared to submersed assemblages, where the majority of the  
346 ‘not threatened’ (\*) taxa are found (e.g., Cantonati et al. 2017).

347 Several papers (e.g., van Rees et al. 2020) list invasive alien species among the main  
348 anthropogenic threats faced by freshwater ecosystems. Supplementary Information Table 3 lists the  
349 ten species that have been suggested in the literature to be neophytes for central Europe according to  
350 Hofmann et al. (2018). However, extreme caution is necessary in considering a diatom species as non-  
351 native and invasive. For instance, Hofmann et al. (2018) discovered that the putative tropical invasive  
352 species *Diadlesmis confervacea*, was present in various historical Kützing samples from a habitat in  
353 north-west Germany, where the species persisted unnoticed for many years (Cantonati et al. 2017). A  
354 classification of this species as a neophyte has therefore to be viewed with reservation, also because  
355 the reasons for its immigration, whether natural or anthropogenic, cannot be assessed. Only one of the  
356 ten potential neobiota listed in Supplementary Information Table 3 doesn’t have North America as the  
357 region of origin but neighbouring northern regions of Eurasia: *Didymosphenia geminata*. This species  
358 which forms conspicuous nuisance growths, and by its large cells is also very noticeable under the



359 light microscope was not found in Germany at all up to the middle of the 20<sup>th</sup> century. At the end of  
360 the last century, while RL96 was still being processed, there were few, undocumented records from  
361 the German area south of the Danube. After the turn of the millennium, while *Didymosphenia* blooms  
362 were becoming a global problem (Bothwell et al. 2014), massive growths were noted in various  
363 tributaries from the Alps (Germany and Austria), rather unexpectedly in view of its apparent absence  
364 previously. Similarly, mass developments were observed in recent years in western Germany of  
365 *Achnantheidium rivulare*, *A. subhudsonis*, and *A. delmontii*. Six other species are suspected of being  
366 neobiota due to their distribution and spreading: *Achnantheidium druartii*, *Capartogramma crucicula*,  
367 *Encyonema triangulum*, *Gomphoneis minuta*, *G. transsilvanica*, and *Gomphosphenia oahuensis*.  
368 *Achnantheidium*, known to include many pioneer species (Rimet and Bouchez 2012, Cantonati et al.  
369 2020c), is the genus with the majority of species amongst the ten taxa discussed as potential  
370 neophytes. However, Hofmann et al. (2018) prefer not to classify any of these species as neophytes  
371 for the time being, since it cannot be ascertained that their spread resulted from human intervention.  
372 Thus, though it is certain that these are opportunistic and relatively tolerant taxa that can spread  
373 rapidly under favourable conditions, it still remains to be demonstrated if they also are neophytes in  
374 Central Europe.

375 In this study we observed a markedly negative association between the cumulative percentage of  
376 diatom taxa in threat categories of the Red List and nitrate concentrations. Nitrates were shown to be  
377 important environmental determinants of diatom assemblages in different types of ecosystems, such  
378 as springs (e.g., Cantonati 1998), high-mountain lakes (e.g., Tolotti 2001), limestone-precipitating  
379 springs (e.g., Denys and Oosterlynck 2015), large peri-Alpine lakes (e.g., Spitale et al. 2011) and  
380 streams (e.g., Smucker et al. 2020). Werum (2001) observed a significant reduction of the proportion  
381 of the RL96 species in threat categories when springs were affected by acid deposition with higher  
382 nitrate concentrations or in springs with intermittent discharge, while this did not occur in springs of  
383 slightly higher trophic status. Werum and Lange-Bertalot (2004) noted that the relative portion of  
384 endangered Red List species was more sensitive to the impact caused by spring-capturing than the  
385 Shannon-Wiener entropy index.

386 Diatom communities always react to multiple stressors that can act in combination. Nevertheless,  
387 the negative relation of nitrates to the cumulative percentage of diatom taxa in threat categories of the  
388 Red List persisted throughout a wide range of habitats, geographic regions differing in human impact,  
389 and neo- as well as paleolimnological records, making it particularly noteworthy.

390 Denys (2000) observed that, if some sensitive habitat, e.g. dystrophic mires, is not represented in a  
391 geographic area of more limited extent, this should be taken into due account when applying a  
392 specific diatom Red List for the wider region. On a similar note, Denys (2000) pointed out the  
393 relevance of developing specific lists for regions in which naturally-eutrophic waters are particularly  
394 common. Cantonati et al. (2020c), during a tentative application of a Red-List approach to streams in  
395 the water-stressed island of Cyprus, found, somewhat unexpectedly, that both species from threat  
396 categories of RL18 and putative threat-category species of a possible future Red List tailored for  
397 Cyprus occurred more frequently and were more relevant in assemblages from sites in intermittent  
398 streams. Since temporary streams are more characteristic than permanent ones in the Mediterranean  
399 climatic setting, this points to the necessity of developing diatom red lists tailored for the different  
400 biogeographic and climatic ecoregions.

401

402

### 403 **Conclusions and way forward**

404 The main conclusions that can be drawn from our study are:

- 405 • Literature on algal Red Lists is very limited, and there are basically only two editions of a  
406 single diatom-specific Red List (developed for Germany), in spite of the paramount  
407 importance of this group of protists for biodiversity and global ecosystem functioning.
- 408 • We have gathered evidence to show that this approach is useful, and should be extended to  
409 many more geographic areas. It is also an efficient way to preserve expert knowledge, and  
410 make it available in an objectively ordered way to future scholars.

- 411 • There is excellent agreement between the two editions of the Red List for Germany, which  
412 confirms that an approach already grounded in robust expert knowledge (Lange-Bertalot  
413 1996) was supported and validated by a large and taxonomically harmonized database  
414 (Hofmann et al. 2018).
- 415 • The association between RL18 threat categories and RL18 ecological groups reveals that: -  
416 threatened taxa concentrate in dystrophic-oligotrophic environments, - 'not threatened' taxa  
417 (= tolerant and opportunistic) are most frequent in eutrophic and saline ecosystems, - most  
418 regional extinctions occurred in carbonate oligotrophic habitats.
- 419 • The high frequency of 'not threatened' taxa (\*) in eutrophic and saline ecosystems reflects  
420 two of the current main impacts on freshwaters: eutrophication (pollution) and salinization.
- 421 • The significantly more frequent than expected occurrence of 'threatened' and 'extremely rare'  
422 taxa among pseud- and eu-aerial species probably reflects the fact that studies on terrestrial  
423 diatoms are still relatively rare when compared to submersed assemblages, which include the  
424 majority of the 'not threatened' taxa.
- 425 • Nitrates have a highly significant negative association with the cumulative percentage of  
426 diatom taxa in threat categories of the Red List. Having included heterogeneous studies, this  
427 points to the high potential of diatom-Red-List based approaches with regard to nitrogen  
428 management in freshwaters.

429 The main suggestions emerging from our study for the future development of freshwater diatom  
430 Red Lists are:

- 431 • In future applications of diatom-based Red Lists, if affordable, approaches applying Red  
432 Lists to large numbers of individual samples, rather than to databases reflecting the overall  
433 composition of the species pool, should be preferred.
- 434 • We suggest to ground future Red Lists on collations of databases adequately representing  
435 all types of inland-water habitats occurring in the region of interest and not only those  
436 considered in monitoring networks. Any future diatom Red List approaches should be  
437 primarily data-driven, and additionally validated by expert knowledge.

- 438           • The applications of the diatom Red List so far point out the importance to develop diatom  
439           Red Lists tailored for the different biogeographic and climatic ecoregions.

440

441

442 **Acknowledgements** The authors (other than HL-B) are most glad to use the occasion of the  
443 publication of this paper to celebrate Prof. Horst Lange-Bertalot, distinguished diatomist and pioneer  
444 of diatom Red Lists, on the occasion of his 85<sup>th</sup> birthday.

445 We thank the Editors and Reviewers for useful suggestions.

446

447 **Author contributions** MC: Conceptualization, Data curation, Funding acquisition, Investigation,  
448 Methodology, Project administration, Resources, Visualization, Writing - original draft, Writing -  
449 review & editing. GH: Conceptualization, Data curation, Funding acquisition, Methodology, Writing -  
450 review & editing. DS: Data curation, Formal analysis, Visualization, Writing - original draft, Writing  
451 - review & editing. MW: Data curation, Methodology, Resources, Writing - review & editing. HLB:  
452 Validation, Writing - review & editing.

453

454 **Funding** The work was financed entirely by the authors.

455

456 **Compliance with ethical standards**

457

458 **Conflict of interest** The authors have no conflict of interest to declare that are relevant to the content  
459 of this article.

460

461 **Ethical issues** None.

462

463 **References**

464 Angeli N, Cantonati M, Spitale D, Lange-Bertalot H (2010) A comparison between diatom  
465 assemblages in two groups of carbonate, low-altitude springs with different levels of  
466 anthropogenic disturbances. *Fottea* 10(1):115–128. <https://doi.org/10.5507/fot.2010.006>

467 Bertrand J, Renon, JP, Monnier O (1999) Les diatomées des sources du rebord karstique de la Beauce  
468 de la région orléanaise. *Symbioses* 1:3–14

469 Bothwell ML, Taylor BW, Kilroy C (2014) The Didymo story: the role of low dissolved phosphorus  
470 in the formation of *Didymosphenia geminata* blooms, *Diatom Res* 29:229–236. <https://doi.org/10.1080/0269249X.2014.889041>

471

472 Bowler C, Allen AE, Badger JH, Grimwood J, Jabbari K, Kuo A, Maheswari U, Martens C, Maumus  
473 F, Otilar RP, Rayko E, Salamov A, Vandepoele K, Beszteri B, Gruber A, Heijde M, Katinka M,  
474 Mock T, Valentin K, Verret F, Berges JA, Brownlee C, Cadoret JP, Chiovitti A, Choi CJ, Coesel  
475 S, De Martino A, Detter JC, Durkin C, Falciatore A, Fournet J, Haruta M, Huysman MJ, Jenkins  
476 BD, Jiroutova K, Jorgensen RE, Joubert Y, Kaplan A, Kroger N, Kroth PG, La Roche J, Lindquist  
477 E, Lommer M, Martin-Jezequel V, Lopez PJ, Lucas S, Mangogna M, McGinnis K, Medlin LK,  
478 Montsant A, Oudot-Le Secq MP, Napoli C, Obornik M, Parker MS, Petit JL, Porcel BM, Poulsen  
479 N, Robison M, Rychlewski L, Rynearson TA, Schmutz J, Shapiro H, Siau M, Stanley M,  
480 Sussman MR, Taylor AR, Vardi A, von Dassow P, Vyverman W, Willis A, Wyrwicz LS, Rokhsar  
481 DS, Weissenbach J, Armbrust EV, Green BR, Van de Peer Y, Grigoriev IV (2008) The  
482 *Phaeodactylum* genome reveals the evolutionary history of diatom genomes. *Nature* 456:239–44

483 Cantonati M (1998) Diatom communities of springs in the Southern Alps. *Diatom Res* 13:201–220.  
484 <https://doi.org/10.1080/0269249X.1998.9705449>

485 Cantonati M (2004) Le diatomee di tre sorgenti del Parco Naturale delle Prealpi Giulie (Italia nord-  
486 orientale). *Gortania – Atti Mus Friul di Storia Nat* 25:95–108

487 Cantonati M, Angeli N, Bertuzzi E, Spitale D, Lange-Bertalot H (2012b) Diatoms in springs of the  
488 Alps: spring types, environmental determinants, and substratum. *Freshw Sci* 31:499–524.  
489 <https://doi.org/10.1899/11-065.1>

490 Cantonati M, Kelly MG, Demartini D, Angeli N, Dörflinger G, Papatheodoulou A, Armanini D  
491 (2020c) Overwhelming role of hydrology-related variables and river types in driving diatom

492 species distribution and community assemblage in streams in Cyprus. *Ecol Ind* 117, 106690.  
493 <https://doi.org/10.1016/j.ecolind.2020.106690>

494 Cantonati M, Kelly MG, Lange-Bertalot H (2017) Freshwater benthic diatoms of Central Europe:  
495 over 800 common species used in ecological assessment. Koeltz Botanical Books, Schmittens-  
496 Oberreifenberg, Germany (942 pp).

497 Cantonati M, Lange-Bertalot H (2010) Diatom biodiversity of springs in the Berchtesgaden National  
498 Park (northern Alps, Germany), with the ecological and morphological characterization of two  
499 species new to science. *Diatom Res* 25:251–280. <https://doi.org/10.1080/0269249X.2010.9705849>

500 Cantonati M, Lange-Bertalot H (2011) Diatom monitors of close-to-pristine, very-low alkalinity  
501 habitats: three new *Eunotia* species from springs in Nature Parks of the south-eastern Alps. *J*  
502 *Limnol* 70:209–221. <https://doi.org/10.4081/jlimnol.2011.209>

503 Cantonati M, Lange-Bertalot H, Decet F, Gabrieli J (2011) Diatoms in very-shallow pools of the site  
504 of community importance Danta di Cadore Mires (south-eastern Alps), and the potential  
505 contribution of these habitats to diatom biodiversity conservation. *Nova Hedwigia* 93:475–507.  
506 <https://doi.org/10.1127/0029-5035/2011/0093-0475>

507 Cantonati M, Poikane S, Pringle CM, Stevens LE, Turak E, Heino J, Richardson JS, Bolpagni R,  
508 Borrini A, Cid N, Čtvrtlíková M, Galassi DMP, Hájek M, Hawes I, Levkov Z, Naselli-Flores L,  
509 Saber AA, Di Cicco M, Fiasca B, Hamilton PB, Kubečka J, Segadelli S, Znachor P (2020a)  
510 Characteristics, Main Impacts, and Stewardship of Natural and Artificial Freshwater  
511 Environments: Consequences for Biodiversity Conservation. *Water* 12:260.  
512 <https://doi.org/10.3390/w12010260>

513 Cantonati M, Scola S, Angeli N, Guella G, Frassanito R (2009) Environmental controls of epilithic  
514 diatom depth-distribution in an oligotrophic lake characterised by marked water-level fluctuations.  
515 *Eur J Phycol* 44:15–29. <https://doi.org/10.1080/09670260802079335>

516 Cantonati M, Segadelli S, Spitale S, Gabrieli J, Gerecke R, Angeli N, De Nardo MT, Ogata K, Wehr  
517 JD (2020b) Geological and hydrochemical prerequisites of unexpectedly high biodiversity in  
518 spring ecosystems at the landscape level. *Sci Total Environ* 740:140157.  
519 <https://doi.org/10.1016/j.scitotenv.2020.140157>

520 Cantonati M, Spitale D (2009) The role of environmental variables in structuring epiphytic and  
521 epilithic diatom assemblages in springs and streams of the Dolomiti Bellunesi National Park  
522 (south-eastern Alps). *Fundam Appl Limnol* 174:117–133. [https://doi.org/10.1127/1863-](https://doi.org/10.1127/1863-9135/2009/0174-0117)  
523 9135/2009/0174-0117

524 Cantonati M, Stevens LE, Segadelli S, Springer AE, Goldscheider N, Celico F, Filippini M, Ogata K,  
525 Gargini A (2020d) Ecohydrogeology: The interdisciplinary convergence needed to improve the  
526 study and stewardship of springs and other groundwater-dependent habitats, biota, and  
527 ecosystems. *Ecol Ind* 110. <https://doi.org/10.1016/j.ecolind.2019.105803>

528 Cantonati M., Van de Vijver B., Lange-Bertalot H (2009) *Microfissurata* gen. nov. (Bacillariophyta),  
529 a new diatom genus from dystrophic and intermittently-wet terrestrial habitats. *J Phycol* 45:732–  
530 741. <https://doi.org/10.1111/j.1529-8817.2009.00683.x>

531 Cantonati M, Zorza R, Bertoli M, Pastorino P, Salvi G, Platania G, Prearo M, Pizzul E (2021a) Recent  
532 and subfossil diatom assemblages as indicators of environmental change (including fish  
533 introduction) in a high-mountain lake. *Ecol Ind* 125:107603.  
534 <https://doi.org/10.1016/j.ecolind.2021.107603>

535 Cohen J (1960) A coefficient of agreement for nominal scales. *Educational and Psychological*  
536 *Measurement* 20:37–46

537 Cohen J (1968) Weighted kappa: Nominal scale agreement with provision for scaled disagreement or  
538 partial credit. *Psychological Bull* 70:213–220

539 Davies TJ (2019) The macroecology and macroevolution of plant species at risk. *New Phytologist*  
540 222:708–713. <https://doi.org/10.1111/nph.15612>

541 Denys L (2000) Historical distribution of 'Red List Diatoms' (Bacillariophyceae) in Flanders  
542 (Belgium). *Systematics Geogr Plants* 70 (2):409–420. <https://doi.org/10.2307/3668653>

543 Denys L, Oosterlynck P (2015) Diatom assemblages of non-living substrates in petrifying  
544 *Cratoneurion* springs from lower Belgium. *Fottea*, 15 (2), 123–138.  
545 <https://doi.org/10.5507/fot.2015.014>

546 Downing JA (2014) Limnology and oceanography: two estranged twins reuniting by global change.  
547 *Inland Waters* 4, 215–232. <https://doi.org/10.5268/iw-4.2.753>.

548 European Union (2000) Directive 2000/60/EC of the European Parliament and of the Council of 23rd  
549 October 2000 establishing a framework for community action in the field of water policy. Official  
550 Journal of the European Communities, L327/1. Brussels, European Commission.

551 Falasco E, Bona F (2011) Diatom community biodiversity in an Alpine protected area: a study in the  
552 Maritime Alps Natural Park. *J Limnol* 70(2):157–167.

553 Falasco E, Piano E, Bona F (2016) Diatom flora in Mediterranean streams: flow intermittency  
554 threatens endangered species. *Biodivers Conserv* 25 (14):2965–2986.  
555 <https://doi.org/10.1007/s10531-016-1213-8>

556 Fell SC, Carrivick JL, Kelly MG, Füreder L, Brown LE (2018) Declining glacier cover threatens the  
557 biodiversity of alpine river diatom assemblages. *Glob Chang Biol* 24(12):5828-5840.  
558 <https://doi.org/10.1111/gcb.14454>

559 Finck P, Heinze S, Raths U, Riecken U, Ssymank A (2017) Rote Liste der gefährdeten Biotoptypen  
560 Deutschlands. Dritte fortgeschriebene Fassung 2017. *Natursch Biol Vielf* 156:637 pp.

561 Foets J, Stanek-Tarkowska J, Teuling AJ, Van de Vijver B, Wetzel CE, Pfister L (2021) Autecology  
562 of terrestrial diatoms under anthropic disturbance and across climate zones. *Ecol Ind* 122:107248.  
563 <https://doi.org/10.1016/j.ecolind.2020.107248>

564 Foissner W (2008) Protist diversity and distribution: some basic considerations. *Biodivers Conserv*  
565 17:235–242

566 Fránková M, Bojková J, Poulíčková A, Hájek M (2009) The structure and species richness of the  
567 diatom assemblages of the Western Carpathian spring fens along the gradient of mineral richness.  
568 *Fottea* 9:355–368. <https://doi.org/10.5507/fot.2009.035>

569 Friendly M 2021 Package ‘vcdExtra’, extension and addition. R package version 0.7-5.

570 Friendly M, Meyer D (2015) Discrete data analysis with R: visualization and modelling techniques  
571 for categorical and count data (Vol. 120). CRC Press.

572 Gärdenfors U, Hilton-Taylor C, Mace G, Rodríguez JP (2001) The application of IUCN Red List  
573 Criteria at Regional levels. *Conserv Biol* 15(5):1206–1212

574 Geissler U (1988) Some changes in the flora and vegetation of algae in freshwater environments.  
575 *Helgoländer Meeresuntersuchungen* 42 (3-4):637–643. <https://doi.org/10.1007/BF02365632>



576 Gesierich D, Kofler W (2010) Epilithic diatoms from rheocene springs in the eastern Alps  
577 (Vorarlberg, Austria). *Diatom Res* 25:43–66

578 Hindák F, Hindáková A (2001) Červený zoznam siníc/cyanobaktérií a rias Slovenska. In: Baláž D,  
579 Marhold K, Urban P (eds) Červený zoznam rastlín a živočíchov Slovenska. Štátna ochrana prírody  
580 Slovenskej republiky and Centrum ochrany prírody a krajiny, Banská Bystrica, pp 14–22

581 Hofmann G, Lange-Bertalot H, Werum M, Klee R (2018) Rote Liste der limnischen Kieselalgen.  
582 *Naturschutz und Biologische Vielfalt* 70(7):601–708

583 Hofmann G, Werum M, Lange-Bertalot H (2013) Diatomeen im Süßwasser-Benthos von  
584 Mitteleuropa. Bestimmungsflora Kieselalgen für die ökologische Praxis. Über 700 der häufigsten  
585 Arten und ihre Ökologie. 2. korrigierte Auflage. – Ruggell (A.R.G. Gantner):908 pp.

586 IUCN (2012) Guidelines for Application of IUCN Red List Criteria at Regional and National Levels:  
587 Version 4.0. Gland, Switzerland and Cambridge, UK: IUCN. iii + 41pp. ISBN: 978-2-8317-1247-  
588 5

589 Juráň J, Kaštovský J (2019) The procedure of compiling the Red List of microscopic algae of the  
590 Czech Republic. *Biodivers Conserv* DOI: 10.1007/s10531-019-01792-x

591 Kamberović J (2020) Springs Ecosystem of the Konjuh Mountain. Tuzla. B&H. OFF – SET  
592 Štamparija. 176 pp.

593 Kamberović J, Plenković-Moraj A, Kralj Borojević K, Gligora Udovič M, Žutinić P, Hafner D,  
594 Cantonati M (2019) Algal assemblages in springs of different lithologies (ophiolithes vs.  
595 limestone) of the Konjuh Mountain (Bosnia and Herzegovina). *Acta Botanica Croatica* 78(1):66–  
596 81. <https://doi.org/10.2478/botcro-2019-0004>

597 Kaushal SS, Likens GE, Pace ML, Utz R, Haq S, Gorman J, Grese M (2018) Freshwater salinization  
598 syndrome on a continental scale. *Proceedings of the National Academy of Sciences of the United*  
599 *States of America* <https://doi.org/10.1073/pnas.1711234115>

600 Kociolek JP, Stoermer EF (2009) Oligotrophy: the forgotten end of an ecological spectrum.  
601 *Proceedings of the 20th International Diatom Symposium (Dubrovnik, Croatia)*. *Acta Botanica*  
602 *Croatica* 68:465–472

603 Lange-Bertalot H, Metzeltin D (1996) Indicators of Oligotrophy. In: H. Lange-Bertalot (Ed.), *Iconogr*  
604 *Diatomol* 2. Koeltz, Koenigstein: 1–390

605 Lange-Bertalot H (1996) Rote liste der limnischen Kieselalgen (Bacillariophyceae) Deutschlands.  
606 *Schriftenreihe für Vegetationskunde* 28:633–677

607 Lange-Bertalot H (1997) A first ecological evaluation of the diatom flora in Central Europe.  
608 *Lauterbornia* 31:117–123

609 Lilleleht V, Ernits P, Hang V et al. (2008). Red data book of Estonia. Commission for nature  
610 conservation of the Estonian academy of sciences. <http://www.zbi.ee/punane/englis/index.html>.  
611 Accessed 28 Jan 2018

612 Mann DG (1999) The species concept in diatoms. *Phycological Reviews* 18. *Phycologia* 38:437–495

613 Mann DG, Vanormelingen P (2013) An Inordinate Fondness? The Number, Distributions, and  
614 Origins of Diatom Species. *J Eukaryot Microbiol* 60:414–420. <https://doi.org/10.1111/jeu.12047>

615 Medlin LK, Kooistra WHCF, Gersonde R, Sims PA, Wellbrock U (1997) Is the origin of the diatoms  
616 related to the end-Permian mass extinction? *Nova Hedwigia* 65:1–11

617 Meyer D, Zeileis A, Hornik K, Gerber F, Friendly M, Meyer MD (2020) Package ‘vcd’. Visualizing  
618 Categorical Data. R package version, 1-4-8.

619 Mogna M, Cantonati M, Andreucci F, Angeli N, Berta G, Miserere L (2015) Diatom communities and  
620 vegetation of springs in the south-western Alps. *Acta Botanica Croatica* 74:265–285.  
621 <https://doi.org/10.1515/botcro-2015-0024>

622 Neméth J (2005) Red list of algae in Hungary. *Acta Bot Hung* 47:379–417. [https://doi.org/10.1556/](https://doi.org/10.1556/ABot.47.2005.3-4.7)  
623 [ABot.47.2005.3-4.7](https://doi.org/10.1556/ABot.47.2005.3-4.7)

624 Paini DR, Mokany K (2016) Birds of a feather: using species assemblages to assess vulnerability to  
625 extinction. *Ecol Appl* 26(2):415–423

626 R Core Team (2020) R: A language and environment for statistical computing. R Foundation for  
627 Statistical Computing, Vienna, Austria. <https://www.R-project.org/> (accessed 07 March 2020).

628 Reichardt E (1997) Taxonomische Revision des Artenkomplexes um *Gomphonema pumilum*  
629 (Bacillariophyceae). *Nova Hedwigia* 65:99–129

630 Reichardt E (2006) Bemerkenswerte Diatomeenfunde aus Bayern. V. Neue und seltene Arten aus der  
631 Schwarzachklamm. Berichte der Bayerischen Botanischen Gesellschaft 76:41–51

632 Reichardt E (2012) Der Artenkomplex um *Gomphonema occultum* E. Reichardt & Lange-Bertalot  
633 (Bacillariophyceae): Variabilität und drei neue Arten. Nova Hedwigia, Beiheft 141:155–168

634 Ricciardi A, Rasmussen JB (1999) Extinction Rates of North American Freshwater Fauna. Cons Biol  
635 13:1220-1222. <http://dx.doi.org/10.1046/j.1523-1739.1999.98380.x>

636 Rimet F, Bouchez A (2012) Life-forms, cell-sizes and ecological guilds of diatoms in European  
637 rivers. Knowledge Manag Aquat Ecosyst 406:01–12

638 Siemińska J, Bąk M, Dziejczak J, Gąbka M, Gregorowicz P et al. (2006) Red list of the algae in Poland  
639 – Czerwona lista glonów w Polsce. In: Z. Mirek et al. (Eds) Red list of plants and fungi in Poland  
640 – Czerwona lista roślin i grzybów Polski. Polish Academy of Sciences, Kraków, 35–52

641 Sims PA, Mann DG, Medlin LK (2006) Evolution of the diatoms: insights from fossil, biological and  
642 molecular data. Phycologia 45:361–402

643 Smol JP, Stoermer EF (2010) The Diatoms: Applications for the Environmental and Earth Sciences,  
644 Second Edition. Cambridge University Press, 667 pp.

645 Smucker NJ, Pilgrim EM, Nietch CT, Darling JA, Johnson BR (2020) DNA metabarcoding  
646 effectively quantifies diatom responses to nutrients in streams. Ecol Appl 30(8):e02205.  
647 [10.1002/eap.2205](https://doi.org/10.1002/eap.2205)

648 Soininen J (2007) Environmental and spatial control of freshwater diatoms – a review. Diatom Res  
649 22:473–490

650 Spitale D, Scalfi A, Lange-Bertalot H, Cantonati M (2011) Using different epilithic-diatom  
651 assemblage metrics for an ecological characterization of the shores of Lake Garda. J Limnol  
652 70:197–208. <https://doi.org/10.4081/jlimnol.2011.197>

653 Taxböck L, Karger DN, Kessler M, Spitale D, Cantonati M (2020) Diatom species richness in Swiss  
654 springs increases with habitat complexity and elevation. Water 12:449.  
655 <https://doi.org/10.3390/w12020449>

656 Taxböck L, Linder HP, Cantonati M (2017) To what extent are Swiss springs refugial habitats for  
657 sensitive and endangered diatom taxa? Water 9(12):967. <https://doi.org/10.3390/w9120967>

658 Taxböck L, Preisig HR (2007) The diatom communities in Swiss springs: a first approach.  
659 Proceedings of the 1st Central European Diatom Meeting, Kusber. Pages 163–168 in W.-H.  
660 Kusber and R. Jahn (editors). Botanic Garden and Botanical Museum Berlin-Dahlem. Freie  
661 Universität Berlin, Berlin, Germany.

662 Tolotti M (2001) Littoral diatom communities in high mountain lakes of the Adamello–Brenta  
663 Regional Park (Trentino, Italy) and their relation to acidification. 327–352 in R. Jahn, J. P.  
664 Kociolek, A. Witkowski, and P. Compère (editors). Lange–Bertalot Festschrift. A.R. G. Gantner  
665 Verlag K. G., Ruggell, Liechtenstein.

666 van Dam H, Mertens A, Sinkeldam J (1994) A coded checklist and ecological indicator values of  
667 freshwater diatoms from the Netherlands. *Netherlands J Aquat Ecol* 28(1):117–133.  
668 <https://doi.org/10.1007/bf02334251>

669 van Rees CB, Waylen KA, Schmidt-Kloiber A, Thackeray SJ, Kalinkat G, Martens K, Domisch S,  
670 Lillebø AI, Hermoso V, Grossart H, Schinegger R, Declerck K, Adriaens T, Denys L, Jarić I, Janse  
671 JH, Monaghan MT, De Wever A, Geijzendorffer I, Adamescu MC, Jähnig SC (2020)  
672 Safeguarding Freshwater Life Beyond 2020: Recommendations for the New Global Biodiversity  
673 Framework from the European Experience. *Cons Lett* 2020;e12771.  
674 <https://doi.org/10.1111/conl.12771>

675 Vanormelingen P, Verleyen E, Vyverman W (2008) The diversity and distribution of diatoms: from  
676 cosmopolitanism to narrow endemism. *Biodiversity and Conservation* 17: 393–405.

677 Werum M (2001) Die Kieselalgen-Gesellschaften in Quellen. Abhängigkeit von Geologie und  
678 anthropogener Beeinflussung in Hessen (Bundesrepublik Deutschland) – Hessisches Landesamt  
679 für Umwelt und Geologie, pp. 273. ISBN 3-89026-331-3.

680 Werum M, Lange-Bertalot H (2004) Diatoms in springs from Central Europe and elsewhere under the  
681 influence of hydrogeology and anthropogenic impacts. *Iconogr Diatomol* 13. A. R. G. Gantner  
682 Verlag K. G., Ruggell (480 pp.).

683 **Tables**

684 **Table 1** Agreement between RL96 and RL18. Computations were done only on taxa with congruent  
 685 taxonomy. 1 = perfect association, 0 = no association. RL18 threat categories are as follows: 0 = presumed  
 686 extinct, 1 = threatened with extinction, 2 = strongly threatened, 3 = threatened, G = threat of unknown extent, R  
 687 = extremely rare, V = declining, D = data insufficient, \* = not threatened

688

	<b>1</b>	<b>2</b>	<b>3</b>	<b>G</b>	<b>R</b>	<b>V</b>	<b>*</b>	<b>D</b>
<b>1</b>	0.62	0.03	0.00	0.01	0.00	0.00	0.00	0.00
<b>2</b>	0.04	0.36	0.01	0.04	0.00	0.02	0.00	0.00
<b>3</b>	0.12	0.37	0.58	0.06	0.02	0.00	0.01	0.02
<b>G</b>	0.00	0.06	0.13	0.39	0.00	0.00	0.01	0.02
<b>R</b>	0.17	0.16	0.06	0.24	0.91	0.00	0.03	0.06
<b>V</b>	0.00	0.03	0.15	0.14	0.01	0.75	0.05	0.03
<b>*</b>	0.00	0.01	0.07	0.07	0.01	0.21	0.87	0.11
<b>D</b>	0.04	0.00	0.00	0.05	0.06	0.02	0.04	0.76

689

690

691

692 **Table 2** Association (Cramer's V) between the RL18 threat categories and the current-distribution situation of  
 693 the diatom taxa. RL18 threat categories are as in Table 1 (or see the Methods section). RL18 current-distribution  
 694 categories are as follows: ex = extinct or lost, er = extremely rare, vr = very rare, r = rare, mf = moderately  
 695 frequent, f = frequent, vf = very frequent, ? = unknown

696

	<b>ex</b>	<b>er</b>	<b>vr</b>	<b>r</b>	<b>mf</b>	<b>f</b>	<b>vf</b>	<b>?</b>
<b>0</b>	1	0	0	0	0	0	0	0
<b>1</b>	0	1	0	0	0	0	0	0
<b>2</b>	0	0.3	0.7	0	0	0	0	0
<b>3</b>	0	0	0.1	0.9	0	0	0	0
<b>G</b>	0	0	0.8	0.2	0	0	0	0
<b>R</b>	0	1	0	0	0	0	0	0
<b>V</b>	0	0	0	0.7	0.3	0	0	0
<b>*</b>	0	0	0	0.3	0.4	0.2	0.05	0
<b>D</b>	0	0	0.1	0.1	0	0	0	0.86

697

698

699

700

701

702 **Table 3** Main Red List attributes of the 55 most menaced taxa of RL18 (4 taxa in category “0” and 51 taxa in  
703 cat. “1”). RL18 (and RL96) threat categories are as in Table 1, RL18 current-distribution categories are as in  
704 Table 2, and ecological groups are as in Fig. 4 (or see the Methods section). Long-term development trends are  
705 as follows: (<) decline of unknown extent, < moderate decline

Taxon (name)	threat category	ecol. group	distribution	RL96	long-term dev.		threat category	ecol. group	distribution	RL96	long-term dev.
<i>Cyclotella lemanensis</i>	0	oc	ex	*	=	<i>Frustulia krammeri</i>	1	od	er	D	(<)
<i>Cymbella hustedtii</i> var. <i>compacta</i>	0	oc	ex	D	=	<i>Geissleria declivis</i>	1	od	er	1	(<)
<i>Cymbopleura</i> <i>citriiformis</i>	0	o	ex	D	=	<i>Geissleria thingvallae</i>	1	?	er	R	(<)
<i>Encyonema latens</i>	0	oc	ex	D	=	<i>Hippodonta costulatiformis</i>	1	?	er	D	(<)
<i>Achnanthes nodosa</i>	1	od	er	1	(<)	<i>Kobayasiella madumensis</i>	1	od	er	D	(<)
<i>Achnantheidium</i> <i>trinode</i>	1	oc	er	3	(<)	<i>Kobayasiella okadae</i>	1	od	er	1	(<)
<i>Chamaepinnularia</i> <i>soehrensoides</i>	1	od	er	D	(<)	<i>Navicula detenta</i>	1	o	er	1	(<)
<i>Cyclotella schroeteri</i>	1	oc	er	D	(<)	<i>Navicula diabolica</i>	1	?	er	R	(<)
<i>Cyclotella socialis</i>	1	o	er	D	(<)	<i>Nitzschia garrensis</i>	1	od	er	1	(<)
<i>Encyonema hophense</i>	1	oc	er	D	(<)	<i>Pinnularia biceps</i> var. <i>gibberula</i>	1	od	er	D	(<)
<i>Encyonema procerum</i>	1	?	er	D	(<)	<i>Pinnularia inculcata</i>	1	?	er	D	(<)
<i>Encyonopsis tiroliana</i>	1	oc	er	D	(<)	<i>Pinnularia nobilis</i> var. <i>nobilis</i>	1	o	er	D	(<)
<i>Eunotia</i> <i>alkalibiontica</i>	1	oc	er	D	(<)	<i>Pinnularia persudetica</i> var. <i>silvatica</i>	1	od	er	D	(<)
<i>Eunotia bactriana</i>	1	od	er	D	(<)	<i>Pinnularia renatiformis</i>	1	o	er	D	(<)
<i>Eunotia biconstricta</i>	1	od	er	D	(<)	<i>Pinnularia ruttneri</i> var. <i>lauenburgiana</i>	1	?	er	R	(<)
<i>Eunotia braendlei</i>	1	o	er	D	(<)	<i>Pinnularia simonsenii</i>	1	o	er	D	(<)
<i>Eunotia</i> <i>circumborealis</i>	1	od	er	D	(<)	<i>Pinnularia stidolphii</i>	1	o	er	D	(<)
<i>Eunotia cisalpina</i>	1	od	er	D	(<)	<i>Pinnularia subrhombica</i> var. <i>subrhombica</i>	1	od	er	D	(<)
<i>Eunotia denticulata</i>	1	od	er	D	(<)	<i>Pinnularia subrhombica</i> var. <i>angusta</i>	1	od	er	D	(<)
<i>Eunotia diadema</i>	1	od	er	1	(<)	<i>Placoneis navicularis</i>	1	o	er	1	(<)
<i>Eunotia elegans</i>	1	od	er	1	(<)	<i>Platessa rupestris</i>	1	o	er	1	(<)
<i>Eunotia faba</i>	1	od	er	1	(<)	<i>Psammothidium kuelbsii</i>	1	od	er	3	<
<i>Eunotia hexaglyphis</i>	1	od	er	1	(<)	<i>Psammothidium lacus-vulcani</i>	1	od	er	2	<
<i>Eunotia lapponica</i>	1	od	er	D	(<)	<i>Tetracyclus emarginatus</i>	1	od	er	R	(<)

<i>Eunotia pseudopectinalis</i>	1	od	er	1	(<)	<i>Tetracyclus glans</i>	1	od	er	3	(<)
<i>Eunotia renata</i>	1	od	er	D	(<)						
<i>Eunotia rhynchocephala</i>	1	od	er	1	(<)						
<i>Eunotia serra</i>	1	od	er	1	(<)						
<i>Eunotia silvahercynia</i>	1	od	er	1	(<)						
<i>Eunotia superpaludosa</i>	1	od	er	D	(<)						

---

706

707



708 **Table 4** Different inland-waters habitats ranked by decreasing percentage of threatened RL96 (shaded  
709 background) and RL18 diatom taxa. %RL = % diatom taxa belonging to the sum of all threatened Red List  
710 categories, N = N. of sampling stations, TNT = total number of taxa found, org. pol. = organic pollution, str. =  
711 streams. ABNP = Adamello-Brenta Natural Park (Trentino, Italy), CRENODAT Project (Autonomous Province  
712 of Trento = Trentino, Italy), DBNP = Dolomiti Bellunesi National Park (Veneto Region, Italy), CESSPA Project  
713 (Province of Verona, Veneto Region, Italy), BeNP = Berchtesgaden National Park (Bavaria, Germany), GNP =  
714 Gesäuse National Park (Styria, Austria), JPNP = Julian Prealps National Park (Friuli Venezia Giulia Autonomous  
715 Region, Italy). lm = lithic material, b = bryophytes, ss = surface sediment, fga = filamentous green-algae, ll =  
716 leaf litter, sa = sand

Habitat type (substrata sampled)	Location	N	[N-NO <sub>3</sub> <sup>-</sup> ] µg L <sup>-1</sup>				Impacts	Reference
			%RL	Mean	Med.	TNT		
Mire pools (b, ss)	Danta di Cadore, south-eastern Alps	5	<b>72</b>	<b>5</b>	<b>6</b>	86	trails, roads	Cantonati et al. (2011)
Springs of different ecomorphological types on various geological substrata (lm, b, ss)	Emilia-Romagna Region, Northern Apennines, Italy	16	<b>60</b>	<b>186</b>	<b>196</b>	285	Agriculture and industrial activities in the lowlands, water abstraction	Cantonati et al. (2020a)
High-mountain lakes on holocrystalline rocks (lm, b, ss)	ABNP, south-eastern Alps	16	<b>56</b>	<b>192</b>	<b>196</b>	204	used as reservoirs for hydropower	Tolotti (2001)
Shallow (max depth = 6.4 m) high mountain lake (neolimnology: lm, paleolimnology: sediment core)	Balma Lake, Orsiera Rocciavè Nature Park, Piedmont, northwestern Italy	14	<b>55</b>	<b>271</b>	<b>284</b>	103	long-distance airborne airborne pollutants, grazing & cattle watering, fishing	Cantonati et al. (2021a)
Mountain carbonate springs (lm, b, ss)	BeNP, north-eastern Alps	9	<b>54</b>	<b>303</b>	<b>248</b>	104	forestry, hunting, cattle breeding	Cantonati and Lange-Bertalot (2010)
Springs (all main lithologies) (lm, b)	Trentino, CRENODAT Project, s.-e. Alps	110	<b>53</b>	<b>735</b>	<b>559</b>	370	water abstraction, pastures, roads, agricultural runoff	Cantonati et al. (2012)
Low-elevation siliceous-metamorphic mountain springs (lm, b, ss)	BFNP 2018	15	<b>53</b>	<b>760</b>	<b>780</b>	120	in the recent past: acidification, forestry	Cantonati et al. unpublished
High-mountain lake on holocrystalline rocks (lm)	Trentino, Lake Cornisello	8	<b>69</b>	<b>170</b>	<b>173</b>	96		MC & M. Segnana unpublished
Carbonate and holocrystalline mountain springs (lm, b, ss)	ABNP, south-eastern Alps	30	<b>48</b>	<b>516</b>	<b>468</b>	254	pastures	Cantonati (1998)

Mountain carbonate springs (lm, b, ss)	BeNP 2018	15	<b>47</b>	<b>754</b>	<b>761</b>	162	forestry, hunting, cattle breeding	Cantonati et al. unpublished
Siliceous metamorphic springs (lm, b, ss)	South-western Alps springs	48	<b>46</b>	<b>302</b>	<b>230</b>	223	cattle watering	Mogna et al. (2015)
Swiss springs: 7 spring types over a wide altitudinal range & wide range of anthropogenic alterations (lm, ss, b, fga, ll)	springs in central and eastern Switzerland as well as the Jura Mountains	74	<b>45</b>	<b>878</b>	<b>512</b>	504	eutrophication, morphology alteration	Taxböck et al. (2017, 2020)
Carbonate springs and streams	DBNP, south-eastern Alps	21	<b>41</b>	<b>497</b>	<b>429</b>	131	org. pol. (str.)	Cantonati and Spitale (2009)
Low-altitude carbonate springs (lm, b, ss)	JPNP, south-eastern Alps	3	<b>40</b>	<b>509</b>		60	org. pol.	Cantonati (2004)
Lake Tovel (epilithon euphotic z.)	ABNP, south-eastern Alps	50	<b>40</b>	<b>350</b>	<b>340</b>			Cantonati et al. (2009)
Springs (all main lithologies) (lm)	Vicinities of Frankfurt/M. (Süd Hessen)	96	<b>37</b>	<b>1580</b>		416	water abstraction, airborne pollution, roads, agricultural runoff	Werum and Lange-Bertalot (2004)
Helocene springs (spring fens)	Western Carpathians	13	<b>33</b>	<b>587</b>		188		Fránková et al. (2009)
Lake Garda (lm)	South-eastern Alps	24	<b>33</b>	<b>420</b>	<b>440</b>	75	(NO <sub>3</sub> <sup>-</sup> ), shore-morphology alteration	Spitale et al. (2011)
Pre-Alpine carbonate springs (lm)	Areas of Basel and Zürich, CH	17	<b>30</b>	<b>2851</b>	<b>2210</b>	118		Taxböck and Preisig (2007)
Mountain springs (lm)	Springs in Vorarlberg, Austria, Alps	27	<b>28</b>	<b>2300</b>	<b>1600</b>	197	water abstraction, pastures, deforestation	Gesierich and Kofler (2010)
Mediterranean island streams (lm)	Cyprus	65	<b>27</b>	<b>985</b>	<b>380</b>	290	water scarcity and abstraction	Cantonati et al. (2020b)
springs on ophiolites and limestone)	Konjuh Mountain, Bosnia and Herzegovina	20	<b>27</b>	<b>1309</b>	<b>1230</b>	187	alterations of the morphology, water abstraction, deforestation, trampling by cattle	Kamberović et al. (2019), Kamberović et al. (2020)
Cratoneurion limestone-precipitating springs (sa, lm)	lower Belgium	13	<b>25</b>	<b>7550</b>	<b>8070</b>	310	nitrate-enriched GW	Denys and Oosterlynck (2015)
Carbonate springs (lm)	Verona Province, pre-Alpine	25	<b>24</b>	<b>1971</b>	<b>1242</b>	138	NO <sub>3</sub> <sup>-</sup> , capturing	Angeli et al. (2010)
Carbonate springs (lm)	Beauce region, Orléanais, N France	14	<b>23</b>	<b>14266</b>	<b>8465</b>	135	NO <sub>3</sub> <sup>-</sup> ,	Bertrand et al. (1999)

## 718 **Figures**

719 **Fig. 1** Observer Agreement Chart between RL96 and RL18 (only taxa with congruent taxonomic concept in  
720 the two Red Lists). In this chart black squares show observed agreement, and are positioned within larger  
721 rectangles (the large rectangle shows the maximum possible agreement). When there is perfect agreement, the  
722 rectangles are all squares, completely filled by the shaded squares. Threat categories are as in Table 1 (or see the  
723 Methods section)

724

725 **Fig. 2** Number of taxa (with percentages on each bin) in each of the threat categories of RL96 and RL18.  
726 Threat categories are as in Table 1 (or see the Methods section)

727

728 **Fig. 3** Current distribution situation of the diatom taxa of the Red List (RL18). RL18 current-distribution  
729 categories are as in Table 2 (or see the Methods section)

730

731 **Fig. 4** Association plot between RL18 threat categories and RL18 ecological groups. Please see the Methods  
732 section for correct interpretation of the association plot. ae = aerial, o = oligotraphentic, oc = oligotraphentic  
733 carbonate, od = oligotraphentic dystrophic, eu = eutrathentic to tolerant, hal = halophilic, ? = unknown. Threat  
734 categories are as in Table 1 (or see the Methods section)

735

736 **Fig. 5** Association plot between Red List categories and pH (van Dam et al., 1994). Please see the Methods  
737 section for correct interpretation of the association plot. Please see the Methods section for correct interpretation  
738 of the association plot. 1 = acidobiontic, 2 = acidophilous, 3 = circumneutral, 4 = alkaliphilous, 5 =  
739 alkalibiontic, 6 = indifferent. Threat categories are as in Table 1 (or see the Methods section)

740

741 **Fig. 6** Association plot between Red List categories and trophic status (T; van Dam et al., 1994). Please see the  
742 Methods section for correct interpretation of the association plot. 1 = oligotraphentic, 2 = oligo-mesotraphentic,  
743 3 = mesotraphentic, 4 = meso-eutrathentic, 5 = eutrathentic, 6 = hypereutrathentic, 7 = oligo- to eutrathentic  
744 (hypereutrathentic). Threat categories are as in Table 1 (or see the Methods section)

745

746 **Fig. 7** Association plot between Red List categories and moisture (M; van Dam et al., 1994). Please see the  
747 Methods section for correct interpretation of the association plot. 1 = never, or only very rarely, occurring  
748 outside water bodies; 2 = mainly occurring in water bodies, sometimes on wet places; 3 = mainly occurring in  
749 water bodies, also rather regularly on wet and moist places; 4 = mainly occurring on wet and moist or  
750 temporarily dry places; 5 = nearly exclusively occurring outside water bodies. Threat categories are as in Table  
751 1 (or see the Methods section)

752

753

754 **Supplementary Information (SI)**

755 **Tables**

756 **Supplementary Information Table 1** Main Red-List inland-water habitats of Germany. nG = national long-  
757 term threat (1 = threatened with complete destruction, 2 = strongly endangered, 3 = endangered, ★ = currently  
758 no recognizable threat, # = risk classification does not make sense), TE = current development trend (↓ =  
759 decrease, → = constant / stable, ↑ = increase, # = classification does not make sense), SE = rarity (X = very  
760 rare), RLD = Red-List status (1 = threat of complete destruction, 1-2 = endangered up to threatened with  
761 complete destruction, 2-3 = endangered to strongly threatened, 3-V = acute pre-warning list, ★ = currently no  
762 risk of loss, # = risk classification does not make sense, V = pre-warning list), RE = restorability [N = cannot be  
763 restored, K = hardly regenerable (>150 years), S = difficult to regenerate (15-150 years), B = conditionally  
764 regenerable (approx. up to 15 years), X = no classification useful] (selected and modified from Finck et al.,  
765 2017)

766

767 **Supplementary Information Table 2** Agreement between RL96 and RL18. Computations were done only on  
768 taxa with congruent taxonomy. 1 = perfect association, 0 = no association. K = Cohen's Kappa; ASE:  
769 approximate standard error; CI = confidence interval

770

771 **Supplementary Information Table 3** Taxa listed as potential neophytes in Hofmann et al. (2018). RL18  
772 attributes are as in Table 3 (or see the Methods section). Size class, and growth forms / ecological guilds are  
773 those available in Rimet and Bouchez (2012). Long-term development indications: > clear increase, ? data  
774 insufficient

775

776 **Supplementary Information Table 4** Association between motile and not-motile (Rimet and Bouchez, 2012)  
777 and threatened (1,2,3, G,R, V,) an not-threatened (\*) RL18 diatom taxa

778

779 **Supplementary Information Table 5** Parameters of the linear model for the relation of the logarithm of  
780 nitrate-nitrogen averages and the cumulative percentage of diatom species in Red List threat categories  
781 (dependent variable)

782

783 **Figures**

784 **Supplementary Information Fig. 1** Association plot between Red List categories and nitrogen uptake  
785 metabolism (N; van Dam et al., 1994). Please see the Methods section for correct interpretation of the  
786 association plot. 1 = nitrogen-autotrophic taxa, tolerating very small concentrations of organically bound  
787 nitrogen; 2 = nitrogen-autotrophic taxa, tolerating elevated concentrations of organically bound nitrogen; 3 =  
788 facultatively nitrogen-heterotrophic taxa, needing periodically elevated concentrations of organically bound  
789 nitrogen; 4 = obligately nitrogen-heterotrophic taxa, needing continuously elevated concentrations of  
790 organically bound nitrogen. Threat categories are as in Table 1 (or see the Methods section)

791

792 **Supplementary Information Fig. 2** Association plot between Red List categories and saprobity (S; van Dam  
793 et al., 1994). Please see the Methods section for correct interpretation of the association plot. 1 = oligosaprobic,  
794 2 = beta-mesosaprobic, 3 = alpha-mesosaprobic, 4 = alpha-meso-/polysaprobic, 5 = polysaprobic. Threat  
795 categories are as in Table 1 (or see the Methods section)

796

797 **Supplementary Information Fig. 3** Linear model applied to the association between the cumulative  
798 percentages of threat categories and nitrate concentrations in diverse inland-water habitats (both neo- and  
799 paleolimnological data)

800

801

802 **Authors and Affiliations**

803 **Marco Cantonati** <sup>1,\*</sup>, **Gabriele Hofmann** <sup>2</sup>, **Daniel Spitale** <sup>1</sup>, **Marcus Werum** <sup>3</sup>, **Horst Lange-**  
804 **Bertalot** <sup>4</sup>

805

806 Gabriele Hofmann

807 GM.Hofmann@t-online.de

808 Daniel Spitale

809 spitale.daniel@gmail.com

810 Marcus Werum

811 werum@aglh.de

812 Horst Lange-Bertalot

813 lange-bertalot@web.de

814

815 <sup>1</sup> MUSE – Museo delle Scienze, Limnology & Phycology Section, Corso del Lavoro e della Scienza

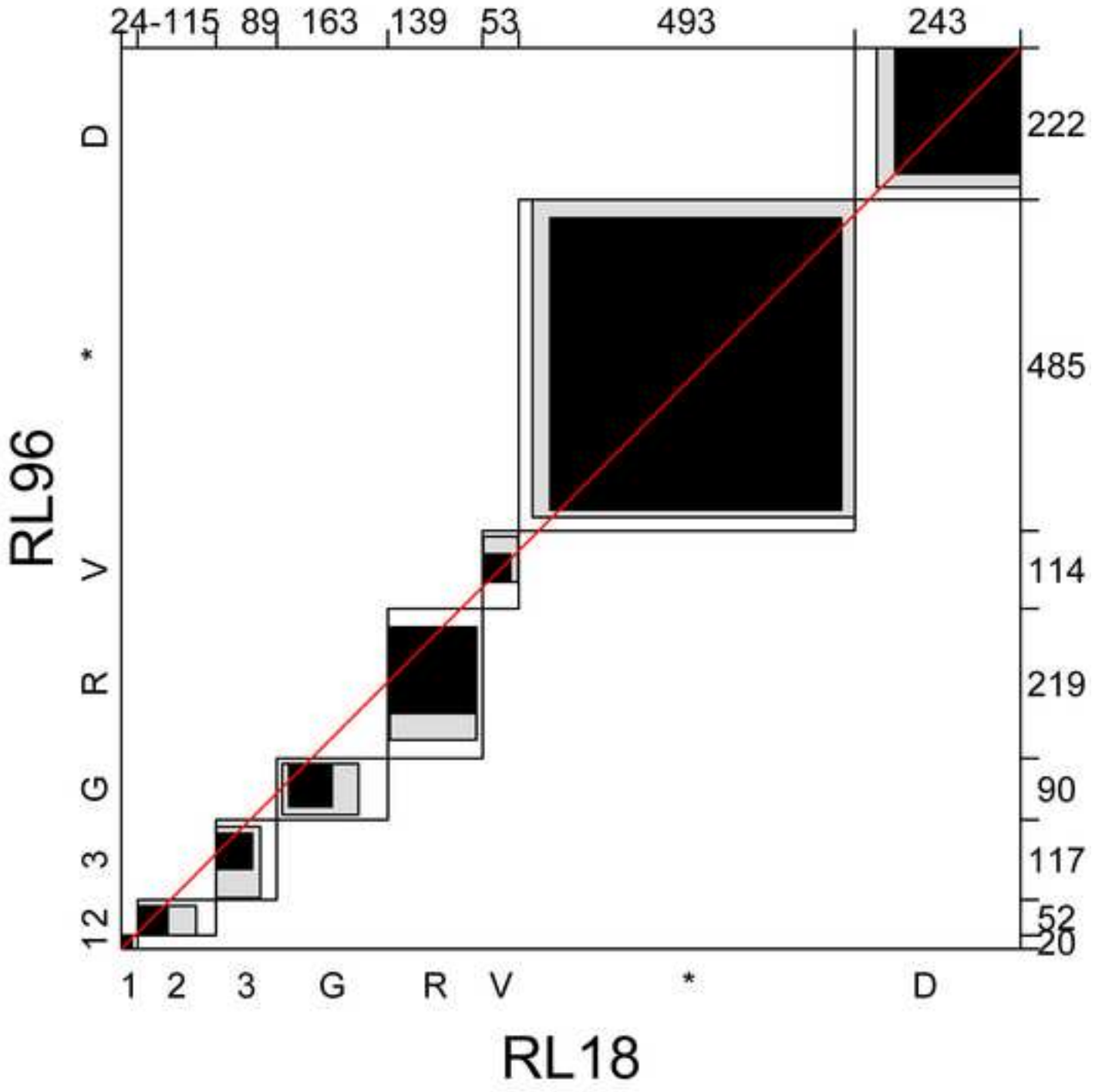
816 3, I-38123 Trento, Italy

817 <sup>2</sup> Hirtenstraße 19, 61479 Glashütten, Germany

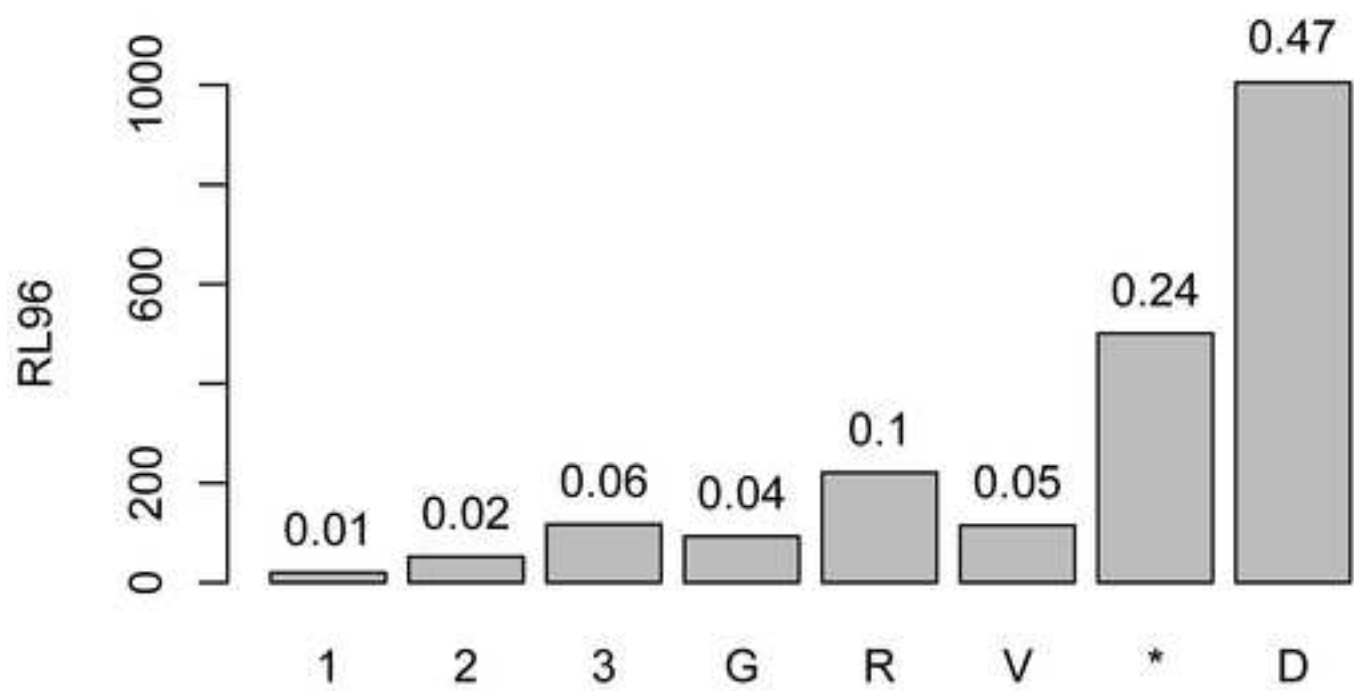
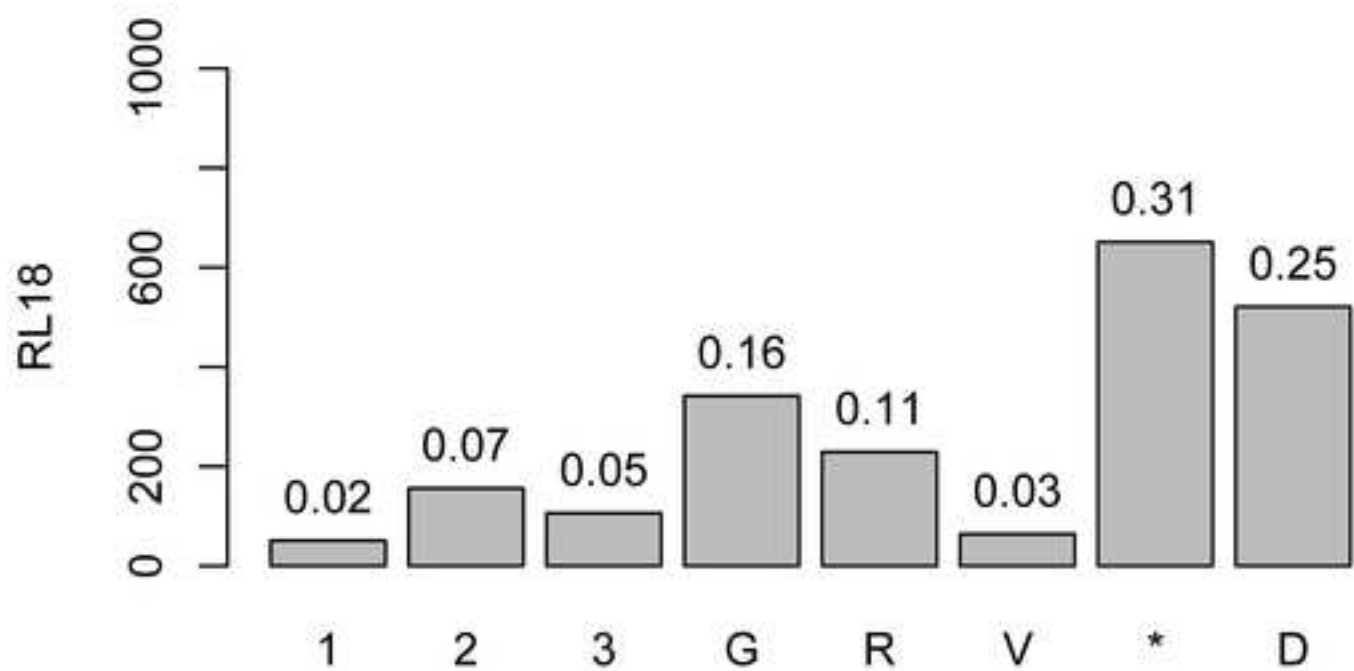
818 <sup>3</sup> Karl-Bieber-Höhe 23, 60437 Frankfurt am Main, Germany

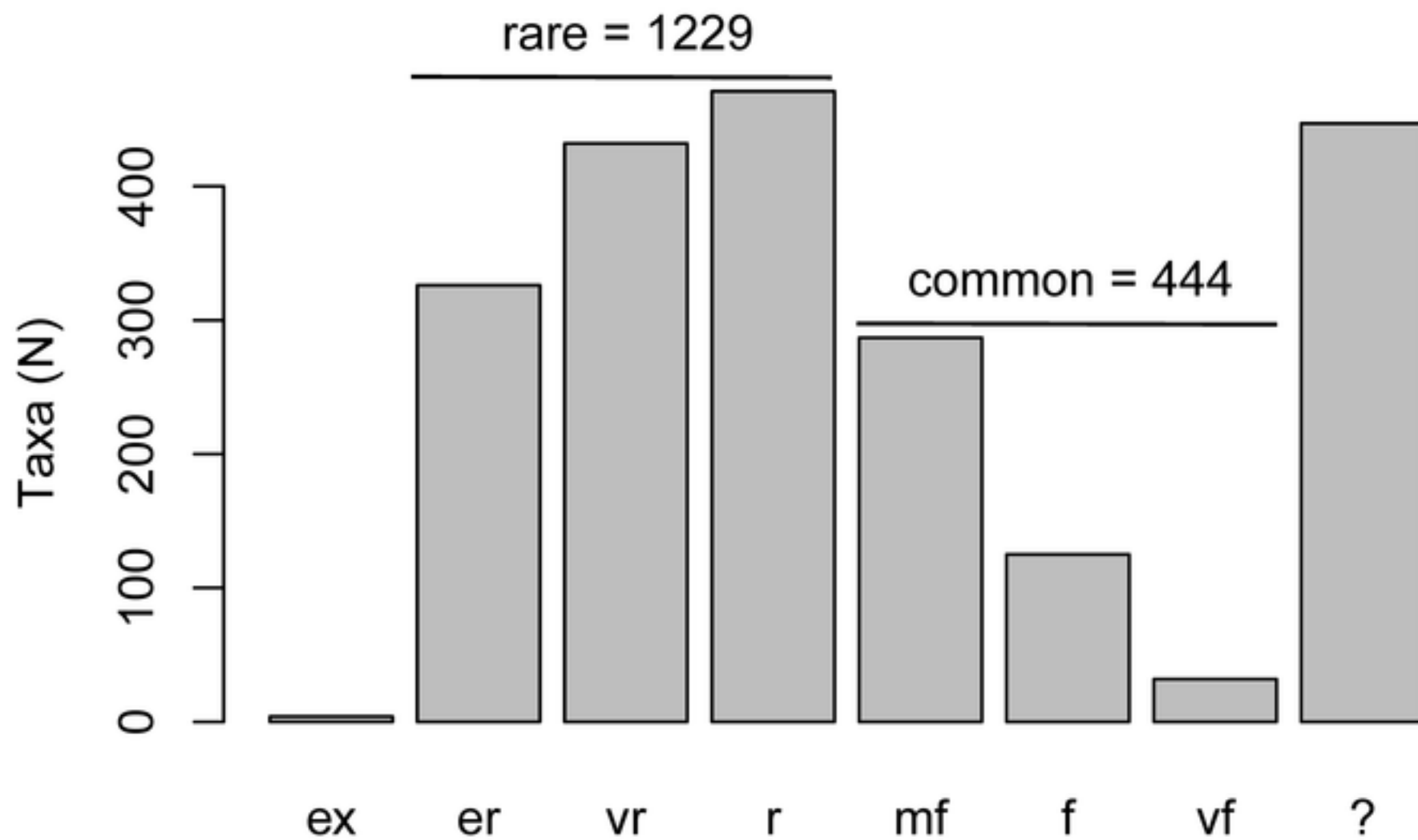
819 <sup>4</sup> Goethe Universität Frankfurt, Biologicum, Max-von-Laue Straße 13, 60438 Frankfurt, Germany

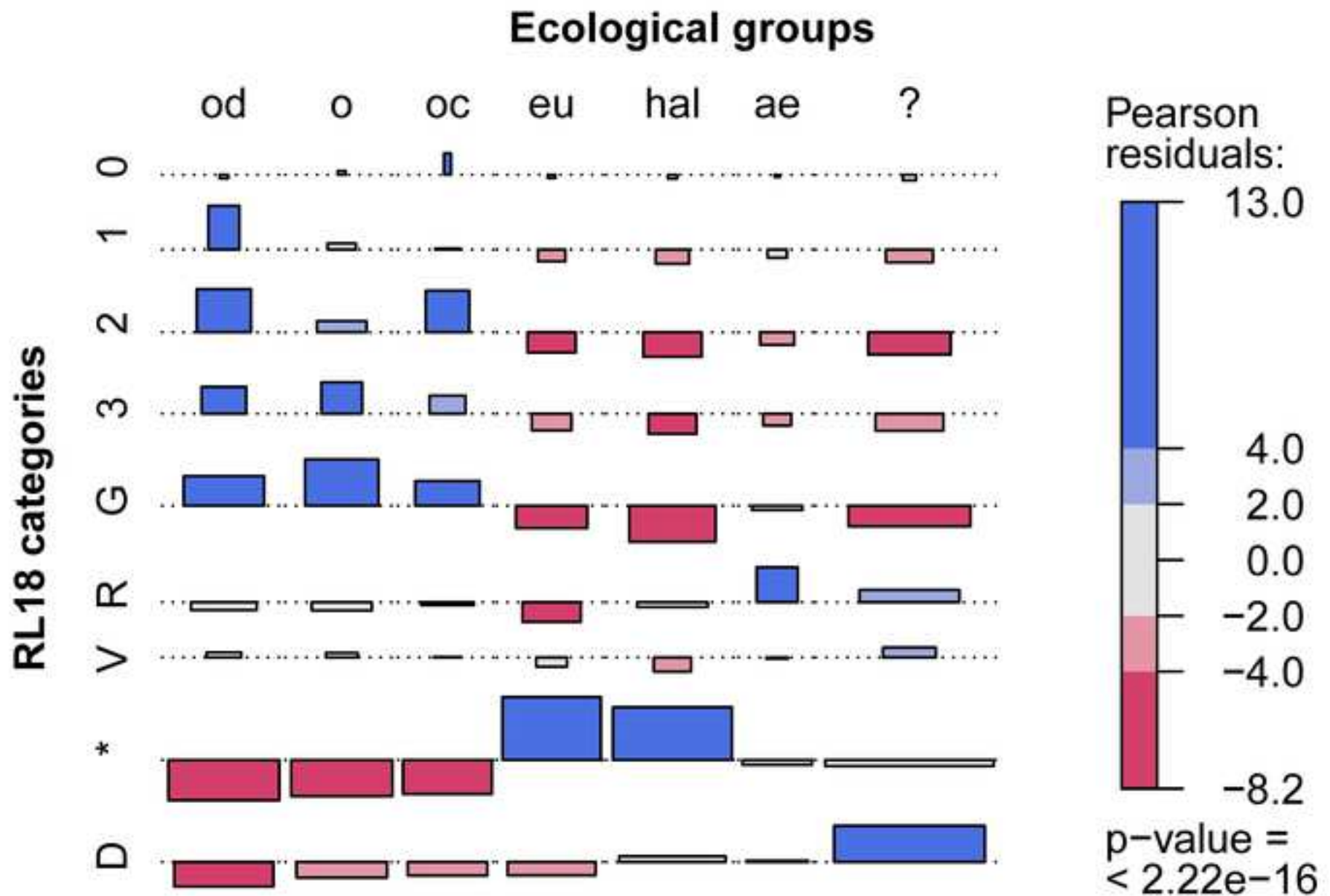
820

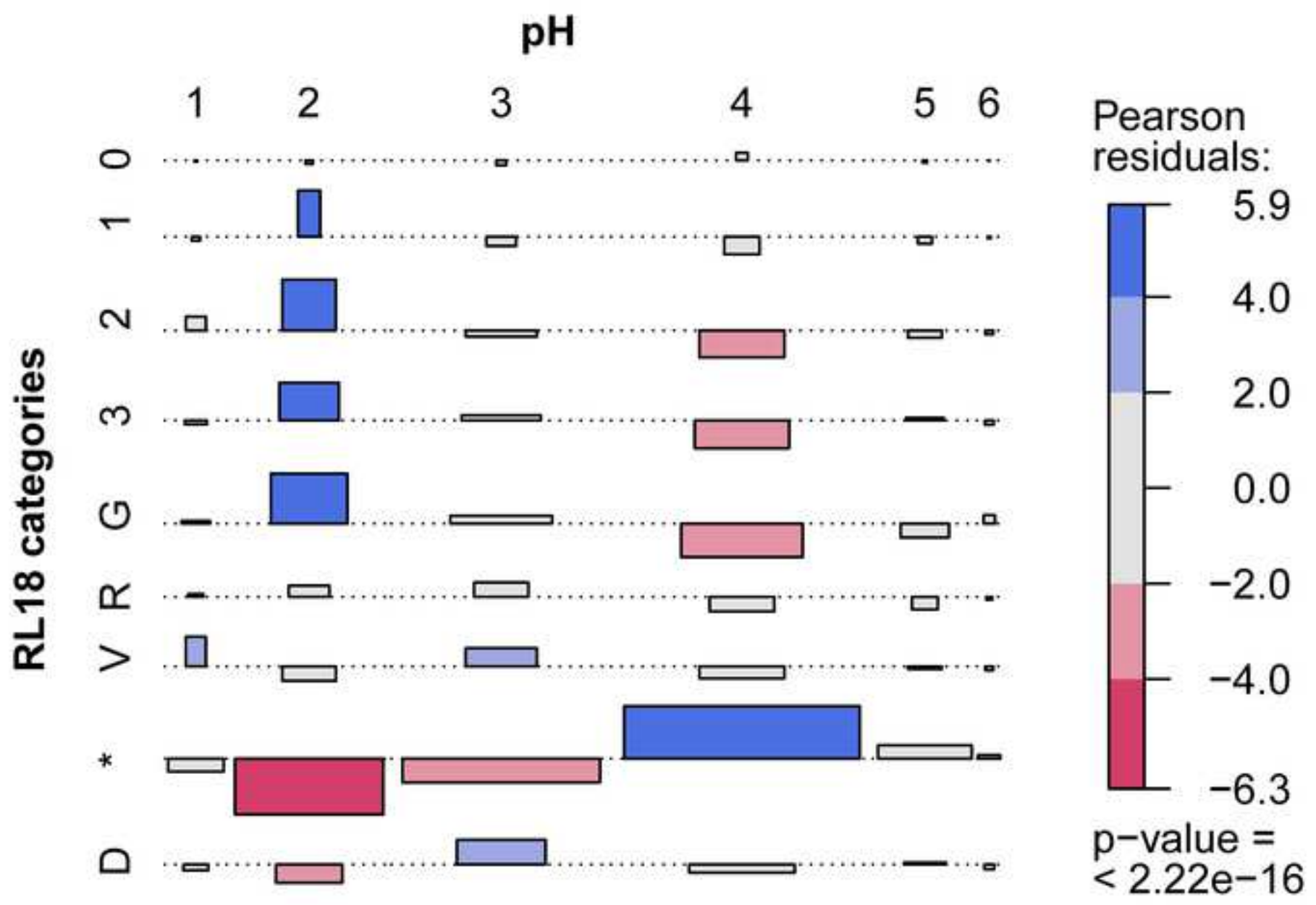


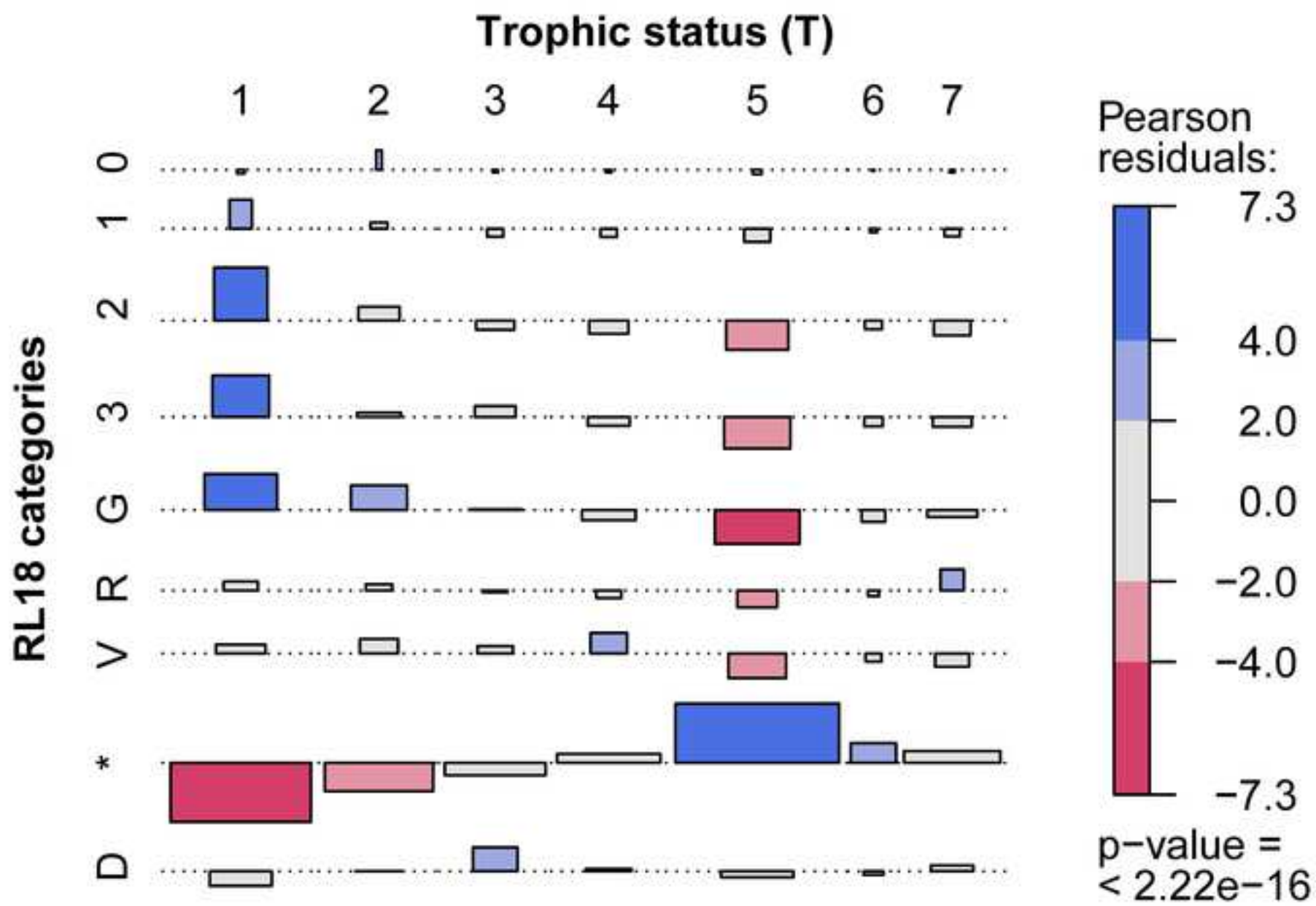


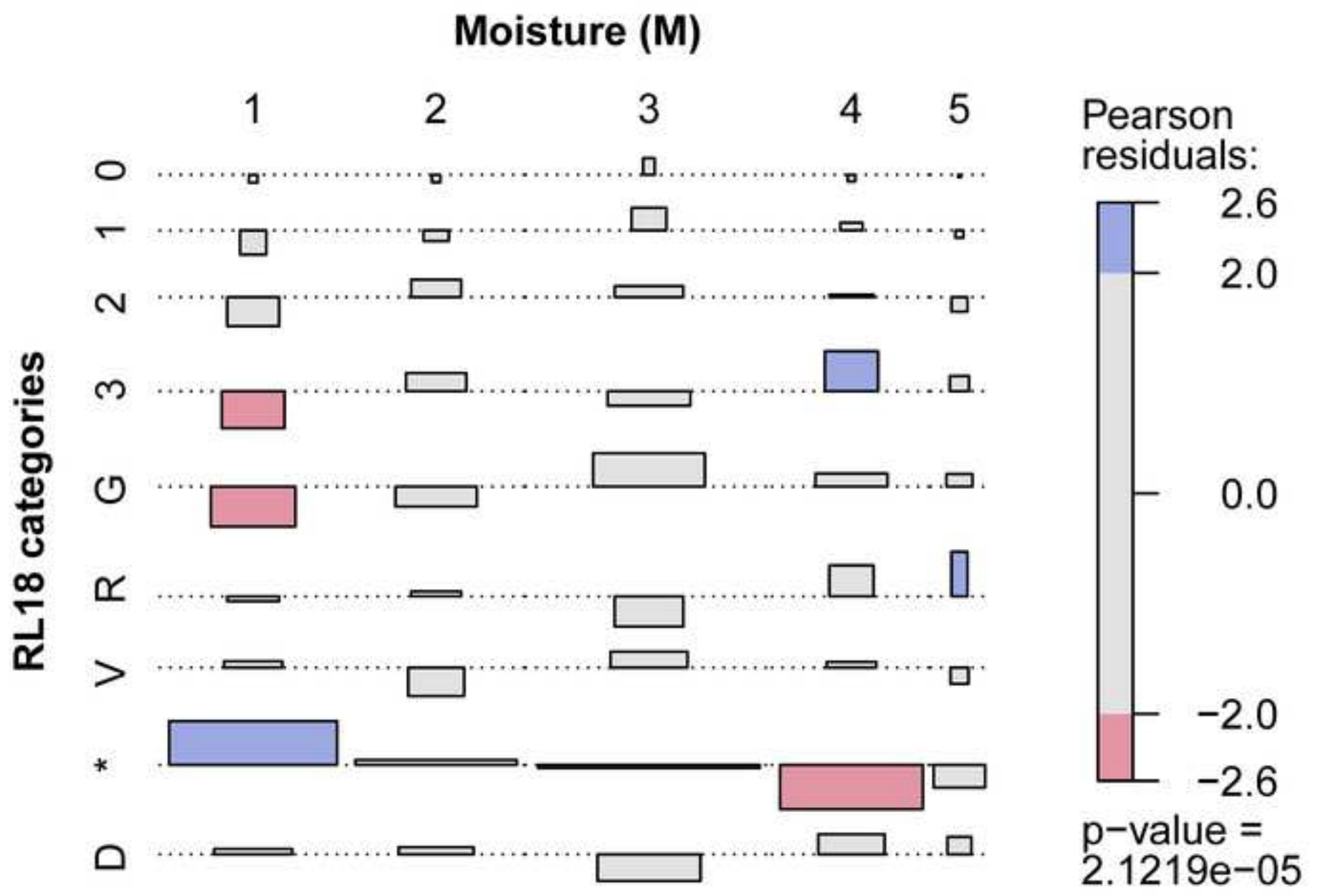












**Supplementary Information Table 1** Main Red-List inland-water habitats of Germany. nG = national long-term threat (1 = threatened with complete destruction, 2 = strongly endangered, 3 = endangered, ★ = currently no recognizable threat, # = risk classification does not make sense), TE = current development trend (↓ = decrease, → = constant / stable, ↑ = increase, # = classification does not make sense), SE = rarity (X = very rare), RLD = Red-List status (1 = threat of complete destruction, 1-2 = endangered up to threatened with complete destruction, 2-3 = endangered to strongly threatened, 3-V = acute pre-warning list, ★ = currently no risk of loss, # = risk classification does not make sense, V = pre-warning list), RE = restorability [N = cannot be restored, K = hardly regenerable (>150 years), S = difficult to regenerate (15-150 years), B = conditionally regenerable (approx. up to 15 years), X = no classification useful] (selected and modified from Finck et al., 2017)

Code	Biotope type	nG	TE	SE	RLD	RE
22.01	Seepages and wetland springs (helocrenes)	2	↓		1-2	K
22.02	Pool springs (Limnocrenes)	2	→		2-3	K
22.03	Flowing springs (Rheocrenes)	2	↓		1-2	B
22.04	Saline or brine springs	1	→	X	1	N
22.05	Tapped (captured) springs	★	#		★	X
23.01	RUNNING WATERS	2	↓		1-2	K
23.01.01	Natural and near-natural rhithral	2	↓		1-2	K
23.01.02	Natural and near-natural potamal	1	→		1-2	K
23.02	Running waters with moderate anthropogenic impact	2	→		2-3	S
23.03	Running waters with strong anthropogenic impact	★	#		★	X
23.04	Running waters with very strong anthropogenic impact	★	#		★	X
23.05.01	Ditches with a flowing-water character all year round	3	→		3-V	B
23.05.02	Technical -gutter and half-round ditches	★	#		★	X
23.05.03	Piped ditch	#	#		#	X
23.06	Deltas in inland waters	3	→		3-V	K
23.07.01	Waterfall	2	→		2-3	B
23.07.02	Oxbow lake	1	→		1-2	S
23.07.03	Lake runoff	3	→		3-V	S
23.07.04	Dammed river stretch	★	#		X	X
23.07.05	Saline stream	1	→	X	N	N
23.08	Intermittently wet habitats below the mean water level in running waters	2	↓		1-2	S
23.09	Natural and near-natural temporary running waters	2	↓		1-2	S
24.01	Dystrophic standing waters / Mire pools	2	↓		1-2	K
24.02	Oligotrophic standing waters	2	↓		1-2	S
24.03	Mesotrophic standing waters	2	↓		1-2	B
24.04	Eutrophic standing waters	3	→		3-V	B
24.05	Poly-hypertrophic standing waters	★	#		★	X
24.06	Saline inland waters	2	↓	X	1	N
24.07	Standing waters of anthropogenic origin					X
24.07.04	Ditches with very slow flowing to standing water	3	↓		2-3	X
35.01	Forest-free, oligo- to mesotrophic fens and swamps	1	↓		1!	K
35.02	Grassland in wet to (intermittently) moist locations					
36.01	Raised bogs (intact to a large extent)	1	↓		1!	N
36.02	Transitional and intermediate mires	2	↓		1-2	N
36.03	Mire degeneration stages	3	→		3-V	X
36.05	Mire regeneration areas	★	↑		★	X
38.01	Club-rush reed	2	↓		1-2	S

38.02.01	Reeds	2	↓		1-2	S
60.01	Springs of the subalpine to alpine elevation belt	V	→		V	K
60.01.01	Wetland (helocrenic) spring (seepage) of the subalpine to alpine elevation belt	V	→		V	K
60.01.02	Pool (limnocrenic) spring of the subalpine to alpine elevation belt	★	→		★	K
60.01.03	Flowing (rheocrenic) springs & gushets of the subalpine to alpine elevation belt	3	→		3-V	K
60.02	Running waters of the subalpine to alpine elevation belt	3	→		3-V	K
60.02.01	Glacial stream	3	↓	X	2	K
60.02.02	Upper part of the running-water system (rhithral) of the subalpine to alpine elevation belt	3	→		3-V	K
60.03	Standing waters of the subalpine to alpine elevation belt	3	↓		2-3	K
60.03.01	Lake of the subalpine to alpine elevation belt	3	→		3-V	K
60.03.02	Tarn of the subalpine to alpine elevation belt	3	→		3-V	K
60.03.03	Pools of the subalpine to alpine elevation belt	3	↓		2-3	K
65.01	Raised and transitional mires of the subalpine to alpine elevation belt	3	→		3-V	N
65.02	Fen or swamp of the subalpine to alpine elevation belt	3	→		3-V	N



**Supplementary Information Table 2** Agreement between RL96 and RL18. Computations were done only on taxa with congruent taxonomy. 1 = perfect association, 0 = no association. K = Cohen's Kappa; ASE: approximate standard error; CI = confidence interval

	<b>K</b>	<b>ASE</b>	<b>z</b>	<b>P</b>	<b>lower</b> <b>CI</b>	<b>upper</b> <b>CI</b>
<b>Unweighted</b>	0.645	0.015	43.6	<0.0001	0.616	0.674
<b>Weighted</b>	0.744	0.014	55.0	<0.0001	0.718	0.771



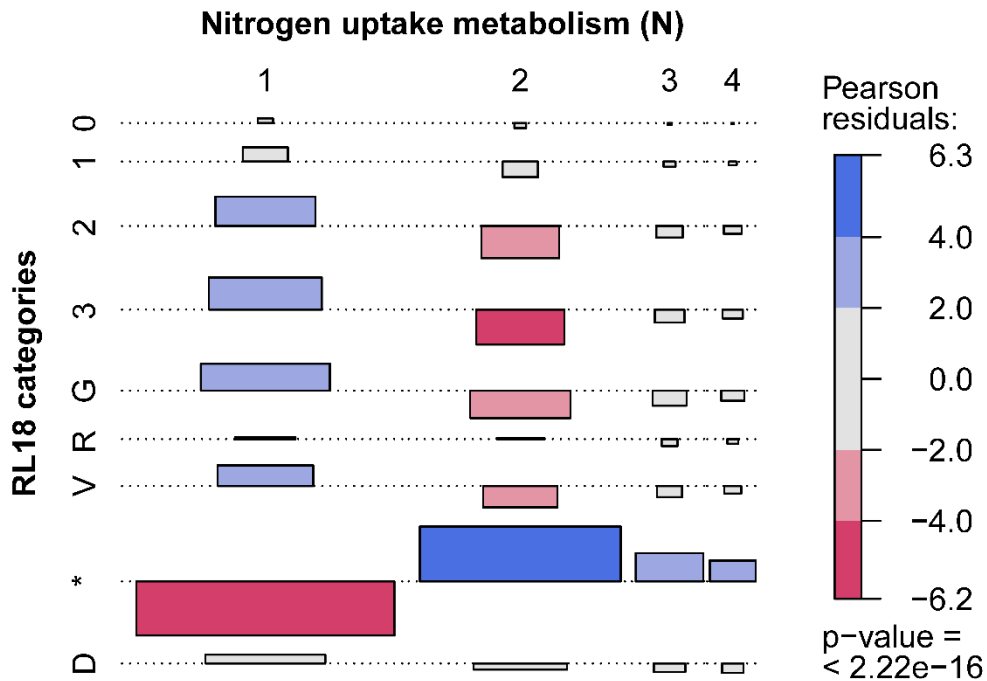
**Supplementary Information Table 4** Association between motile and not-motile (Rimet and Bouchez, 2012) and threatened (1,2,3, G,R, V,) an not-threatened (\*) RL18 diatom taxa

<i>N (%)</i>	<b>threatened</b>	<b>not-threatened</b>
<b>not-motile</b>	12 (16.4)	148 (37.5)
<b>motile</b>	61 (83.6)	247 (62.5)

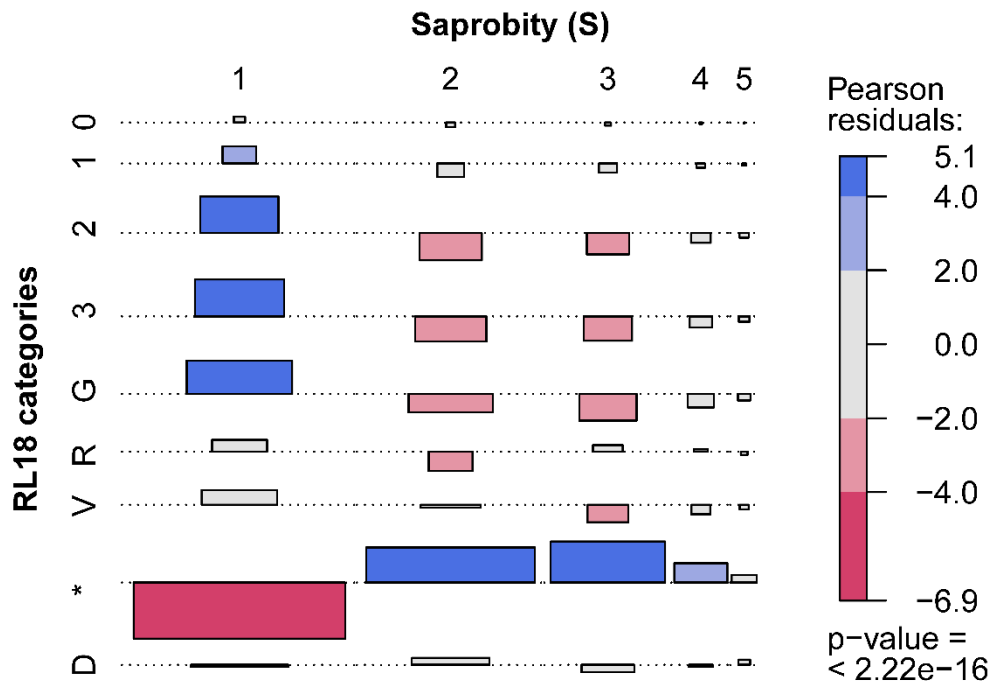
**Supplementary Information Table 5** Parameters of the linear model for the relation of the logarithm of nitrate-nitrogen averages and the cumulative percentage of diatom species in Red List threat categories (dependent variable)

	<b>Min</b>	<b>Q1</b>	<b>Median</b>	<b>Q3</b>	<b>Max</b>
<b>Residuals:</b>	-12.7	-7.1	1.5	5.7	16.3
<b>Coefficients:</b>	<b>Estimate</b>	<b>Std. Error</b>	<b><i>t</i> value</b>	<b><i>p</i> (&gt; <i>t</i> )</b>	
<b>Intercept</b>	92.3	7.5	12.4	1.2x10 <sup>-11</sup>	
<b>logmean</b>	-7.7	1.1	-6.8	5.8x10 <sup>-07</sup>	

**Supplementary Information Fig. 1** Association plot between Red List categories and nitrogen uptake metabolism (N; van Dam et al., 1994). Please see the Methods section for correct interpretation of the association plot. 1 = nitrogen-autotrophic taxa, tolerating very small concentrations of organically bound nitrogen; 2 = nitrogen-autotrophic taxa, tolerating elevated concentrations of organically bound nitrogen; 3 = facultatively nitrogen-heterotrophic taxa, needing periodically elevated concentrations of organically bound nitrogen; 4 = obligately nitrogen-heterotrophic taxa, needing continuously elevated concentrations of organically bound nitrogen. Threat categories are as in Table 1 (or see the Methods section)



**Supplementary Information Fig. 2** Association plot between Red List categories and saprobity (S; van Dam et al., 1994). Please see the Methods section for correct interpretation of the association plot. 1 = oligosaprobic, 2 = beta-mesosaprobic, 3 = alpha-mesosaprobic, 4 = alpha-meso-/polysaprobic, 5 = polysaprobic. Threat categories are as in Table 1 (or see the Methods section)



**Supplementary Information Fig. 3** Linear model applied to the association between the cumulative percentages of threat categories and nitrate concentrations in diverse inland-water habitats (both neo- and paleolimnological data)

