TITLE PAGE

Title: Combined ecological risks of nitrogen and phosphorus in European freshwaters

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ABSTRACT

Eutrophication is a key water quality issue triggered by increasing nitrogen (N) and phosphorus (P) levels and potentially posing risks to freshwater biota. We predicted the probability that an invertebrate species within a community assemblage becomes absent due to nutrient stress as the ecological risk (ER) for European lakes and streams subjected to N and P pollution from 1985 to 2011. The ER was calculated as a function of species-specific tolerances to NO_3^- and total P concentrations and water quality monitoring data. Lake and stream ER averaged 50% in the last monitored year (i.e. 2011) and we observed a decrease by 22% and 38% in lake and stream ER (respectively) of river basins since 1985. Additionally, the ER from N stress surpassed that of P in both freshwater systems. The ER can be applied to identify river basins most subjected to eutrophication risks and the main drivers of impacts.

Keywords: ecological risk; nitrogen; phosphorus; lake; stream; river basin;

Capsule: Quantifying the ecological risk of invertebrate losses due to N and P pollution

Introduction

The "limiting nutrient" concept, following Liebig's Law of the Minimum, was based on the effects of added nutrients on crop performance (van der Ploeg et al., 1999). Later, the concept was extended to productivity-based experiments for eutrophication, such as those testing the effects of nutrient surplus (mainly nitrogen – N – and phosphorus – P) on chlorophyll concentration or biomass productivity (Allgeier et al., 2011; Elser et al., 2007). Despite the benefit prompted by the increase in the availability of a resource, such as increase in productivity, a further increase in the same resource level could cause ecosystem damage, such as a shift in species composition (Odum et al., 1979).

Freshwater eutrophication is triggered by agricultural and urban discharges of N and P as well as atmospheric emissions of N (Harrison et al., 2010; van Drecht et al., 2005; van Drecht et al., 2009). On one hand, the increase in nutrient availability generally increases primary production and, thus, the availability of food to planktivores and herbivores (Carpenter et al., 1985). On the other hand, it may also lead to increased predation by secondary consumers and decreases in food quality (Carpenter et al., 1985; Grimm and Fisher, 1989), water transparency and light availability, thereby eliciting competitive exclusion of autotrophic species and the release of allelochemicals by competing phytoplankton, particularly cyanobacteria (Havens et al., 2001; Havens et al., 2003; Leflaive and Ten-Hage, 2007). Furthermore, enhanced decomposition of nuisance algae and macrophytes may generate hypoxic or (in extreme cases) anoxic conditions in aquatic systems (Carpenter et al., 1998). Ultimately, the presence of oxygen depleted conditions, exposure to toxins released by phytoplankton, and shifts in food availability may be harmful to invertebrates (Camargo and Alonso, 2006; Correll, 1998) (Figure 1). Therefore, the same nutrient stimulating autotrophic productivity and food availability may, in turn, instigate ecosystem damage at increasing concentrations. Accordingly, defining the nutrient as a resource or as a stressor depends as to whether its concentration prompts a benefit or damage to ecosystems.

Ecological theory models detect this dual aspect of N and P. The intermediate disturbance hypothesis (IDH) conveys that species richness is maximized at intermediate levels of stress and minimized at the two extremes (Grime, 1973). Underlying the IDH, the physiological tolerance hypothesis (Currie et al., 2004) conveys that species richness is the upshot of the tolerance of each individual species to specific local conditions. Currie et al. (2004) use the hypothesis to explain species tolerance to climatic variables and we expand it so as to describe species tolerance to the upper end of nutrient levels, i.e. the level of the stressor which triggers species loss.

Eutrophication is a complex issue as it encompasses potential feedback mechanisms (van Donk and van de Bund, 2002), non-linear responses of primary production to trophic conditions (Genkai-Kato and Carpenter, 2005), and synergistic effects of N and P on primary production (Elser et al., 2007). The extent to which they drive primary productivity can be examined by analyzing past nutrient level patterns (Anderson, 1998) or nutrient stoichiometry changes (Glibert, 2012), ecological modeling (Genkai-Kato and Carpenter, 2005), or via nutrient addition experiments (Schindler, 1977). Nonetheless, the development and the application of eutrophication models which include all the various pathways through which N and P influence individual invertebrate species occurrence may be troublesome due to lack of data and of insights on all relevant mechanisms of impact.

Alternatively to mechanistic models, statistical models coupled with available monitoring data of water bodies may be used to underpin biodiversity effects of eutrophication and provide environmental protection agencies with guidelines for the improvement and the maintenance of water quality (Smith et al., 2007). We circumvent the uncertainties within each of the different ecological mechanisms by developing a probabilistic model of invertebrate species occurrences with the upper observed stressor tolerance in field observations (Figure 1).

Eutrophication indicators based on the performance of invertebrates may be less certain than those on autotrophs since consumers are not directly affected by N and P concentrations as are photosynthesizing organisms (Johnson et al., 2014). However, invertebrates are convenient to environmental agencies because they are extensively monitored (Growns et al., 1997) and their monitoring can be easily employed as water quality indicators, such as the ecological quality ratio (EQR). In the case of the EQR, the composition of invertebrates is compared with a reference representing minimum impairment (Clarke, 2013). Nevertheless, indicators usually do not detect the main stressor driving the eutrophication impact.

In the case of eutrophication, the estimation of the overall health quality of freshwater needs also to uncover what the main cause of impairment is. Therefore, an ecological indicator that allows for estimation of the ecosystem health as well as for identification of the driving stressor of eutrophication impairment may provide environmental agencies with the tools to recognize impaired areas and to target the stressor of concern. In this study, we propose the ecological risk (ER) to identify the areas and the main drivers of eutrophication impairment. This framework is compatible with risk assessments proposed for toxicants (Beketov et al., 2013; Fedorenkova et al., 2012; Malaj et al., 2014; van Straalen, 2002).

Material and methods

Ecological risk

The ER posed to a group of species depends upon the sensitivity of each of its species and the probability that the group of species is subjected to the stressor (Figure 2a). Thus, ER (dimensionless) is the definite integral

$$ER_i = \int_{-\infty}^{\infty} CDF_i(x) \cdot PDF_i(x) dx \qquad (1)$$

where PDF is the probability density function of the stressor i of ^{10}log concentration x and CDF is the cumulative distribution function of the sensitivity of species to increasing x (Fedorenkova et al., 2012; van Straalen, 2002) (see study outline in Figure 3). The ER can be interpreted as the probability that an invertebrate species within a community in a river-basin and in a given year is exposed to a stressor level above its threshold of occurrence in the environment.

The dual effects of the two stressors can be combined in order to estimate the total ER to species as

$$ER_T = 1 - \prod_i (1 - ER_i) \qquad (2)$$

where ER_i is the individual ER of stressor i (Fedorenkova et al., 2012). In our case, the ecological risks are calculated for two stressors (NO_3^- and TP) and in two freshwater types (i.e. lakes and streams) in different European river basins per year. The ecological risks posed by NO_3^- and TP are hereafter referred to ER_N and ER_P, respectively. In the absence of ER_N or of ER_P, ER_T was not determined. We focus on TP and NO_3^- as indicators for P and N stress since they are commonly monitored by water quality programs (Lewis et al., 2011). Our study considers the ecological risk to lakes and streams separately because they differ considerably with respect to the cycling of water and nutrients as well as their sources of energy supply (Doi, 2009). Finally, we used linear regression to test if the ER_T changed over time in each river basin.

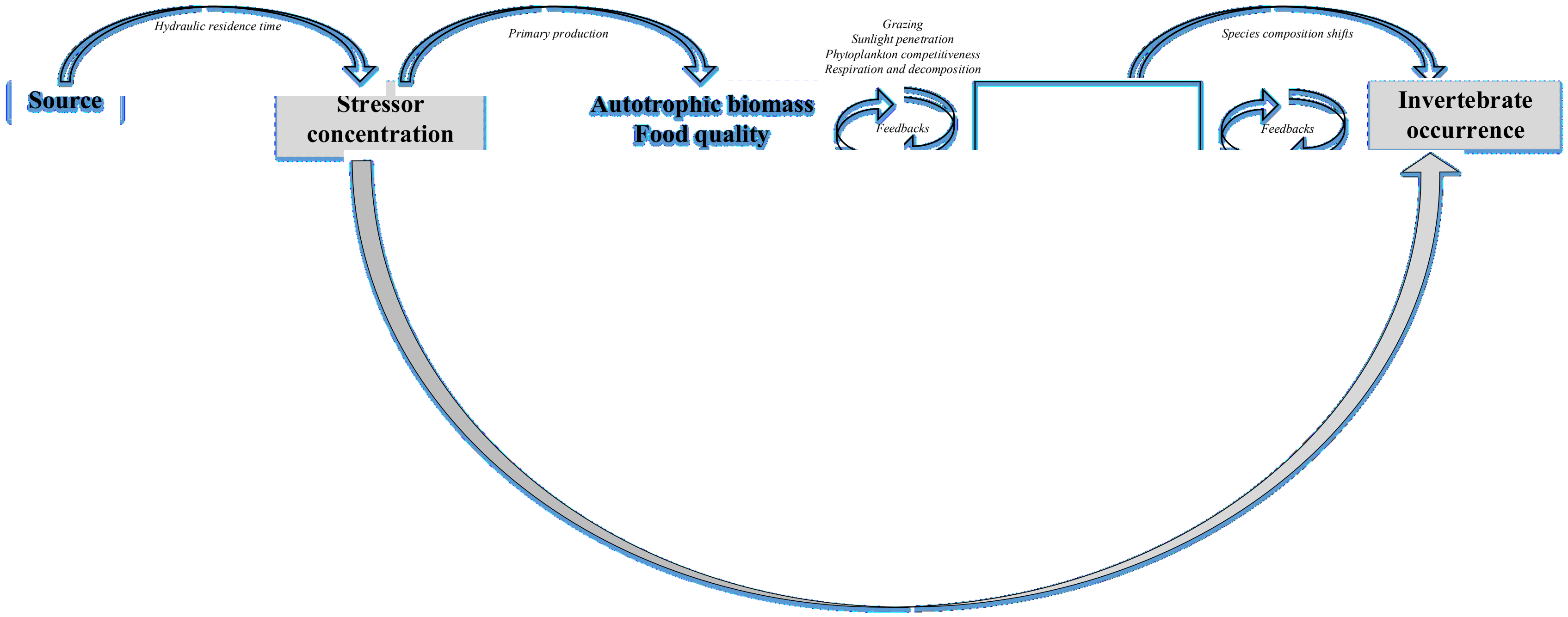

Figure 1 (2-column figure image) Probabilistic model of our study: the linkage between stressor concentrations and invertebrate species occurrences (grey fill). The full impact pathway of freshwater eutrophication includes the discharge of stressors (nitrogen and phosphorus) into freshwater bodies, thereby influencing N and P levels, primary productivity, and autotrophic biomass which, in turn, may set off various ecological feedbacks, including the occurrence of invertebrates in the field. The graph illustrates the upper boundary of the stressor concentration (x-axis) at which individual invertebrate species occurs under field conditions (y-axis, listed as I_1 to I_n, ordered in descending tolerance to a stressor).

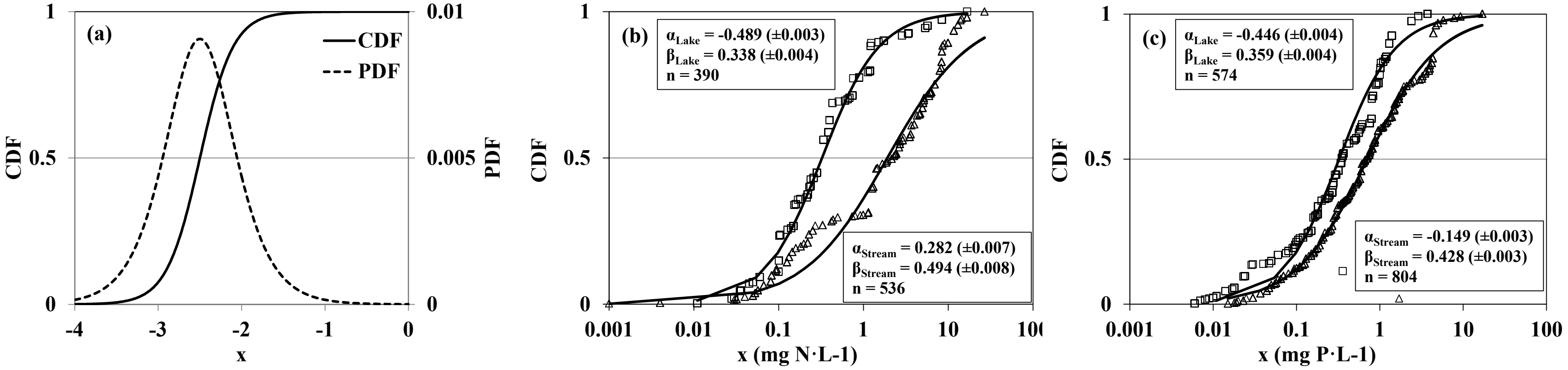

Figure 2 (2-column figure image) (a) Illustration of ecological risk (ER) defined as the area under the probability density function (PDF) of stressor ^{10}log concentration x and the cumulative distribution function of the cumulative fraction of absent species (CDF) with x, adapted from Fedorenkova et al. (2012). In this example, the α_c and β_c coefficients of the PDF are -0.25 and 0.28 and the α and β coefficients of the CDF are -2.5 and 0.20, respectively. Cumulative distribution functions of invertebrate species losses and **(b)** nitrogen and **(c)** phosphorus concentration x in lakes (squares) and streams (triangles). Standard error is shown in brackets and sample size as n.

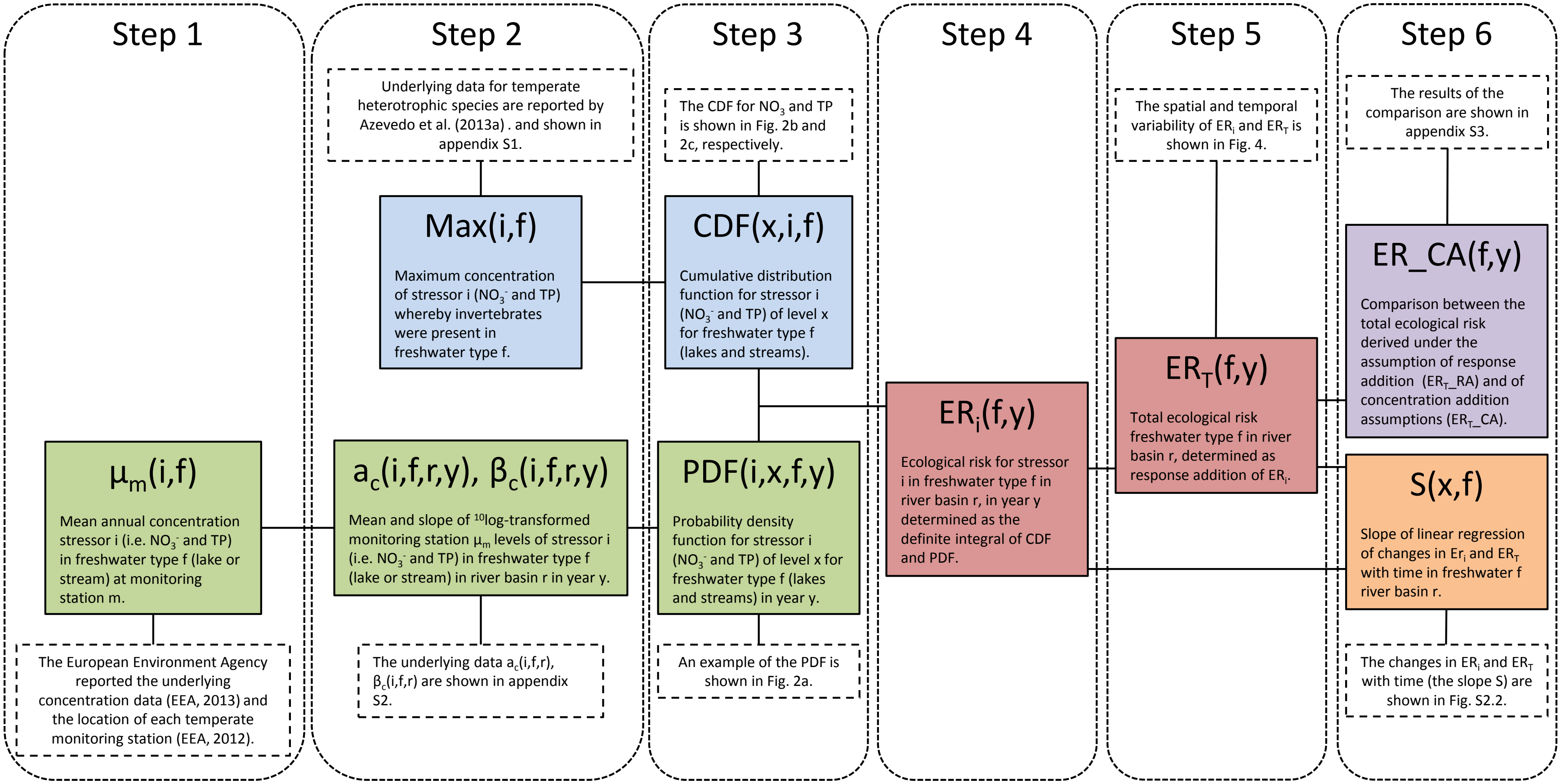

Figure 3 (2-column figure image) Derivation of the cumulative distribution function, CDF (Step 1), of the probability density function, PDF (Step 2), and of the ecological risk, ER (Step 3). The dashed line boxes show the underlying data and the results of our study.

Cumulative distribution function (CDF)

The CDF describes the cumulative number of species no longer tolerant to high stressor levels (Figure 2). Increasing sensitivity to stressors can be illustrated in different ways, such as decreasing frequency of occurrence or reproduction, or increasing mortality. Ultimately, a species subjected to high enough stressor level will no longer be able to subsist in the environment. Here, the tolerance to the stressor is defined as the threshold level at which an individual species is no longer present in field surveys.

To attain the CDF, species-specific maximum tolerance concentration levels were ordered from low to high values (highest being the species most tolerant to the stressor) and ranked from zero to one. The CDF was then fitted to a logistic function describing the fraction of species becoming absent with increasing levels of a stressor as

$$CDF_i(x) = \frac{1}{1+exp^{-\left(\frac{x_i-\alpha_i}{\beta_i}\right)}} \tag{3}$$

where x is the ^{10}log concentration of stressor i, the coefficients α represents the ^{10}log concentration at which 50% of the all species become absent because of excess of stressor i, also called location parameter, and β represents the slope of the CDF. (Both coefficients are dimensionless.) The β depicts the variability in maximum tolerance concentration levels across species (i.e. the lower its value, the more similar the species are with respect to their sensitivity to high stressor levels). The CDF was determined for the two stressors (i.e. NO_3^- and TP) in two freshwater types (i.e. lake or stream) but we do not account for differentiated species composition within a given freshwater type, e.g. the differences between shredder or collector species, or between stream orders.

We use the same CDF expressing the vulnerability of species towards high nutrient levels (i.e. α coefficient) and their sensitivity to changes (i.e. β coefficient) across the years and across the same freshwater type. We expect that this function is unlikely to change in such a short period of time as its parameters describe characteristics inherited during years of evolution and are driven by differentiated exposures to hydrological and biogeochemical patterns in the two freshwater types (Azevedo et al., 2013a).

Probability density function (PDF)

The probability of a stressor being found at a ^{10}log concentration x can be described by a PDF of a logistic curve as

$$PDF(x) = \frac{exp^{-\left(\frac{x_i-\alpha_c}{\beta_c}\right)}}{\beta_c\cdot\left[1+exp^{-\left(\frac{x_i-\alpha_c}{\beta_c}\right)}\right]^2} \qquad (4)$$

where α_c and β_c are, respectively, mean and the slope of ^{10}log-transformed mean annual stressor concentrations at each monitoring station, μ_m, in a freshwater type (i.e. lake or stream) in a given year (i.e. from 1985 to 2011). The slope β_c is equal to $\frac{\sigma\sqrt{3}}{\pi}$ (Kooijman, 1981), where σ is the standard deviation of ^{10}log-transformed μ_m values.

Model input data

For the derivation of CDF for NO_3^- and TP for a typical lake and stream in Europe, we used invertebrate species-specific maximum TP tolerance obtained from a collection of field surveys, whereby the presence of heterotrophs was recorded in the field alongside the TP concentration in temperate lakes or streams (Azevedo et al., 2013a). The maximum level at which invertebrate species was confirmed to be present was employed in the derivation of the CDF and it illustrates the highest stressor concentration of a temperate lake or stream in which the species was confirmed to occur at any given time. The same procedure described by Azevedo et al. (2013a) was employed in our study in order to determine the maximum tolerance to NO_3^- of species inhabiting temperate lakes and streams and, thus, to derive the CDF for NO_3^-. The number of times a species was spotted in the field depended on the scale of each field survey and on how frequent the species is found in the environment. Species-specific data on maximum concentrations of NO_3^- and TP at which each species was present are not differentiated across river-basins and across years. The list of species and their respective maximum concentrations are available in appendix S1 of the Supporting Information.

For the derivation of PDF for NO_3^- and TP for each river basin (total of 88) and per year (from 1985 to 2011), mean annual concentration μ_m per monitoring station within each river basin of the European temperate zone are reported by the European Environment Agency (EEA, 2013). We employed the river basin delineation reported by the EEA (2012) and temperate freshwaters as defined by the major freshwater habitat types (MHT) of the Freshwater Ecoregions of the World (FEOW). Each monitoring station was allocated to its respective river basin based on its geographic coordinate. The number of monitored stations within each basin depended on the size of the basin and it varied from 1 to 738 (averaging 112) and the number of times each station was monitored in a given year varied from 1 to 513 (averaging 11.4), see Figure S2.1. The variables α_c and β_c required for the derivation of each PDF are river-basin and year specific. From 1985 to 2011, the number of river basins which had both NO_3^- and TP lake or streams monitored at any given time during a one-year period increased from 2 to 48 and from 8 to 68 (see appendix S2 for the summary statistics with α_c and β_c results in each river basin).

Testing assumptions

In our study, we assume that the total ecological risk to species (ER_T) is a result of the added response of N and P, i.e. response addition (RA, equation 2) (van Straalen, 2002). However, if they have similar metabolic pathways (in ecotoxicology, this is defined to as having similar modes of action), the concentration of the mixture of stressors affecting the organism is given as the added concentrations of the individual stressors, thereby defined as concentration addition (CA) (Backhaus et al., 2000). We tested how the assumptions of RA and CA affect ER_T (appendix S3 for details).

Results

The number of species available for the derivation of the CDFs varied from 390 (for NO_3^- in lakes) to 804 (for TP in streams). The species-specific maximum tolerance levels varied from 0.001 mg N/L to 27 mg N/L for NO_3^- (Tables S1.1a and S1.2a) and from 0.01 to 17 mg P/L for TP (Tables S1.1b and S1.2b). Our results show that the tolerance to N and P levels is lower in lakes than in streams ($\alpha_{Lake,TP} < \alpha_{Stream,TP}$ and $\alpha_{Lake,NO_3} < \alpha_{Stream,NO_3}$, Figure 2b,c). Likewise, the sensitivity to increasing nutrient levels is higher in lakes than in streams, as shown by steeper slopes of the CDF ($\beta_{Lake,TP} < \beta_{Stream,TP}$ and $\beta_{Lake,NO_3} < \beta_{Stream,NO_3}$).

The number of stations monitored for nutrient levels increased in both lake and stream systems over the monitored period. Still, the derivation of ER_T depended upon the availability of both ER_N and ER_P for a given year and river-basin. Of 51 river basins for which lake ER_N was available over time, 10 basins comprised decreasing ER_N (slope $S < 0$ at a 95% confidence level, Figure S2.2a) and 39 basins were not subjected to temporal changes in ER_N. Likewise, the number of basins whereby lake ER_P decreased with time was rather low compared with the number of unchanging ER_P (11 of 42), Figure S2.2b. Accordingly, most basins show no temporal changes in lake ER_T (total of 33) or stream ER_T (total of 42), Figure S2.2c. Nevertheless, the number of basins with decreasing stream ER_N, ER_P, and ER_T relative to the total number of estimated basins was considerably higher than that in lakes. For example, 11 of 56 basins were subjected to a decrease in lake ER_P but the same was only observed in 29 of 79 basins for stream ER_P (Figure S2.2b).

The ER_N was predominantly higher than ER_P in streams (Figure 4a,b). For example, from 2001 to 2011, 46 to 77% of river basins comprised ER_N higher than ER_P in a given year. However, the opposite pattern is observed in streams. Over the same period, 11 to 52% of river basins in a given year comprised ER_N above ER_P. We also found a strong variability in the ER across European river basins, especially for ER_N. This can be seen as the average range of 95th and 5th percentiles of ER_N in basins in a given year was 58.1% in lakes and 45.1% in streams (Figure 4a) while the average range of 95th and 5th percentiles of ER_P was 25.3% in lakes and 31.3% in streams (Figure 4b). The increase in monitoring efforts over the years also prompted the increasing variability across river basins, Figure 4c.

The difference between the ER_T derived under the assumption of RA and CA was minor, although the difference increased with increasing ER_T (Figure S3.1). The average difference in river basin ER_T derived using the RA and CA methods was of 0.005 (for lakes) and 0.033 (for streams).

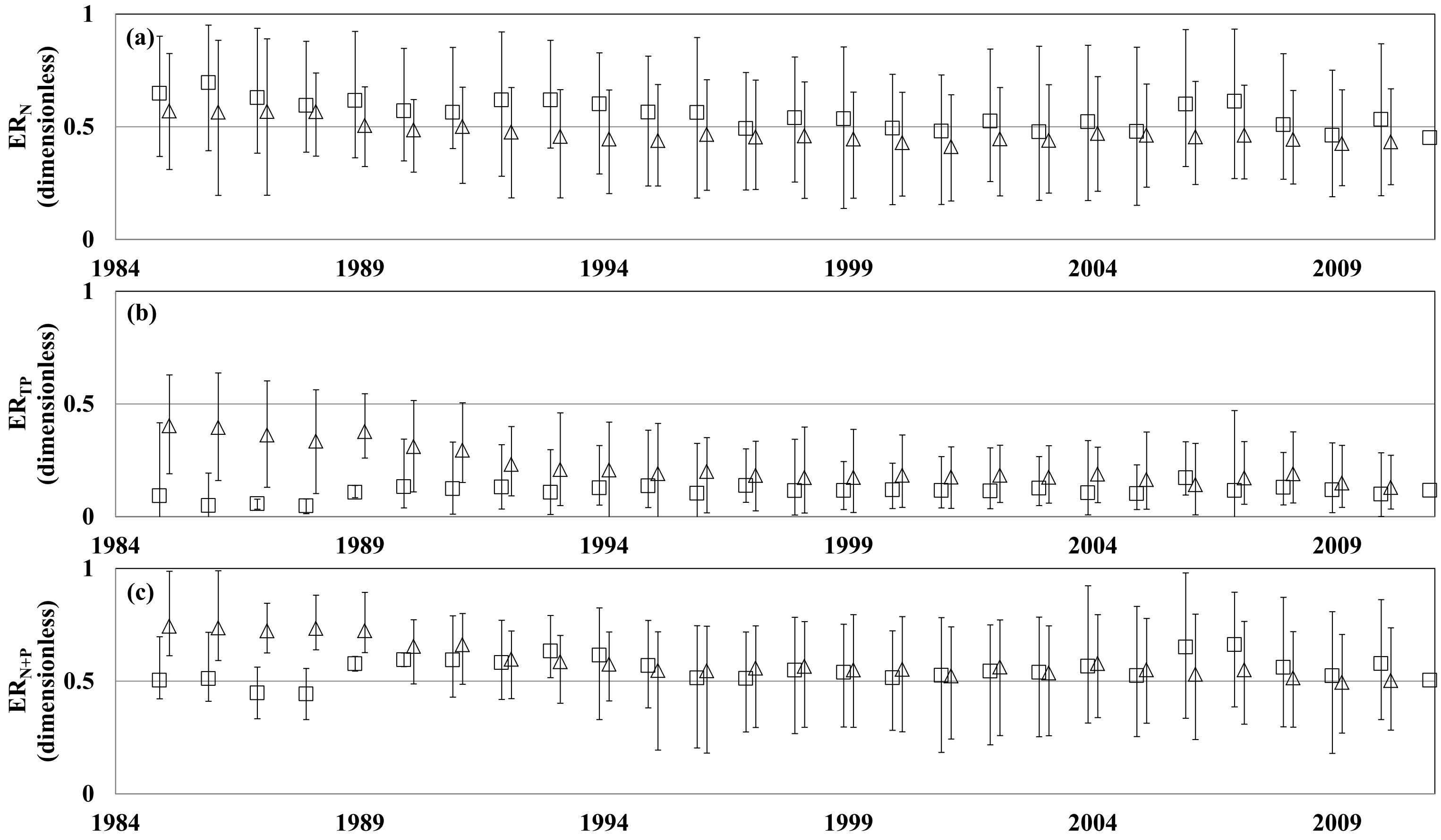

Figure 4 (2-column figure image) Ecological risk (ER) due to **(a)** N, **(b)** P, and **(c)** N+P from 1985 to 2011. Square and triangle symbols represent the median of ER in lakes and streams, respectively, and bars represent the 5th and 95th percentiles.

Discussion

Lakes versus streams

Streams have a shorter hydraulic residence time than lakes; thereby the former may serve as an instantaneous indicator of recent nutrient discharges. The decrease in ER_P in streams can be attributed to successful efforts to reduce P discharges to freshwater by controlling wastewater emissions and by banning phosphates in detergents (EEA, 2010; Glibert, 2012). By contrast, since lakes are subjected to continuous recycling and may release P from sediments which were enriched in the past (Marsden, 1989), the decrease in lake ER_P was observed in only 20% of European river basins.

Despite the success in reducing P discharges from wastewater, this management strategy alone may not solve issues of freshwater eutrophication in Europe since ER_P and ER_N remained constant in a large number of basins. As agricultural emissions account for an important share of freshwater eutrophication impacts, controlling emissions from non-point sources (e.g. agricultural fertilizers) is an important step towards achieving further reductions in nutrient discharges (EEA, 2010).

Stream fauna appeared to be less vulnerable to high nutrient levels ($\alpha_{Lake} < \alpha_{Stream}$). This also corresponds with the lower N and P levels defining trophic state thresholds for lakes than for streams (Smith et al., 1999). Additionally, lake invertebrates are more sensitive to increasing nutrient levels compared to streams ($\beta_{Lake} < \beta_{Stream}$). Here, we propose biogeochemical and ecological reasons for this trend. The long residence time of lentic waters may prevent the decrease in organic matter following N and P discharges and primary production in lakes (Holtan et al., 1988). Furthermore, convective mixing of anoxic hypolimnetic waters with surface waters may cause fast decreases in dissolved oxygen levels (Baehr and DeGrandpre, 2004). Azevedo et al. (2013a) suggest that this higher sensitivity is due to the fact that insects in lakes (corresponding to 42% of the invertebrates) respond to increasing nutrient stress more similarly to one another than those in streams (corresponding to 64% of invertebrates) due to the higher β-diversity in the latter (see also Hof et al., 2008). Additionally, since the surface area of streams in contact with adjacent areas (namely, terrestrial systems) is larger than that of lakes (Johnson et al., 2014), streams may be subjected to higher nutrient input from terrestrial systems relative to the area they occupy, there is a possibility that species may have adapted to corresponding nutrient levels. The adaptation of species to the environmental conditions they are exposed to through time is depicted by the evolutionary species pool hypothesis (Pither and Aarssen, 2005).

Despite the higher sensitivity of lake invertebrates to increasing nutrient concentrations, efforts to monitor nutrient levels in lakes were considerably less than in streams. We attribute that to the higher level of spatial detail required in the guidelines of monitoring data, whereby the sampling per covered area in streams is more frequent than in lakes (Nixon et al., 1998). The increase in monitoring efforts over the last two decades in both lakes and streams is evident (appendix S2). Ultimately, the monitoring of stressor concentrations by the EEA (or by another environmental agency) is an obligatory step for the derivation of the stressors probability density functions and, thus, for the estimation of the ecological risks.

Nitrogen versus phosphorus

The ecological risk posed by N stress is estimated to be considerably higher than the ecological risk of P stress in both streams and lakes (Figure 4a,b). Here, we propose two reasons for this trend. In low primary production systems, nutrient demand by autotrophs is reduced. The lessened uptake by N by autotrophs may cause accumulation of N. High NO_3^- values were associated to low primary production rates in a low productivity temperate lake (Sterner, 2011). Second, the excess of N supply compared to P was also identified in stoichiometric analysis of tissues of herbivores and of tissues of herbivore food supplies (Elser et al., 2000). In their study, the average N:P ratio of herbivore tissue was 22.2 while the average N:P ratio in herbivory feed was 30.2. An excess of N in lakes is expected if N:P ratios are lower in the tissue of consumer organisms than in the tissue from which they feed (Elser et al., 2000; Glibert, 2012).

The relatively high ER_N in both lakes and streams can be possibly attributed to high atmospheric N deposition rates in midwestern Europe compared with the global average (Dentener et al., 2006) and the emphasis in controlling emissions of P, not of N. As environmental programs emphasized the reduction of P discharges to freshwater, N emissions from wastewater (and particularly from non-point sources) remained fairly unchanged from 1975 to 1995 in the Netherlands, for example (van der Molen et al., 1998). Nonetheless, the impact of unchanging N emissions compared to those of P may be offset by the faster removal rates of N compared to those of P in lakes (van der Molen et al., 1998). The differentiated atmospheric deposition patterns across European landscapes (Dentener et al., 2006) may have also prompted the higher variability in river basin ER_N compared to that of ER_P.

Contrariwise to our findings, Weijters et al. (2009) found that macroinvertebrate family richness was more affected by P than by N. This may have been caused by the difference in spatial coverage, taxonomic level, and taxonomic coverage. Our study focused on a wide range of invertebrate species in Europe while Weijters et al. (2009) perform a global scale meta-analysis for family richness of mayflies, stoneflies, and caddisflies. Because our study uses species-specific NO_3^- and TP concentration thresholds, an individual species is either subjected to N or to P stress, not both at the same time. Nonetheless, at the species community level, the differentiated sensitivity across species essentially characterizes dual nutrient management.

Limitations

In our study, we assume that NO_3^- and TP have additive responses on potential invertebrate species losses. Although the difference between ER derived under the assumptions of RA and CA was low, this difference increases with increasing stressor levels and when the slope coefficient β is lower than one (Drescher and Boedeker, 1995). Strong deviations between the ER derived with the two methods could point towards non-additive effects between stressor mixtures (Drescher and Boedeker, 1995). Non-additive effects on primary productivity or autotrophic biomass have been repeatedly confirmed for combined N and P stress (Allgeier et al., 2011; Dodds, 2006; Elser et al., 2007). However, as opposed to effects on primary productivity, we are not aware of observational studies confirming interacting effects of N and P on diversity of invertebrate species in freshwaters. Similarly, Harpole & Tilman (2007) observed interactive effects of stressors on plant species losses in experimental studies but only an additive effect in observational studies, which is likely a result of the independent detrimental effects of N and P on species richness, as observed for north-western European grasslands (Ceulemans et al., 2013). In our study, we assume additive effects of N and P since the mechanisms behind mixed stressors effects on organisms are often unidentified or unknown (Backhaus et al., 2000; Backhaus et al., 2004) and, in the case of N and P, the mechanisms are multiple, e.g. effects on the synthesis of nucleic acids and of phospholipids. As environmental concentrations in observational studies are not deliberately designed as are those in controlled experiments (Tilman, 1987), it is not possible to test this interaction based on our field survey study. If the interaction between N and P would be non-additive, the increase of one stressor would not affect the total ecological risk of the mixture. For example, following the limiting nutrient concept for responses based on primary productivity (Schindler, 1977; Schindler et al., 2008), increasing NO_3^- concentrations would not cause changes in primary productivity if productivity is limited by P deficiency. Thereby, ER_N would not have any influence in the ER_T. We do not rule out the possibility of non-additive effects of N and P on invertebrate losses because of the few comparisons between effect model outcomes up to now.

Our study uses the maximum NO_3^- and TP concentrations at which each invertebrate species was confirmed present and they represent the threshold whereby species are no longer tolerant to higher nutrient levels. However, field surveys may simply have been unable to detect the species at levels higher than this threshold (Azevedo et al., 2013a), a characteristic that is common to presence-only studies of species occurrence (Azevedo et al., 2013b; Walker and Cocks, 1991). In more controlled experiments, Hickey and Martin (2009) found that effective and lethal concentrations of NO_3^- for invertebrates were generally higher than those of our study. This discrepancy may be attributed to the higher tested NO_3^- concentrations than those generally found in European freshwater bodies (see average $^{10}\log$ concentrations a_c in appendix S2). If higher species-specific maximum tolerance levels were employed for all species instead and if the monitored concentrations remained unchanged, α in the CDF would be higher than those reported in our study, thereby decreasing the ecological risk posed by the stressor. Additionally, we perform our risk assessment not with pre-selected (indicator) species but with all species reported in the environment. In the former, the CDF would likely indicate a higher sensitivity of the ecosystem to stress since target or indicator species tend to be more sensitive to stress (Smetanová et al., 2014).

Freshwater systems comprise many different stressors which may cause detrimental effects to organisms, e.g. N, P, oxygen, temperature, pH, etc. Given that we gathered data on environmental concentrations and organism threshold levels of two (NO_3^- and TP) but no other stressor, we are unable to test for potential auto-correlation between stressors. Such correlations might exist between NO_3^- and NO_2^-, which affects oxygen-carrying hemocyanin in crustaceans (Alonso and Camargo, 2008) and NH_3, which affects the secretion of byssus in bivalves (U.S. Environmental Protection Agency, 2013), as well as between other stressors involved in eutrophication impacts, e.g. dissolved oxygen and temperature.

The underlying data for the derivation of the yearly river basin PDF are the annual mean concentrations of NO_3^- and TP at different monitoring stations reported by the EEA. Thus, the σ_c used in the PDF describes the spatial variability across monitoring stations within the basin but not the intra-annual variability in nutrient levels. Occasional high concentrations at individual monitoring stations, common during peak rainfall events or upon fertilization at upstream farms, would shift the mean annual concentration of the monitoring station towards higher values. Ultimately, if many monitoring stations in a river basin would be subjected to peak concentrations, the year-based river basin ER would be higher than the basins comprising fewer high annual mean concentrations.

A final limitation of our study is the underlying data used for the derivation of the CDF and of the PDF. While the PDF is derived with spatially and temporally differentiated α_c and β_c variables, the α_i and β_i of the CDF are not differentiated across river-basins or across years but solely across temperate freshwater types (i.e., lakes and streams). This lack of differentiation could have underestimated the ER of basins which are particularly sensitive to eutrophication as well as overestimated the ER of the less sensitive ones. Nonetheless, spatial and temporal differentiation of the CDF may be obtained once surveys of invertebrates presence becomes as widespread as the monitoring of nutrient levels in freshwaters.

Conclusions

Because the monitoring of biodiversity shifts can be costly, ecological indicators of water quality impairment should be an available tool for environmental agencies (Johnson et al., 2014). Here, we estimated the ecological risks in lakes and streams due to dual N and P stress to invertebrates as this species group is frequently monitored (Growns et al., 1997) and because recent studies have shown the importance of dual nutrient management to control algae blooms (Paerl et al., 2011). Other environmental stressors, e.g. temperature or salinity, can be included in further assessments as well if sufficient monitoring data are available. Efforts to improve water quality in Europe should take into account that risks of absence of invertebrates due to excess of nutrients were generally correlated to high N levels, not P. Thus, a stricter control of nitrogen discharges to Europe inland waters is recommendable in order to comply with the ecological status requirements of the Water Framework Directive (James et al., 2005). As water bodies in the European Union are required to achieve ‘good ecological status’ by 2015 (Water Framework Directive, 2000), this framework determines the ecological risks of eutrophication as a function of the sensitivity of individual species in temperate lakes and streams as well the water quality of individual river basins. Furthermore, this framework conveys which stressors related with increasing primary productivity may cause the highest risk of biodiversity losses, which European river basins are most subjected to eutrophication risks, and how have these risks changed over time. These are some of the principal aspects required for environmental policy decisions.

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Supporting Information

Species-specific data on maximum concentrations of NO_3^- and TP are available in appendix S1. Yearly monitoring data on NO_3^- and TP concentrations and ER_N, ER_P, and ER_T per river-basin data are available in appendix S2. Results of the comparison between total ecological risks derived under the assumptions of response and concentration additions are shown in appendix S3.

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