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

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Abstract

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

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

Introduction

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

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

Species at risk are non-randomly distributed across phylogeny (Davies 2019). Diatoms are the most species-rich group of algae (Mann 1999). The global diversity of diatoms may be one or more orders of magnitude higher than the current number of species described (Vanormelingen et al. 2008), with as many as ca. 100,000 but at least 30,000 extant diatom species (Mann and Vanormelingen 2013). They contribute to global primary production with a proportion as large as 20%, possessing global significance in the carbon and silicon cycles (Mann 1999). They can be found wherever there is a little bit of moisture, and in all aquatic habitats. Most of them have very precise preferences for ecological conditions of paramount relevance in environmental quality assessments, such as pH, mineralization, nutrients etc. The frustules allow species identification, and are usually preserved in sediments, thus providing a precious tool for paleo-environmental reconstructions, and diatom mounts are excellent collection objects, present in many natural history museums. All these characteristics have fostered the use of diatoms in a wide array of disciplines (e.g., Smol and Stoermer 2010). A combination of mechanisms ("melting pot genome" of ca. 10.000 genes with many diatom specific genes but also extensive gene transfer from bacteria to diatoms; active urea cycle, probably used to improve nitrogen storage; fatty acids oxydation in mitochondria; chemical information exchange among cells etc.) may underlie the rapid diversification rates, and may explain why diatoms have come to dominate several contemporary systems in a relatively short period of time (Bowler et al. 2008). In oligotrophic habitats, which host diverse and characteristic diatom communities (e.g., Lange-Bertalot and Metzeltin 1996), a detailed taxonomy, capable of identifying and characterizing typical (and, possibly, new) taxa must be applied. Shoe-horning of taxa in aggregates of species common in meso- and eutrophic waters must be avoided. For diatoms, Mann (1999) advocated a so-called "Waltonian" species concept, a total-evidence approach combining data from morphology, genetics, mating systems, physiology, ecology, and crossing behaviour.

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

Oligotrophic aquatic habitats have been neglected also by ecologists and taxonomists, including those working on diatoms (Kociolek and Stoermer 2009), whose attention has been more focussed on aquatic environments affected by human impacts (eutrophication, acidification etc.). The diatom microflora of oligotrophic environments has received renewed attention only since the 1990s. Lange-Bertalot and Metzeltin (1996) found as many as 800 taxa in a small number of samples from three oligotrophic lakes (from carbonate to dystrophic) at a time when the total diatom flora of Central Europe was estimated to comprise 1600 taxa. Oligotrophic habitats are threatened by direct (exploitation for hydropower and drinking water, land-reclamation, nutrient enrichment etc.) and indirect (diffuse airborne pollution, climate change) impacts. Lange-Bertalot (2007) suggests that there are indeed cosmopolitan diatom species, and that these include, in the first instance, about 250 well-known species adapted to thrive in highly eutrophic to strongly saprobic waters and widely distributed in cultural landscapes e.g. in Europe. Unluckily, strictly oligotrophic freshwaters are becoming rarer and rarer because of direct and indirect (diffuse) human impact.

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

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

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

Methods

Diatom Red Lists for Germany

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

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

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

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

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

RL18 data processing and statistical analyses

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

To test for agreement between RL96 and RL18, using only taxa with congruent taxonomy in the two Red Lists, we used Cohen's kappa (k) (Cohen 1960, 1968), a commonly used measure of agreement that compares the observed agreement to the congruency expected by chance if two observers' panels rate independently (k = 1 means perfect agreement). The original (unweighted k) only counts strict agreement (the same category is assigned by both panels). A weighted version of k (Cohen 1968) allows for partial agreement. For example, exact agreements can be given full weight, while a one-category difference might be given a weight of 1/2. This typically makes sense only when the categories are ordered. Cohen's kappa values were used to construct a contingency table. Based on this, an Observer Agreement Chart between RL96 and RL18 was plotted (Fig. 1).

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

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

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

Applications of diatom Red Lists

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

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

Results

Diatom Red Lists

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

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

Agreement between RL96 and RL18

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

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

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

The new diatom Red List (RL18)

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

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

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

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

Association of RL18 with ecological groups

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

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

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

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

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

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

Discussion

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

Using the two diatom Red Lists and studies in which they were used, we gathered examples and evidence supporting the high potential of Red Lists for a sound representation and characterization of the ecological integrity and diversity of the different types of inland-waters' ecosystems. This has also important applied implications, since IUCN Red Lists of e.g. birds and plants are widely used to designate the most relevant habitats and conservation areas, and to identify regions where resident species are most vulnerable (e.g., Paini and Mokany 2016), whereas this remains extremely limited in the case of diatom Red Lists.

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

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

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

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

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

Species at risk are non-randomly distributed across space (Davies 2019). The association between RL18 threat categories and RL18 ecological groups reveals that: - threatened taxa concentrate in dystrophic-oligotrophic environments, - 'not threatened' (= tolerant and opportunistic) taxa are most frequent in eutrophic and saline ecosystems, - most regional extinctions occurred in carbonate oligotrophic habitats. Carbonate inland waters are common also at low and medium elevations, where they are often affected by multiple human impacts, such as water abstraction and morphological alteration, nitrates and other pollutants from intensive agriculture etc. (e.g., Angeli et al. 2010; Cantonati et al. 2020a). Low-conductivity, naturally acidic (organic acids), oligotrophic (including nitrogen), dystrophic environments were singled out for their rich and peculiar diatom microflora by previous works (e.g., Cantonati et al. 2009; 2011; 2020b). Conversely, the fragility of oligotrophic and dystrophic habitats explains the fact that most oligo- and oligo-mesotraphentic diatom taxa are in threat categories of the Red List for central Europe (Lange-Bertalot 1997). The high frequency of 'not threatened' (*) taxa in eutrophic and saline ecosystems reflects two of the current main impacts on freshwaters: eutrophication (pollution) and salinization (e.g., Downing 2014; Kaushal et al. 2018).

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

Several papers (e.g., van Rees et al. 2020) list invasive alien species among the main anthropogenic threats faced by freshwater ecosystems. Supplementary Information Table 3 lists the ten species that have been suggested in the literature to be neophytes for central Europe according to Hofmann et al. (2018). However, extreme caution is necessary in considering a diatom species as non-native and invasive. For instance, Hofmann et al. (2018) discovered that the putative tropical invasive species *Diadesmis confervacea*, was present in various historical Kützing samples from a habitat in north-west Germany, where the species persisted unnoticed for many years (Cantonati et al. 2017). A classification of this species as a neophyte has therefore to be viewed with reservation, also because the reasons for its immigration, whether natural or anthropogenic, cannot be assessed. Only one of the ten potential neobiota listed in Supplementary Information Table 3 doesn't have North America as the region of origin but neighbouring northern regions of Eurasia: *Didymosphenia geminata*. This species which forms conspicuous nuisance growths, and by its large cells is also very noticeable under the light microscope was not found in Germany at all up to the middle of the 20th century. At the end of the last century, while RL96 was still being processed, there were few, undocumented records from the German area south of the Danube. After the turn of the millennium, while *Didymosphenia* blooms were becoming a global problem (Bothwell et al. 2014), massive growths were noted in various tributaries from the Alps (Germany and Austria), rather unexpectedly in view of its apparent absence previously. Similarly, mass developments were observed in recent years in western Germany of *Achnanthidium rivulare, A. subhudsonis,* and *A. delmontii*. Six other species are suspected of being neobiota due to their distribution and spreading: *Achnanthidium druartii*, *Capartogramma crucicula*, *Encyonema triangulum*, *Gomphoneis minuta*, *G. transsilvanica*, and *Gomphosphenia oahuensis*. *Achnanthidium*, known to include many pioneer species (Rimet and Bouchez 2012, Cantonati et al. 2020c), is the genus with the majority of species amongst the ten taxa discussed as potential neophytes. However, Hofmann et al. (2018) prefer not to classify any of these species as neophytes for the time being, since it cannot be ascertained that their spread resulted from human intervention. Thus, though it is certain that these are opportunistic and relatively tolerant taxa that can spread rapidly under favourable conditions, it still remains to be demonstrated if they also are neophytes in Central Europe.

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

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

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

Conclusions and way forward

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

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

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

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

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

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

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Tables

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

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

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

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

Table 3 Main Red List attributes of the 55 most menaced taxa of RL18 (4 taxa in category “0” and 51 taxa in cat. “1”). RL18 (and RL96) threat categories are as in Table 1, RL18 current-distribution categories are as in Table 2, and ecological groups are as in Fig. 4 (or see the Methods section). Long-term development trends are 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.**
Cyclotella lemanensis	0	oc	ex	*	=	*Frustulia krammeri*	1	od	er	D	(<)
Cymbella hustedtii var. *compacta*	0	oc	ex	D	=	*Geissleria declivis*	1	od	er	1	(<)
Cymbopleura citriformis	0	o	ex	D	=	*Geissleria thingvallae*	1	?	er	R	(<)
Encyonema latens	0	oc	ex	D	=	*Hippodonta costulatiformis*	1	?	er	D	(<)
Achnanthes nodosa	1	od	er	1	(<)	*Kobayasiella madumensis*	1	od	er	D	(<)
Achnanthidium trinode	1	oc	er	3	(<)	*Kobayasiella okadae*	1	od	er	1	(<)
Chamaepinnularia soehrensioides	1	od	er	D	(<)	*Navicula detenta*	1	o	er	1	(<)
Cyclotella schroeteri	1	oc	er	D	(<)	*Navicula diabolica*	1	?	er	R	(<)
Cyclotella socialis	1	o	er	D	(<)	*Nitzschia garrensis*	1	od	er	1	(<)
Encyonema hophense	1	oc	er	D	(<)	*Pinnularia biceps* var. *gibberula*	1	od	er	D	(<)
Encyonema procerum	1	?	er	D	(<)	*Pinnularia inculpata*	1	?	er	D	(<)
Encyonopsis tiroliana	1	oc	er	D	(<)	*Pinnularia nobilis* var. *nobilis*	1	o	er	D	(<)
Eunotia alkalibiontica	1	oc	er	D	(<)	*Pinnularia persudetica* var. *silvatica*	1	od	er	D	(<)
Eunotia bactriana	1	od	er	D	(<)	*Pinnularia renatiformis*	1	o	er	D	(<)
Eunotia biconstricta	1	od	er	D	(<)	*Pinnularia ruttneri* var. *lauenburgiana*	1	?	er	R	(<)
Eunotia braendlei	1	o	er	D	(<)	*Pinnularia simonsenii*	1	o	er	D	(<)
Eunotia circumborealis	1	od	er	D	(<)	*Pinnularia stidolphii*	1	o	er	D	(<)
Eunotia cisalpina	1	od	er	D	(<)	*Pinnularia subrhombica* var. *subrhombica*	1	od	er	D	(<)
Eunotia denticulata	1	od	er	D	(<)	*Pinnularia subrhombica* var. *angusta*	1	od	er	D	(<)
Eunotia diadema	1	od	er	1	(<)	*Placoneis navicularis*	1	o	er	1	(<)
Eunotia elegans	1	od	er	1	(<)	*Platessa rupestris*	1	o	er	1	(<)
Eunotia faba	1	od	er	1	(<)	*Psammothidium kuelbsii*	1	od	er	3	<
Eunotia hexaglyphis	1	od	er	1	(<)	*Psammothidium lacus-vulcani*	1	od	er	2	<
Eunotia lapponica	1	od	er	D	(<)	*Tetracyclus emarginatus*	1	od	er	R	(<)

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

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

				[N-NO$_3^-$] μg L^{-1}				
Habitat type (substrata sampled)	**Location**	**N**	**%RL**	**Mean**	**Med.**	**TNT**	**Impacts**	**Reference**
Mire pools (b, ss)	Danta di Cadore, south-eastern Alps	5	**72**	**5**	**6**	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	**60**	**186**	**196**	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	**56**	**192**	**196**	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 Rocciavrè Nature Park, Piedmont, northwestern Italy	14	**55**	**271**	**284**	103	long-distance airborne aiborne pollutants, grazing & cattle watering, fishing	Cantonati et al. (2021a)
Mountain carbonate springs (lm, b, ss)	BeNP, north-eastern Alps	9	**54**	**303**	**248**	104	forestry, hunting, cattle breeding	Cantonati and Lange-Bertalot (2010)
Springs (all main lithologies) (lm, b)	Trentino, CRENODAT Project, s.-e. Alps	110	**53**	**735**	**559**	370	water abstraction, pastures, roads, agricultural runoff	Cantonati et al. (2012)
Low-elevation siliceous-metamorphic mountain springs (lm, b, ss)	BFNP 2018	15	**53**	**760**	**780**	120	in the recent past: acidification, forestry	Cantonati et al. unpublished
High-mountain lake on holocrystalline rocks (lm)	Trentino, Lake Cornisello	8	**69**	**170**	**173**	96		MC & M. Segnana unpublished
Carbonate and holocrystalline mountain springs (lm, b, ss)	ABNP, south-eastern Alps	30	**48**	**516**	**468**	254	pastures	Cantonati (1998)

Mountain carbonate springs (lm, b, ss)	BeNP 2018	15	**47**	**754**	**761**	162	forestry, hunting, cattle breeding	Cantonati et al. unpublished
Siliceous metamorphic springs (lm, b, ss)	South-western Alps springs	48	**46**	**302**	**230**	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	**45**	**878**	**512**	504	eutrophication, morphology alteration	Taxböck et al. (2017, 2020)
Carbonate springs and streams	DBNP, south-eastern Alps	21	**41**	**497**	**429**	131	org. pol. (str.)	Cantonati and Spitale (2009)
Low-altitude carbonate springs (lm, b, ss)	JPNP, south-eastern Alps	3	**40**	**509**		60	org. pol.	Cantonati (2004)
Lake Tovel (epilithon euphotic z.)	ABNP, south-eastern Alps	50	**40**	**350**	**340**			Cantonati et al. (2009)
Springs (all main lithologies) (lm)	Vicinities of Frankfurt/M. (Südhessen)	96	**37**	**1580**		416	water abstraction, airborne pollution, roads, agricultural runoff	Werum and Lange-Bertalot (2004)
Helocrene springs (spring fens)	Western Carpathians	13	**33**	**587**		188		Fránková et al. (2009)
Lake Garda (lm)	South-eastern Alps	24	**33**	**420**	**440**	75	(NO_3^-), shore-morphology alteration	Spitale et al. (2011)
Pre-Alpine carbonate springs (lm)	Areas of Basel and Zürich, CH	17	**30**	**2851**	**2210**	118		Taxböck and Preisig (2007)
Mountain springs (lm)	Springs in Vorarlberg, Austria, Alps	27	**28**	**2300**	**1600**	197	water abstraction, pastures, deforestation	Gesierich and Kofler (2010)
Mediterranean island streams (lm)	Cyprus	65	**27**	**985**	**380**	290	water scarcity and abstraction	Cantonati et al. (2020b)
springs on ophiolites and limestone)	Konjuh Mountain, Bosnia and Herzegovina	20	**27**	**1309**	**1230**	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	**25**	**7550**	**8070**	310	nitrate-enriched GW	Denys and Oosterlynck (2015)
Carbonate springs (lm)	Verona Province, pre-Alpine	25	**24**	**1971**	**1242**	138	NO_3^-, capturing	Angeli et al. (2010)
Carbonate springs (lm)	Beauce region, Orléanais, N France	14	**23**	**14266**	**8465**	135	NO_3^-,	Bertrand et al. (1999)

Figures

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

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

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

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

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

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

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

Supplementary Information (SI)

Tables

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)

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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)

Figures

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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)

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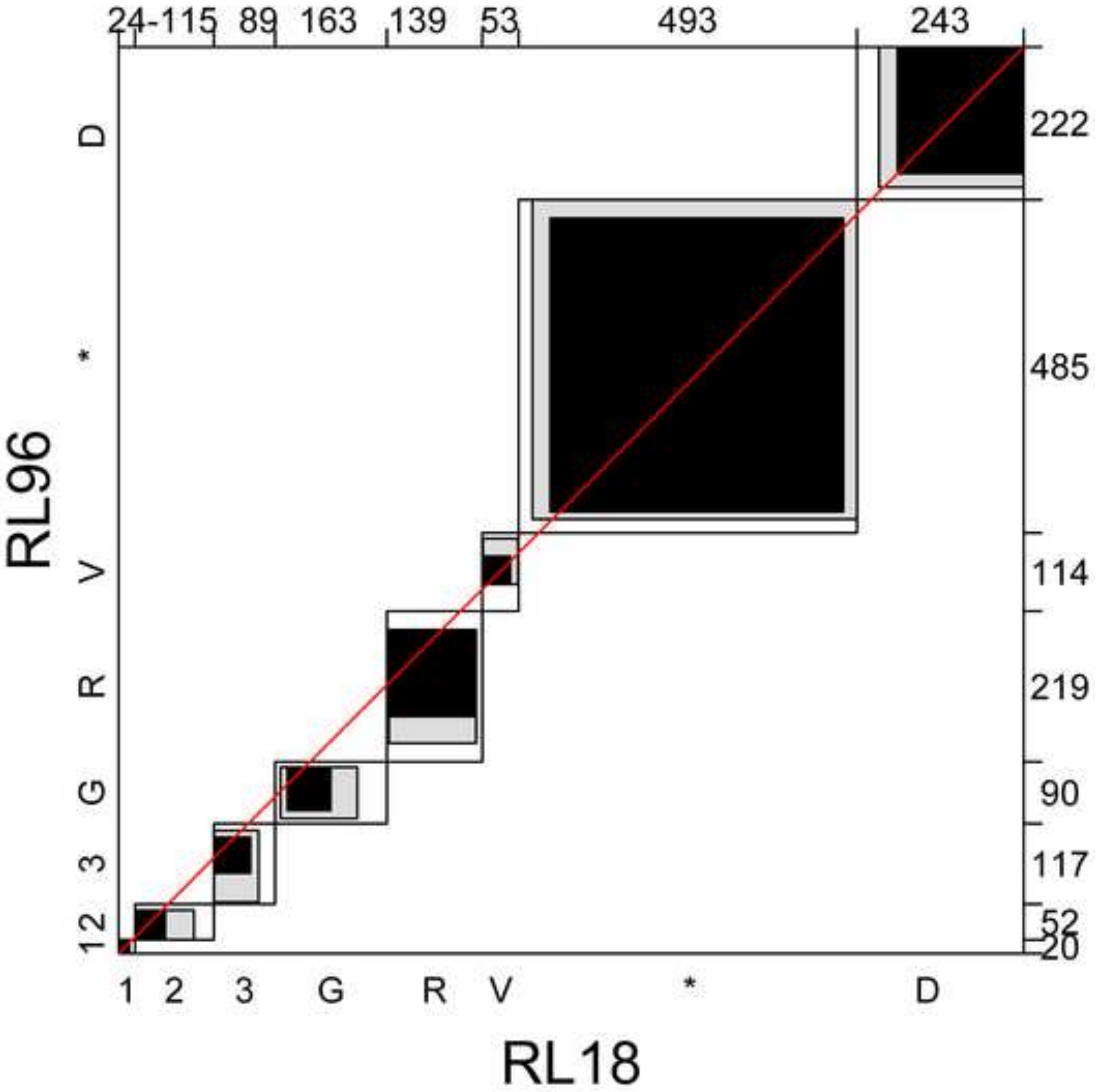
24-115
89
163
139
53
493
243
D
*
V
R
G
3
1 2
RL96
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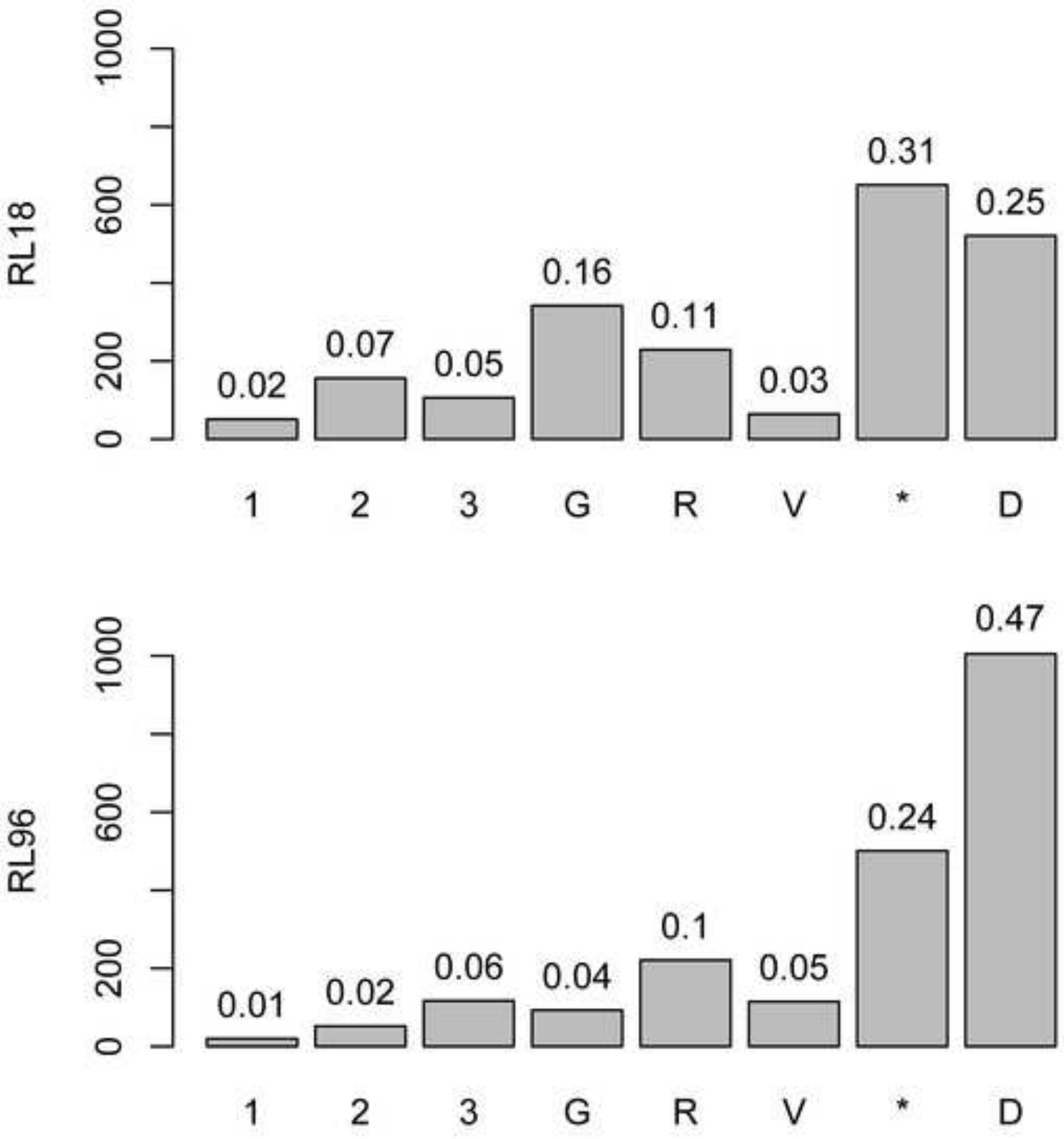
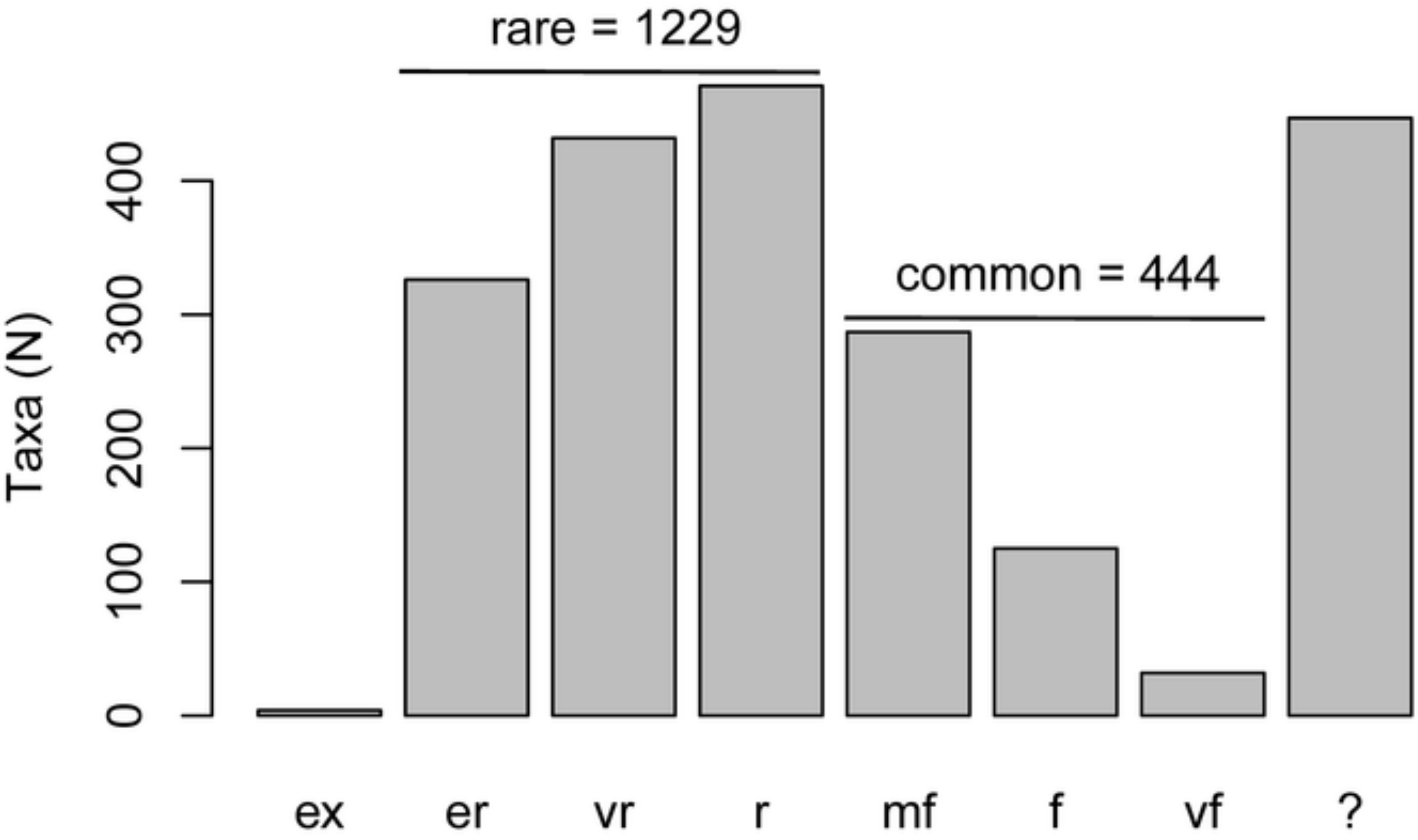
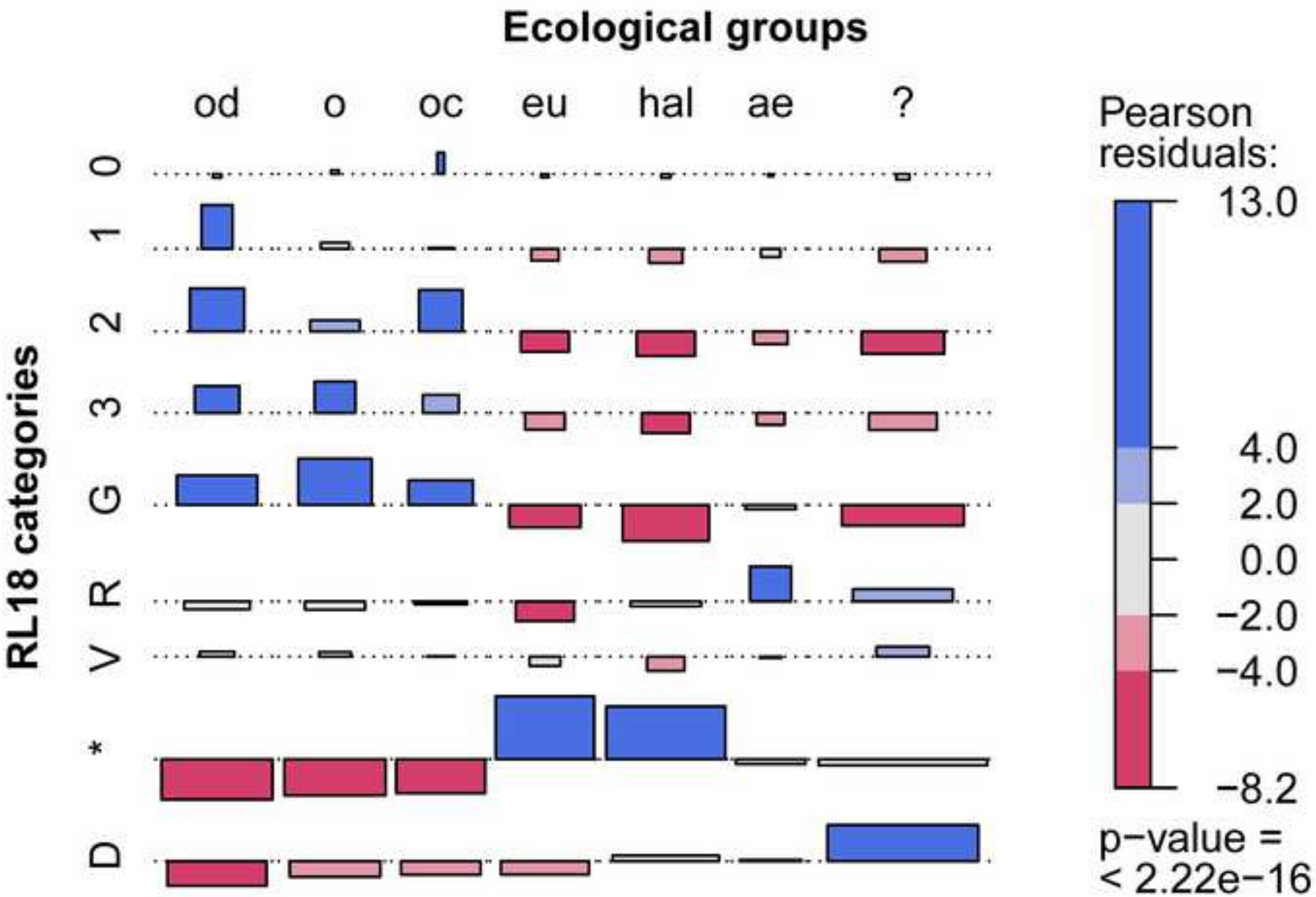
485
114
219
90
117
52
20
1
2
3
G
R
V
*
D
RL18


RL18
0
200
600
1000
0.02
0.07
0.05
0.16
0.11
0.03
0.31
0.25
1
2
3
G
R
V
*
D
RL96
0
200
600
1000
0.01
0.02
0.06
0.04
0.1
0.05
0.24
0.47
1
2
3
G
R
V
*
D


rare = 1229
common = 444
Taxa (N)
0
100
200
300
400
ex
er
vr
r
mf
f
vf
?


Ecological groups
od
o
oc
eu
hal
ae
?
RL18 categories
0
1
2
3
G
R
V
*
D
Pearson residuals:
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4.0
2.0
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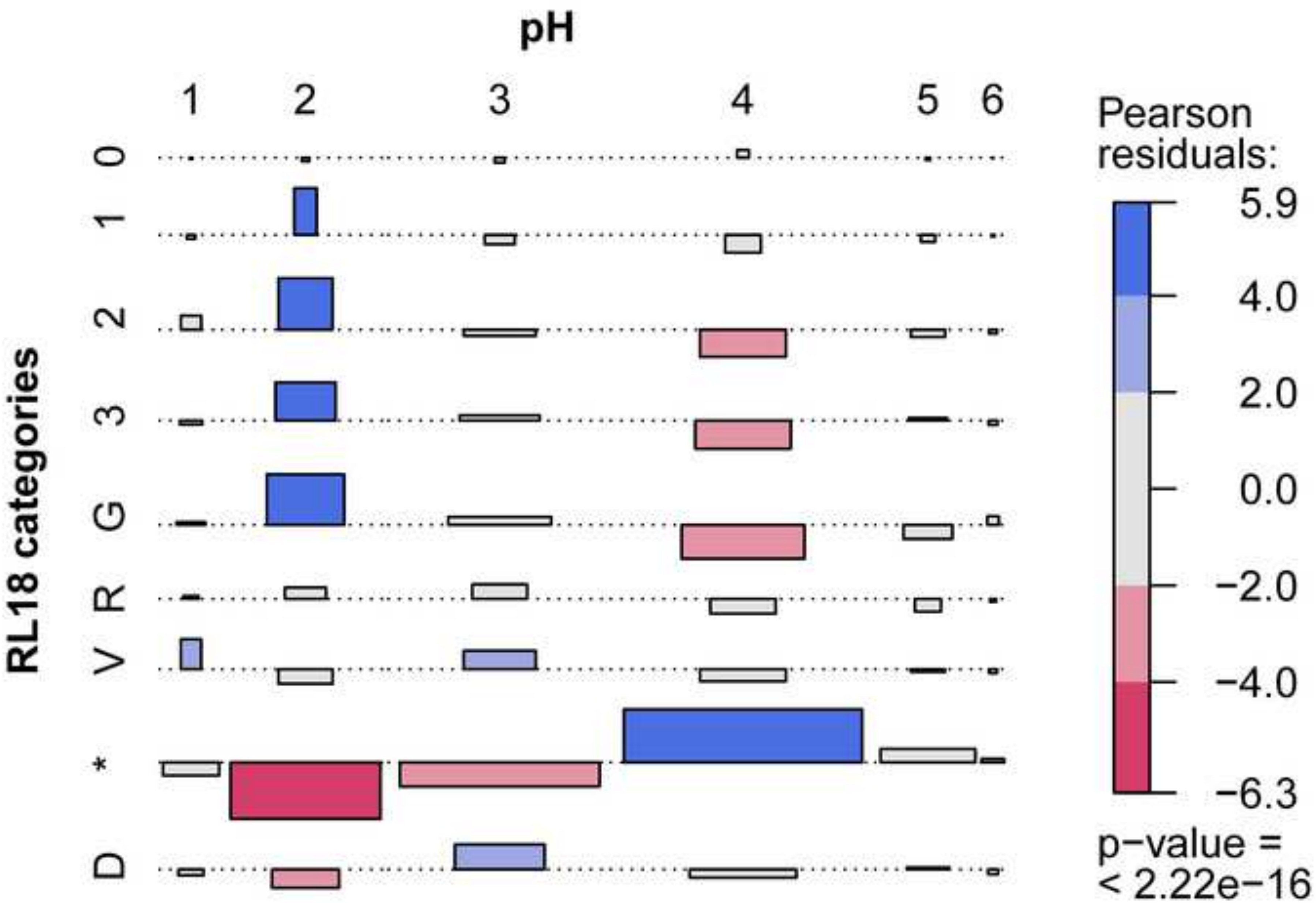
−2.0
−4.0
−8.2
p−value =
< 2.22e−16


pH
1
2
3
4
5
6
RL18 categories
0
1
2
3
G
R
V
*
D
Pearson residuals:
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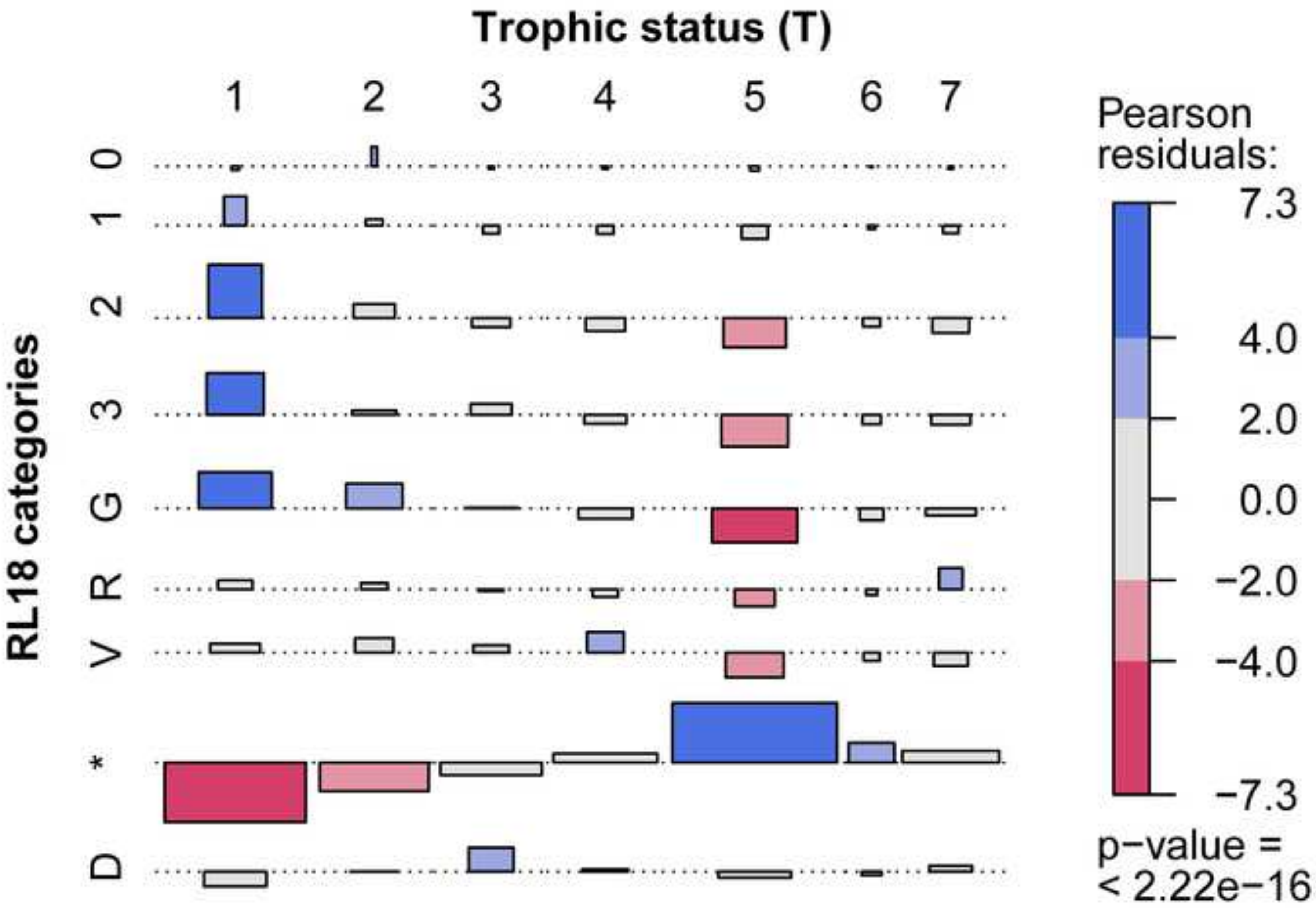
4.0
2.0
0.0
−2.0
−4.0
−6.3
p−value =
< 2.22e−16


Trophic status (T)
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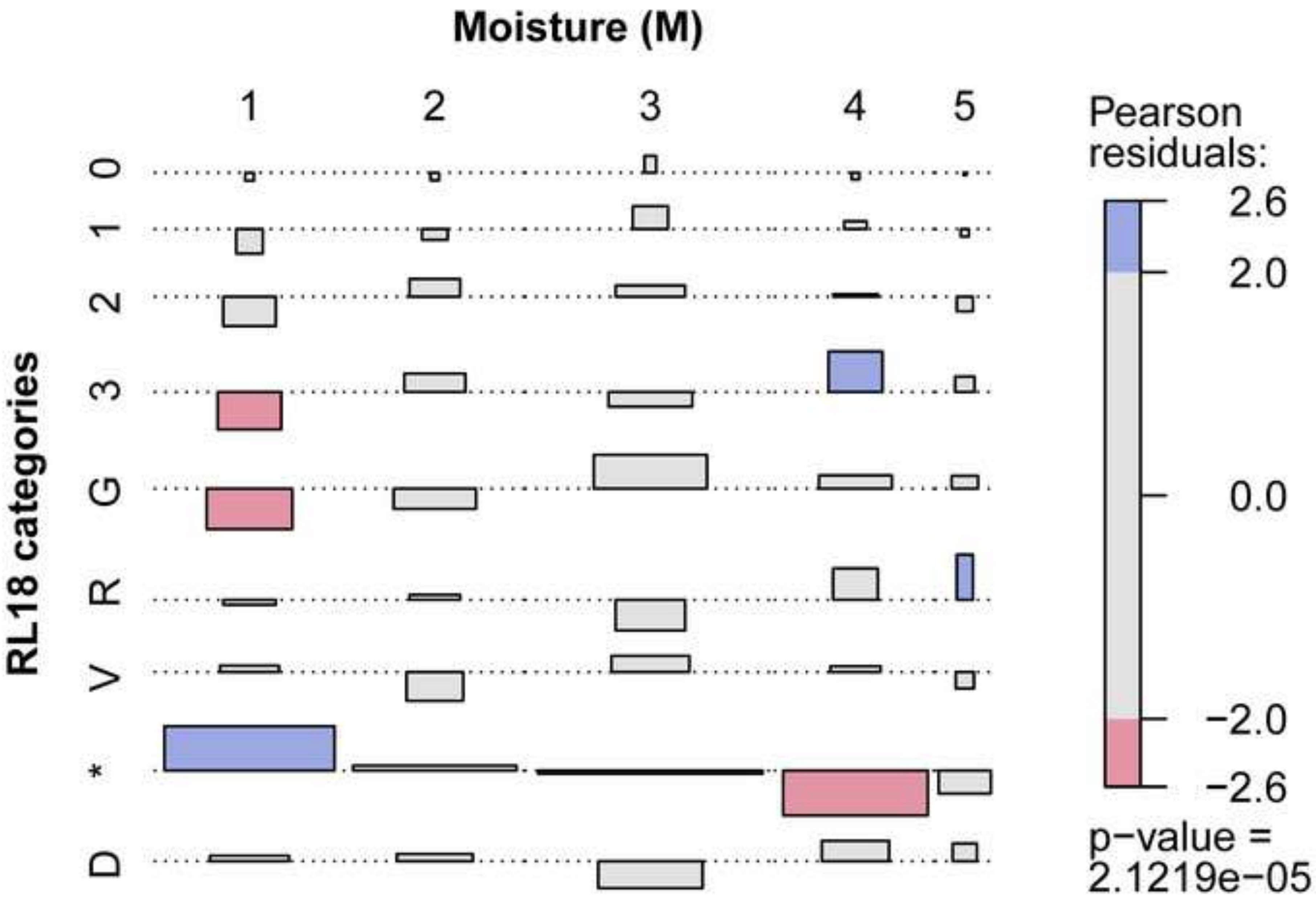
2
3
4
5
6
7
RL18 categories
0
1
2
3
G
R
V
*
D
Pearson residuals:
7.3
4.0
2.0
0.0
−2.0
−4.0
−7.3
p−value =
< 2.22e−16


Moisture (M)
1
2
3
4
5
RL18 categories
0
1
2
3
G
R
V
*
D
Pearson residuals:
2.6
2.0
0.0
−2.0
−2.6
p−value =
2.1219e−05

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

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

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

	RL18				**Rimet and Bouchez (2012)**										
Species	**threat cat.**	**ecol. group**	**distribution**	**long-term d.**	**size class**	**motile**	**pioneer**	**pedunculate**	**stalk**	**colonial**	**mucous**	**arbuscular**	**high-profile**	**low-profile**	**motile guild**
Achnanthidium delmontii	*	eu	mf	>											
Achnanthidium druartii	D	eu	r	?											
Achnanthidium rivulare	*	?	mf	>	1	1	1	1	1	0	0	0	0	1	0
Achnanthidium subhudsonis	*	eu	mf	>	1	1	0	1	1	0	0	0	0	1	0
Capartogramma crucicula	R	hal	er	?											
Didymosphenia geminata	*	?	r	?	5	0	0	1	1	1	0	1	1	0	0
Encyonema triangulum	R	eu	er	?	5	1	0	0	0	1	1	0	1	0	0
Gomphoneis minuta	D	eu	vr	?	5	1	0	1	1	1	0	1	1	0	0
Gomphoneis transsilvanica	D	eu	r	?											
Gomphosphenia oahuensis	R	hal	er	?											

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

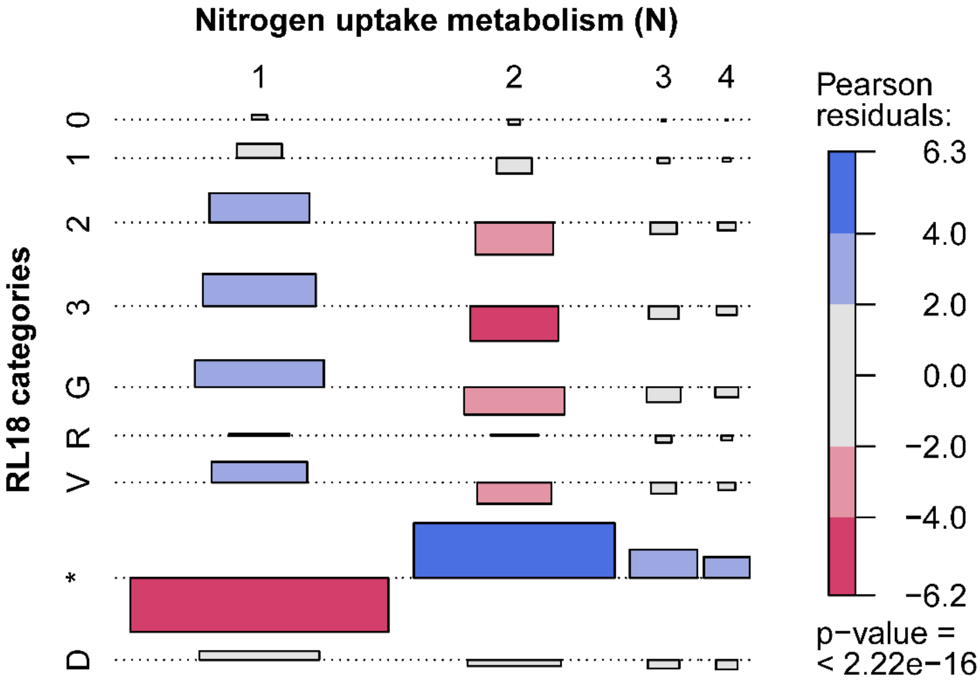
N (%)	**threatened**	**not-threatened**
not-motile	12 (16.4)	148 (37.5)
motile	61 (83.6)	247 (62.5)

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)

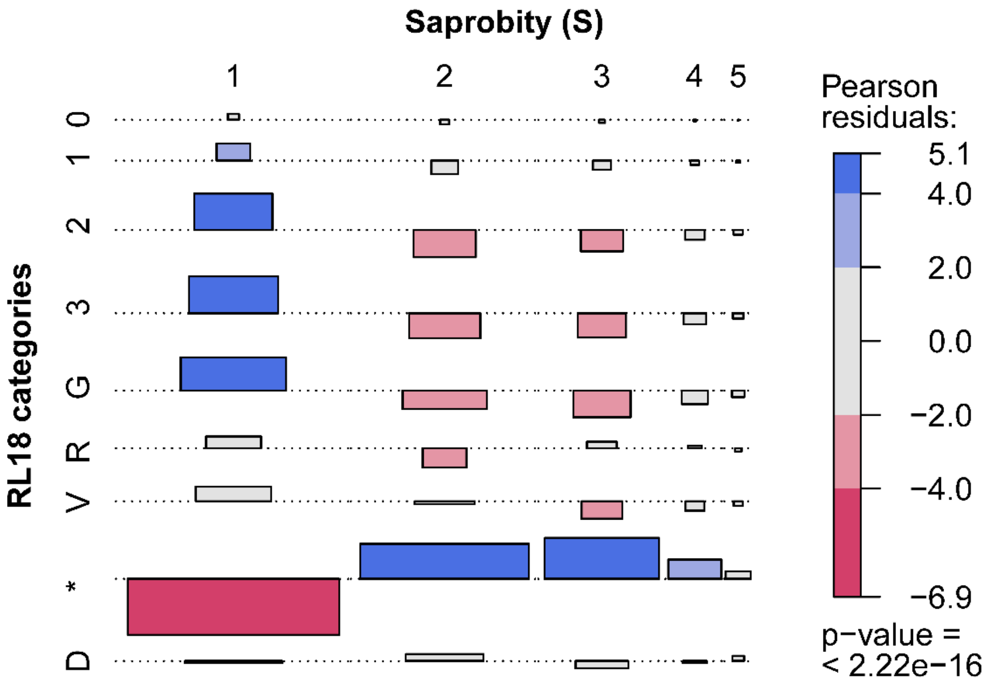
	Min	**Q1**	**Median**	**Q3**	**Max**
Residuals:	-12.7	-7.1	1.5	5.7	16.3

Coefficients:	**Estimate**	**Std. Error**	***t* value**	***p* (>|*t*|)**
Intercept	92.3	7.5	12.4	1.2x10^{-11}
logmean	-7.7	1.1	-6.8	5.8x10^{-07}

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)