Title: Species richness – phosphorus relationships for lakes and streams worldwide

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ABSTRACT

Aim: We investigated the patterns of autotrophic and heterotrophic relative species richness along a total phosphorus (TP) concentration gradient. The relative species richness – TP relationships were calculated separately for four different regions [(sub)tropical, xeric, temperate, and cold] and two types of water bodies (lakes and streams).

Location: Global

Methods: Using data from peer-reviewed articles reporting the occurrence of freshwater species at specific TP concentrations, we determined the species richness along a TP gradient. Using log-logistic regressions, we then estimated the TP concentration at which the potential decrease of relative species richness (RSR) equals 0.5 and the slope at which the decrease occurs (β). The RSR is given as the ratio of species richness to maximized species richness along a TP gradient.

Results: The RSR of streams generally decreased more rapidly than that of lakes with increasing P, as illustrated by the steeper slope of the log-logistic functions for streams (β_{lakes} < β_{streams}). Although there was no consistent trend between autotrophs and heterotrophs in the different regions, we found that the TP concentration at which the RSR equals 0.5 was lower in cold regions (0.04 – 0.22 mg P/L) than in warmer regions (0.28 – 1.29 mg P/L).

Main conclusions: The log-logistic relationships between RSR and TP concentration vary considerably among regions of the world, between freshwater types (lakes and streams) and between species groups (autotrophs and heterotrophs). This variability may be attributed to differences between the two freshwater types in respect to their species groups and evolutionary patterns, nutrient demand, biogeochemical and hydrologic processes. We were not able to derive log-logistic regressions for all combinations of freshwater type or species type and region [e.g., (sub)tropical lakes]. For other areas, our results can be used to assess the potential impact of phosphorus eutrophication on freshwater biota.
Keywords: Autotrophs, heterotrophs, freshwater, log-logistic regression, total phosphorus, species sensitivity, relative species richness

INTRODUCTION

The intensification of agriculture, industrialization, and urbanization has led to increasing fluxes of phosphorus (P) worldwide (Liu & Wang, 2007). In freshwater systems, this nutrient is regarded as the main factor driving primary productivity (Schindler, 1974). In addition to increases in biomass, changes in autotrophic and heterotrophic species richness associated with P concentrations have been reported (McIntyre et al., 2007; Penning et al., 2008; Rumes et al., 2011). For example, high crustacean species richness was found in eutrophic (sub)tropical lakes, while macrophyte species richness in temperate lakes (Rumes et al., 2011) was maximized at intermediate total-phosphorus levels (Penning et al., 2008). The rate of nutrient recycling in tropical streams has also been linked to fish species richness (McIntyre et al., 2007), suggesting that total-phosphorus levels and faunal richness are strongly correlated.

Because aquatic eutrophication is currently considered one of the strongest threats to water quality and stream biodiversity worldwide (Björklund et al., 2009; Vörösmarty et al., 2010), it is important to identify the freshwater types and regions whose biotic communities are most affected by P imbalances. Many studies have focused on the effects of increasing P in one or more specific water bodies, species, or regions (Dodson et al., 2000; Smith et al., 2007; Struijs et al., 2011b). However, to our knowledge, no study has outlined the effects of different P concentrations on relative species richness on a global scale. Understanding how decrease in relative species richness due to P increases may occur in different regions of the world and in different freshwater types is essential for the ecological assessment of eutrophication impacts.
The goal of this study was to compare the relationships between P concentration and the relative species richness of autotrophs and heterotrophs in lakes and streams in different regions of the world. We first performed an inventory of peer-reviewed observational field data, which yielded a large number of studies worldwide. We then derived concentration-response relationships based on this dataset. Given that the cause-effect relationships obtained from nutrient-addition experiments may reflect only small spatial and temporal coverage, observations from surveys make it possible to derive concentration-response relationships for other areas or periods.

Concentration-response relationships are commonly applied in toxicology and can be used to compare the sensitivity of different species groups to changes in the concentration of the stressor of interest. They can also be directly coupled with existing biogeochemical models of the fate of P in inland waters, such as those used in Global NEWS (Harrison et al., 2005; van Drecht et al., 2005), the Millennium Ecosystem Assessment (van Drecht et al., 2009), life cycle impact assessments (Helmes et al., 2012), or estimation of past or expected ecological risk of stressors (Leuven et al., 2011; Fedorenkova et al., 2012), to ultimately determine the relationships between environmental stressors and relative species richness in freshwater systems.

MATERIAL AND METHODS

Species richness – total P relationships

We chose total P (TP) as our stressor of interest because it is the recommended P fraction for water quality monitoring (Lewis et al., 2011) and is commonly reported in water-quality studies. Based on observational studies of species occurrence, we calculated the relative species richness of autotrophs and heterotrophs along a TP gradient. Subsequently, we derived log-logistic regressions of the relative species richness in lakes and streams in (sub)tropical, temperate, cold, and xeric regions. Each step is described in detail below, and
the complete framework is illustrated in Fig. 1.

We computed the overall species richness (SR) as the number of species reported in 0.1 log\(_{10}\)-transformed concentration intervals (\(C_i\), mg P/L) for streams and lakes in different regions as

where \(O_{t,h,g}\) is the occurrence of a given species in species group \(g\) (autotrophs or heterotrophs) at nutrient concentration \(C_i\) in freshwater type \(t\) (stream or lake) in region \(h\). \(O\) is one if the species is reported to be present and zero if the species is not present.

We identified the concentration of TP at which SR\(_{t,t,h,g}\) is at its maximum (SR\(_{\text{max}}\)). This approach has been applied by Struijs et al. (2011b) for genus-level macroinvertebrate richness in Dutch streams as a function of TP-induced stress and by Azevedo et al. (2013) for vascular-plant species richness in biomes as a function of low-pH stress. The TP concentration at which SR equals SR\(_{\text{max}}\) is hereafter referred to as the optimum TP concentration, \(C_{\text{opt}}\). To compare SR – TP concentration relationships between autotrophs and heterotrophs, between lakes and streams, and among regions, we transformed SR into a relative species richness measure as

following Azevedo et al. (2013) and Struijs et al. (2011b), where e\(\text{RSR}\) is the empirical relative species richness (e\(\text{RSR}\)) of species in group \(g\) at concentration \(C_i\) in freshwater type \(t\) in region \(h\). At \(C_{\text{opt}}\), e\(\text{RSR}\) is one. Conversely, an e\(\text{RSR}\) of zero represents the complete absence of species. At \(C_i\) values lower than \(C_{\text{opt}}\), we consider that SR is no longer affected by the excess P (Struijs et al., 2011b). Thus, e\(\text{RSR}\) is here described only for \(C_i\) values larger than \(C_{\text{opt}}\). It is important to note that species richness – TP relationships are typical bell-shaped curves, with species richness also decreasing at limiting nutrient levels (Penning et
In this study, we were interested in how individual species’ tolerance to high TP levels might influence species richness – TP relationships. Therefore, we limited the analysis to the eutrophic side of the curve, i.e. at $C_i$ greater than $C_{opt}$ (Struijs et al., 2011b).

Next, we used a log-logistic model of the calculated RSR ($cRSR$) by fitting it to $eRSR$ as

\[ eRSR = \frac{1}{1 + e^{-\alpha + \beta \log_{10}(TP)}} \]

The coefficient $\alpha$ indicates the $\log_{10}$ TP concentration at which relative species richness is 0.5, and $\beta$ indicates the slope of the log-logistic regression. We fitted both $cRSR$ coefficients ($\alpha$ and $\beta$) using logistic regression in SAS 9.2. The sample size for the log-logistic regression is given by the number of SR$_{i,t,h,g}$ data points (at 0.1 $\log_{10}$ TP-concentration intervals). We considered a log-logistic model to fail to fit the empirical data if it had a $p$ value > 0.05, a $\beta$ coefficient non-different from zero at a 95% confidence level, or an $\alpha$ coefficient outside the concentration range at which $eRSR$ data were available (between $C_{opt}$ and the maximum observed TP level). To test for potential sampling bias resulting from different sampling effort across regions, we tested whether the number of species or the number of studies was correlated with our results for $C_{opt}$, $\alpha$, or $\beta$.

**Sensitivity analysis**

We conducted a sensitivity analysis to investigate how our regressions might vary if smaller spatial units were used. For this analysis, we used the methodology described above, but at smaller spatial levels of detail: freshwater ecoregions [using the map delineated by Abell et al. (2008)], regions within individual realms, and individual studies in our data inventory. Azevedo et al. (2013) have suggested employing a sensitivity analysis based on repeating the procedure using smaller spatial-aggregation levels to identify differences in
We repeated the sensitivity analysis to determine how our results would change if we distinguished among specific taxonomic groups within the autotrophic and heterotrophic groups. Autotrophs were divided into cyanobacteria, silicon-based algae, non-silicon-based algae, and macrophytes, while heterotrophs were divided into fish and invertebrates.

**Collection and management of data**

To acquire data about the TP-concentration ranges at which specific aquatic species were reported to be present, we selected peer-reviewed articles using a combination of relevant keywords in *Web of Science* (lake, freshwater, phosphorus, etc.). The specific keyword combinations can be found in Appendix S1 in the Supporting Information. We included only articles that reported the locations of their field surveys and that recorded TP concentrations and species occurrences at the same sampling location and time. We also included species records from the *Limnodata Neerlandica* database (STOWA, 2010), which includes invertebrate-occurrence data and water-quality measurements for inland waters in the Netherlands. We did not consider brackish or inland saline waters or anthropogenic ecosystems, such as fish ponds or wastewater-treatment plants.

We grouped ponds and reservoirs with freshwater lakes and springs, rivers, and creeks with streams. Wetlands were excluded from this study. Furthermore, based on the geographical location of each study, we assigned each freshwater system to a biogeographical region: (sub)tropical, temperate, cold, or xeric. This division was based on the major freshwater habitat types (MHT) of the world, recently developed by Abell and colleagues of the FEOW project (Freshwater Ecoregions Of The World, [http://www.feow.org](http://www.feow.org)). We combined the regions designated as temperate coastal rivers, temperate floodplain rivers and wetlands, and temperate upland rivers into one temperate region. Likewise, we combined (sub)tropical coastal rivers, (sub)tropical floodplain rivers and wetlands and (sub)tropical
upland rivers into one (sub)tropical region. Although the MHT map delineated by the FEOW includes many freshwater types, such as temperate floodplain rivers and wetlands, we used the freshwater-type classification given by each inventoried study. Montane and polar freshwater systems were combined and referred to as the cold region. Studies located in the MHT classes Oceanic Islands, Large Lakes, and Large River Deltas (such as studies located in the Great Lakes and Lake Peipsi regions, the Paraná River basin, and the Azores Islands) were assigned to the closest adjacent habitat.

We selected species belonging to the following groups: autotrophs (comprising cyanobacteria, silicon-based algae, non-silicon-based algae, and macrophytes) and heterotrophs (comprising aquatic invertebrates and fish). We used the Integrated Taxonomic Information System database (www.itis.gov) for the nomenclature of invertebrates and fish, the PlantList (www.theplantlist.org) for macrophytes, and the AlgaeBase (www.algaebase.org) for cyanobacteria, silicon-based algae, and non-silicon-based algae (for details on the allocation of each organism, see Table S2.1). We included records at taxonomic levels lower than species (i.e., variety and subspecies) when they were available, considering them as species-level records. Next, we standardized the different reporting methods in each study (e.g., biomass and abundance) into an occurrence/non-occurrence dataset for each species. For example, if biomass was greater than zero, then we considered the species as occurring.

We then derived the TP-concentration range at which each species occurred in streams or lakes within each water body in each region following the method described by Azevedo et al. (2013). Each concentration range represents the tolerance of an individual species to TP levels in a given freshwater type and region. Outside of these concentration boundaries, the species was considered absent. The range of occurrence (minimum and maximum concentration values) was calculated using one of the following procedures, in
order of preference: (1) the lognormal variation distribution of 90% of the sample (Slob, 1994), (2) the minimum – maximum reported concentration range, or (3) the mean value alone. Finally, we considered the overall range of occurrence of each species to be represented by the lowest minimum and highest maximum concentrations obtained for that species in a given freshwater type in a given region. Species whose final minimum- and maximum-concentration values were equal were excluded from the dataset because this condition does not represent a true concentration range found in the environment.

Data set

Approximately 6800 studies were found using the keyword combinations, and 186 of these studies were applicable (see list of studies in Table S1.1). Most studies did not fulfill all the requirements of this data inventory, such as identifying organisms at the species level, sampling either lake or stream freshwater systems, and providing spatially explicit, field-observation data on TP concentrations.

Many studies (142) represented North American and European freshwater systems (Fig. 2). The number of studies conducted in lakes and streams was 155 and 35, respectively (see Table S1.1). The inventory included 2294 unique species, with 1318 and 1596 species-occurrence – TP-concentration records for autotrophs and heterotrophs, respectively (see Table S2.2 for specific species ranges). We found no data for autotrophs in cold-region streams or for autotrophs and heterotrophs in xeric-region streams.

RESULTS

We were able to derive log-logistic functions for eight of the thirteen combinations of species group (autotrophs and heterotrophs), freshwater type (lakes and streams), and region for which eRSR data were available (Fig. 3). The number of available species in each combination varied considerably, from 45 autotrophic species in xeric lakes to 835 heterotrophic species in temperate streams (Table 1). Fig. S2.1 shows the scatter-plots of
species richness vs. TP concentration that were used to define $C_{opt}$ and to calculate eRSR.

The TP concentration at which species richness is maximized, $C_{opt}$, was lowest in cold-region lakes (0.02 to 0.03 mg P/L, Table 1). $C_{opt}$ was generally higher in streams (0.07 to 0.20 mg P/L) than in lakes (0.02 to 0.10 mg P/L). We found no clear distinction between the $C_{opt}$ values of heterotrophs and autotrophs across freshwater types and regions.

The log-transformed TP concentration at which RSR equals 0.5, represented by $\alpha$, was highest in temperate streams (for both autotrophs and heterotrophs) and in xeric lakes (for autotrophs) ($10^\alpha = 1.0$ to 1.3 mg P/L, Table 1). $\alpha$ was lowest for heterotrophs in cold-region lakes ($10^\alpha = 0.04$ mg P/L).

The sensitivity of species to increasing nutrient levels is represented by the slope of the log-logistic function, $\beta$ (the higher the $\beta$, the steeper the function). Heterotrophic RSR was systematically more sensitive to increases in P compared to autotrophic relative species richness, except in temperate streams. Autotrophs in temperate and cold lakes were the least sensitive to TP increases ($\beta = -0.53$ to $-0.63$). The optimum concentration $C_{opt}$ was positively correlated with $\alpha$ [Fig. S3.1(a)]. However, we found no correlation between $\alpha$ and the slope of the log-logistic function, $\beta$. In addition, we found no significant correlation between the sampling effort in each region and the results of the log-logistic regressions ($C_{opt}$, $\alpha$, and $\beta$) (appendix S4).

**Sensitivity analysis**

To analyze the robustness of our results, we compared how the choice of spatial scale would affect the log-logistic regression coefficients by aggregating the dataset into realms, ecoregions, and individual studies. We found that there may be high spatial variability across spatial units within a region (Appendix 5). However, the variability of the coefficients obtained for spatial aggregation at the level of regions was within the range of results obtained for these additional levels of aggregation.
The three phytoplankton groups showed similar sensitivity to TP changes within lakes in the same region (Table S5.5). The results for cyanobacterial species were not different for similar freshwater types across regions (Tables S5.5 and S5.6). On the other hand, phytoplankton species of temperate lakes (cyanobacteria, silicon-based and non-silicon-based algae) are less sensitive to TP changes than macrophytes (Table S5.5). However, invertebrates in cold-region lakes were more sensitive to TP changes than invertebrates in temperate lakes (Table S5.7). The overall heterotrophic group also showed greater sensitivity in cold-region lakes than in temperate lakes (Table 1). In the temperate region, the level of P at which heterotrophic lake species maintained 50% of their richness was similar to that obtained for invertebrates but higher than that obtained for fish (Table S5.7).

We were not able to derive log-logistic regressions for all smaller spatial units and specific taxonomic groups. For example, although there were nine ecoregions within the temperate region, we were able to derive regressions for stream autotrophs in only four of these ecoregions (i.e., Central & Western Europe, Northeast US & Southeast Canada Atlantic Drainages, Southeastern Korean Peninsula, and Upper Mississippi, Table S5.2). Likewise, no data were available for macrophytes in (sub)tropical streams; therefore, this species group was not evaluated.

**DISCUSSION**

We derived the concentration-response relationships between TP and the RSR of autotrophic and heterotrophic species in two freshwater types (lakes and streams) in temperate, (sub)tropical, xeric, and cold regions. Below, we explore the main uncertainties of our study and interpret our results.

**Uncertainties**

First, the optimum concentration ($C_{opt}$) reported here corresponds to the maximal species richness found within an observed TP-concentration gradient. Thus, we assume that
the RSR – TP patterns we report are valid for TP levels above but not below $C_{opt}$. This concentration does not necessarily correspond to minimally disturbed, “baseline” conditions. Nonetheless, the calculated $C_{opt}$ values are within the range of target values established by the European Union and the United States Environmental Protection Agency, which range from 0.01 to 0.15 mg P/L (European Commission, 2000; Smith et al., 2003). The species used in the regression above $C_{opt}$ may have been more represented by high-TP-tolerant algae and less by species that are typically present at low nutrient levels. In fact, many species in our inventory were present at TP conditions below $C_{opt}$ (Table S2.3), suggesting that these species may also be adapted to survive at low TP levels.

Second, in our study, the response of organisms to stress was estimated based on phosphorus levels alone because this nutrient is considered the primary limiting nutrient in freshwater systems (Schindler, 1974; Carpenter et al., 1998). We did not consider the influence of other stressors, although these can also influence freshwater species. For example, nitrogen has been reported to influence primary productivity as much as phosphorus, and co-limitation has also been reported to drive eutrophication (Elser et al., 2007). Lower light availability due to increasing turbidity or growth of macrophytes may hinder the influence of P (Le Bagousse-Pinguet et al., 2012). Likewise, grazing pressure, oxygen availability, chlorophyll concentration, substrate texture, stream width or area, and lake depth have been reported as factors to explain the variability in species-richness responses to P (Amarasinghe & Welcomme, 2002; Huszar et al., 2006; Friberg et al., 2010).

In addition to abiotic stressors, the nutrient demands of planktivores or higher-order consumers may vary widely (Hall, 2009). These differences are frequently addressed by biomanipulation experiments (Carpenter et al., 2001). Although the present study did not account for each species’ position in the food chain, we distinguished organisms according to their primary nutrition pathways (i.e., their ability or inability to perform photosynthesis).
Third, the type or number of species representing each species group (autotrophs and heterotrophs) may depend on the research focus. For example, diatoms are commonly used for water-quality monitoring, but the Dutch water-quality database we employed (STOWA, 2010) focuses on macroinvertebrates. In cold- and temperate-region lakes, the number of invertebrate species was more than three times the number of fish species (Table S5.7). One outcome of the high number of invertebrates is that the TP level at which 50% of the species are maintained for the overall heterotrophic group was similar to that for invertebrates but not to that for fish. Although taxa differ in their tolerance to high P levels (Caputo et al., 2008), we did not account for these differences in this study. Our results should therefore be interpreted at the level of the overall autotrophic and heterotrophic species groups but not at lower taxonomic levels.

Fourth, due to the strong research effort in Western Europe and in eastern North America, we found numerous studies in the temperate region (Fig. 2). This prompted the number of species employed to derive the RSR – TP functions in temperate systems to be higher than elsewhere. However, neither the number of studies nor the number of species used to derive our regressions influenced the results we obtained for $C_{opt}$, $\alpha$, or $\beta$ (appendix 4). In any case, the sensitivity analysis showed that there may be strong variability among areas within a specific region. For example, while the $10^\alpha$ and $\beta$ coefficients for temperate lake autotrophs were 0.30 and -0.63 mg P/L, respectively, individual studies yielded $10^\alpha$ values ranging from 0.02 to 4.07 and $\beta$ values ranging from -0.53 to -0.01 (Table S5.1). The choice of spatial units into which localities are aggregated to derive log-logistic regressions (e.g., regions, ecoregions, or individual studies) remains under discussion. For example, Azevedo et al. (2013) derived regressions at the biome level of spatial detail, while Struijs et al. (2011b) and Amarasinghe & Welcomme (2002) defined their species-richness patterns per country and per continent, respectively.
Fifth, we gathered data on TP concentration ranges at which freshwater species were present. In conditions outside this surveyed range, it is uncertain whether the species truly becomes absent because of life-threatening concentration levels. In contrast to controlled experimental studies, field-based observational studies like those surveyed here provide less certainty as to the exact boundaries between tolerable and intolerable stressor conditions (Struijs et al., 2011b). Therefore, we cannot confirm factual species disappearance since the calculations of species richness are not based on verified species loss but on first-encounter species occurrence. Ultimately, there may be an underestimation of relative species richness estimations which are based on first-encounter analysis as opposed to confirmed species loss due to intolerable TP levels. This concern has been recently defined as ‘dark diversity’ (Pärtel et al., 2011) and it is commonly under scrutiny when the stressor of interest is the potential loss of species caused by damage to the species living space as such (He & Hubbell, 2011). In observational studies such as ours, the issue caused by unconfirmed species loss may be alleviated by thorough monitoring of species occurrence and by coverage of a wide TP-concentration gradient, up to highly eutrophic levels.

Finally, to compare the potential impact of phosphorus across regions with evident differences in species richness (e.g., cold vs. (sub)tropical freshwater systems), we employed a relative measure of relative species richness. Despite our effort to gather data from the literature, we were not able to successfully estimate the RSR in regions for which very few or no data were found, such as xeric-region streams and (sub)tropical lakes.

This species-occurrence/non-occurrence approach simplifies an effect type previously described in a continuous manner (e.g., biomass or abundance) into a simpler binary dataset (presence and absence). This standardization has the advantage of combining the different ways in which effects have been reported by different studies. While abundance is more commonly reported for species that are visible to the naked eye, other organisms are more
frequently reported as present or absent in a given freshwater body.

**Interpretation**

**Optimum TP**

Our results show that the optimum TP concentrations are generally higher in streams than in lakes. This finding can be attributed to distinct biotic nutrient demands in the two freshwater types. First, because lakes require lower P levels than streams to reach the same net primary-productivity rate (Smith *et al.*, 1999), the optimum TP can be reached at lower concentrations in lakes than in streams. This pattern was observed across all autotrophic groups (cyanobacteria, silicon-based and non-silicon-based algae, and macrophytes) in the temperate region. Second, while nutrient surpluses in lakes are quickly reduced by widespread, fast-growing algae (Carpenter *et al.*, 1998; Doi, 2009), nutrient recycling in streams, especially those of lower size orders or with strong tree shading, depends primarily on the speed at which heterotrophs assimilate organic matter (Vannote *et al.*, 1980; Merritt *et al.*, 1984).

Another reason for the higher optimum TP levels in streams compared to lakes is the differences in hydrological patterns that influence nutrient removal from the water column. Once nutrients are deposited in the sediment layer via the sinking of soil, animal fecal pellets or algae, they can be either transported back into the water column or immobilized in the sediment for long periods (Holtan *et al.*, 1988). In streams, strong water currents enhance sediment uplift, favoring the maintenance of high TP levels in the water column (Bahnwart *et al.*, 1998). In addition, the short residence times of water in streams, especially lower-order streams, may decrease phytoplankton exposure to nutrients hence hinder autotrophic growth (Vannote *et al.*, 1980). Conversely, lakes, especially cold ones (Carpenter *et al.*, 1999), may be subjected to thermal stratification for many months, confining nutrients to the surface layer, where net primary productivity and nutrient recycling prevails, and hindering the
vertical uplift of nutrient-rich sediments (Tylmann et al., 2012).

Phosphorus loads are lower overall in the cold region than in the temperate and (sub)tropical regions due to lesser anthropogenic nutrient release in cold regions, e.g., agricultural runoff (Harrison et al., 2010). Smaller decomposition rates in colder regions may also decrease nutrient release into water bodies. It is therefore likely that most species in cold regions are adapted to low nutrient concentrations. Because we were not able to derive regressions for both species groups in warmer regions [(sub)tropical and xeric], it is unclear how the climatic gradient represented by the four regions may influence $C_{\text{opt}}$ across the two species groups.

Regression coefficients

Geological and evolutionary processes in freshwater systems will determine how tolerant the biotic community is to a given stressor. As a result, species in nutrient-rich environments will be more adapted to high nutrient levels than those unused to such conditions (Köhler, 1994; Bontje et al., 2011). In this study, we found that the freshwater systems that could maintain half of the relative species richness at higher TP levels were also those with high optimum TP levels, $C_{\text{opt}}$. Nonetheless, this trend does not imply that organisms with high tolerance to elevated phosphorus levels are also less sensitive to changing levels of this nutrient.

Heterotrophs have higher P demands than autotrophs because autotrophs have higher N:P and C:P ratios (Elser et al., 2000). Hence, heterotrophs are expected to be more affected by P surpluses than autotrophs (and more sensitive to changing P levels). In our study, however, this hypothesis was confirmed in temperate and cold-region lakes but not in temperate streams (Table 1). Here, we propose three explanations for the lower sensitivity of autotrophs in lakes compared to streams based on the difference in light availability and photosynthetic rates between the two freshwater types. This analysis is verified once
phytoplankton species (cyanobacteria, silicon-based and non-silicon-based algae) are separated from macrophytes in the autotrophic group in temperate lakes. First, the increased production of N-rich protein and RNA by small organisms with high growth rates suggests that the biological demand for N may surpass that for P (Elser et al., 1996). The lesser effect of P on autotrophs in lakes compared to streams may be due to more intense autochthonous phytoplankton activity in lakes than in streams (Doi, 2009). Second, the lower sensitivity of autotrophic richness to P changes can be attributed to the insensitivity of cyanobacteria to low-oxygen and high-P conditions (Downing et al., 2001). Many cyanobacteria, including toxic groups (e.g., Aphanizomenon), compensate for their light demand in light-abundant lacustrine systems and for their N demand via N fixation; ultimately, P supplementation allows them to overcome their primary growth limitations (Camargo & Alonso, 2006). Third, macrophytes help to maintain algal diversity under increasingly eutrophic conditions by impeding the fast-growing, light-favored phytoplankton that prevail in eutrophic lakes (Le Bagousse-Pinguet et al., 2012). Given that the proportion of macrophytes within the autotrophic group was considerably higher in temperate lakes than in streams, the buffering effects of increasing P may be more intense in lakes compared to streams in the temperate region.

The sensitivity of organisms could be compared across lakes and streams only in the temperate region. We found that heterotrophic species were less sensitive to TP changes in streams than in lakes. This difference may be due to the high dispersal ability of heterotrophs in lakes, generating stronger similarity between species assemblages and thus lower β-diversity in lakes compared to streams (Hof et al., 2008). If lakes contain more similar species assemblages, which are expected to react in a more similar way following stress exposure, then relative species richness will change more rapidly in lakes than in streams because streams encompass more dissimilar species.
The comparison across regions was hindered by the scarcity of data for (sub)tropical lakes and xeric streams. In addition, the influence of P on primary productivity is less apparent in (sub)tropical lakes than in temperate lakes (Huszar et al., 2006), which may explain why we were not able to derive a log-logistic regression for this ecosystem. Huznar et al. (2006) and Abell et al. (2012) have suggested that nitrogen may drive primary production more than P does in (sub)tropical lakes because higher temperatures enhance nitrogen losses, particularly through denitrification, and P transport to water bodies due to weathering (Abell et al., 2012).

Relevance of research and application of results

Global-scale assessments are available for the influence of P on net primary productivity in freshwater systems (Elser et al., 2007). However, changes in species richness due to increasing P concentrations have previously been assessed in studies focusing on a specific freshwater type or covering a smaller area, e.g., Struijs et al. (2011b) and Friberg et al. (2010). The results of the present study show that the patterns of relative species richness along a TP-concentration gradient can be described using logistic regressions.

We identified patterns in the RSR along a TP-concentration gradient for two species groups (autotrophs and heterotrophs), in two freshwater types (lakes and streams), and in four regions [(sub)tropical, xeric, temperate, and cold]. We found that lakes generally, but not always, have lower optimal TP levels and that their species assemblages are less sensitive to TP changes than those in streams. Furthermore, autotrophs and heterotrophs in cold regions have lower optimal concentrations compared to those in other regions. The regressions can be used to describe the potential decrease in relative species richness in a quantitative manner, although it is important to note that factual causal relationships between species losses and P increases are not tackled in this study. In combination with biogeochemical models of the fate of nutrients, this procedure can assist in estimating the ultimate effects of stressors on species
richness maintenance (van Zelm et al., 2007; Struijs et al., 2011a; Verbrugge et al., 2012).

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The research team is involved in ecological risk assessment of environmental stressors with the use of mathematical models (http://www.ru.nl/environmentalscience). Author contributions: L.B.A., R.V.Z., R.S.W.E.L, A.J.H., and M.A.J.H. conceived the ideas and analyzed the data. L.B.A., P.E., J.S. and D.D.Z. collected and managed the data. All authors contributed to the writing of the manuscript.
Table 1 Number of studies, surveyed TP range (mg P/L), number of species ($\text{SR}_{\text{max}}$), optimum TP level in region ($C_{\text{opt}}$) and range of $C_{\text{opt}}$ estimates across ecoregions (in parenthesis), relative species richness (RSR) coefficients $\alpha$ and $\beta$ and 95% confidence intervals (in parenthesis), Pseudo-$R^2$ and p value of log-logistic regressions for two organism groups, in two freshwater types, and four world’s regions.

<table>
<thead>
<tr>
<th>Freshwater type</th>
<th>Species Group</th>
<th>Region</th>
<th>Number of studies</th>
<th>Number of species</th>
<th>$\text{SR}_{\text{max}}$</th>
<th>$C_{\text{opt}}$ in region ($C_{\text{opt}}$ across ecoregions)</th>
<th>$\alpha$</th>
<th>$\beta$</th>
<th>Pseudo-$R^2$ / p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lake</td>
<td>Autotroph</td>
<td>Cold</td>
<td>10</td>
<td>0.002 to 3.155</td>
<td>163</td>
<td>122</td>
<td>0.03</td>
<td>-0.64</td>
<td>-0.53</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Temperate</td>
<td>74</td>
<td>0.001 to 41.970</td>
<td>614</td>
<td>459</td>
<td>0.05</td>
<td>-0.52</td>
<td>-0.63</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Xeric</td>
<td>2</td>
<td>0.009 to 1.180</td>
<td>45</td>
<td>45</td>
<td>0.03 (0.03 to 0.03)</td>
<td>-0.39</td>
<td>-0.39</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Sub)tropical*</td>
<td>7</td>
<td>0.003 to 35.000</td>
<td>95</td>
<td>42</td>
<td>15.85 (0.001 to 15.85)</td>
<td>1.58</td>
<td>-0.19</td>
</tr>
<tr>
<td>Heterotroph</td>
<td></td>
<td>Cold</td>
<td>14</td>
<td>0.002 to 3.155</td>
<td>54</td>
<td>49</td>
<td>0.02 (0.003 to 0.02)</td>
<td>-1.37</td>
<td>-0.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Temperate</td>
<td>61</td>
<td>0.001 to 682</td>
<td>682</td>
<td>488</td>
<td>0.10</td>
<td>-0.19</td>
<td>-0.24</td>
</tr>
<tr>
<td>Stream Type</td>
<td>Habitat</td>
<td>$C_{opt}$ Range</td>
<td>$C$ Range</td>
<td>Species Richness</td>
<td>$a$ Range</td>
<td>$b$ Range</td>
<td>$r^2$</td>
<td>Significance</td>
<td></td>
</tr>
<tr>
<td>-------------</td>
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<td>-----------------</td>
<td>-----------</td>
<td>-----------</td>
<td>-------</td>
<td>--------------</td>
<td></td>
</tr>
<tr>
<td>Xeric</td>
<td>1</td>
<td>0.385 to 4.124</td>
<td>4</td>
<td>4</td>
<td>0.63*</td>
<td>No successful fit with the log-logistic regression</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Sub)tropical*</td>
<td>4</td>
<td>0.001 to 0.220</td>
<td>9</td>
<td>7</td>
<td>0.04 (-0.005 to 0.04)</td>
<td>-0.82 (-0.86 to -0.78)</td>
<td>-0.03 (-0.07 to 0.01)</td>
<td>0.92 / 0.001</td>
<td></td>
</tr>
<tr>
<td>Temperate</td>
<td>11</td>
<td>0.001 to 2.625</td>
<td>182</td>
<td>146</td>
<td>0.20 (0.001 to 0.50)</td>
<td>0.01 (-0.02 to 0.04)</td>
<td>-0.17 (-0.20 to -0.15)</td>
<td>0.99 / &lt; 0.001</td>
<td></td>
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<tr>
<td>(Sub)tropical</td>
<td>6</td>
<td>0.001 to 5.088</td>
<td>203</td>
<td>199</td>
<td>0.07 (0.001 to 0.08)</td>
<td>-0.56 (-0.64 to -0.48)</td>
<td>-0.41 (-0.50 to -0.33)</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>Cold</td>
<td>1</td>
<td>0.010 to 0.044</td>
<td>2</td>
<td>2</td>
<td>0.02*</td>
<td>No successful fit with the log-logistic regression</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperate</td>
<td>16</td>
<td>0.007 to 17.000</td>
<td>835</td>
<td>697</td>
<td>0.10 (0.01 to 0.40)</td>
<td>0.11 (0.06 to 0.15)</td>
<td>-0.32 (-0.36 to -0.29)</td>
<td>0.99 / &lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>(Sub)tropical*</td>
<td>3</td>
<td>0.052 to 1.225</td>
<td>9</td>
<td>8</td>
<td>0.40 (0.1 to 0.40)</td>
<td>0.13 (-0.02 to 0.29)</td>
<td>-0.15 (-0.29 to -0.01)</td>
<td>0.85 / 0.001</td>
<td></td>
</tr>
</tbody>
</table>

* Regression was not successfully derived since either $\beta$ coefficient is not significantly different from zero at a 95% confidence level or $10^\alpha$ coefficient was outside the TP concentration range of relative species richness (defined by $C_{opt}$ to upper boundary of surveyed TP concentration).

** Minimum and maximum range of $C_{opt}$ based on one ecoregion only.

*** As defined by Schabenberger & Pierce (2001).
FIGURE LEGENDS

**Fig. 1** Description of the steps taken for acquisition of data and derivation of response curves.

**Fig. 2** World map (projection: Aitoff) with the location of the freshwater bodies used in this study allocated to four regions. The studies within “Other” areas (11), which represent large freshwater bodies and oceanic islands, were allocated to their closest region. Squares (155) and triangles (35) represent lakes and streams, respectively.

**Fig. 3** Empirical (eRSR, circles) and the calculated (cRSR, lines) relative species richness of autotrophs along a total phosphorus (TP) gradient in lakes (a) and in streams (b) and of heterotrophs in lakes (c) and in streams (d). Log-logistic coefficients used in the cRSR functions are shown in Table 1. Green, orange, rose, and blue represent the (sub)tropical, xeric, temperate, and cold regions, respectively (same color code as in Fig. 2).
FIGURES

<table>
<thead>
<tr>
<th>COLLECTION AND MANAGEMENT OF DATA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1- Keyword selection of peer-reviewed articles (see keyword combination in Box 1, appendix 1) and selected studies in Table S1.1</td>
</tr>
<tr>
<td>2- Allocation of studies into their respective habitat and freshwater type (Fig. 2)</td>
</tr>
<tr>
<td>3- Allocation of species to its respective species group and trophic group (see taxonomic classification method in Table S2.1)</td>
</tr>
<tr>
<td>4- Standardization of species occurrence to presence/absence data</td>
</tr>
<tr>
<td>5- Determining the total P (TP) concentration range of occurrence of each species (Table S2.3)</td>
</tr>
</tbody>
</table>

<table>
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<tr>
<th>RESPONSE CURVES</th>
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<tbody>
<tr>
<td>6- Calculation of the species richness (SR) in each species group and freshwater type (Equation 1)</td>
</tr>
<tr>
<td>7- Identification of optimal concentration ($C_{opt}$) based on maximized species richness ($SR_{max}$)</td>
</tr>
<tr>
<td>8- Calculation of the empirical relative species richness (eRSR) in each species group and freshwater (Equation 2)</td>
</tr>
<tr>
<td>9- Calculation of calculated RSR (cRSR) per species group and freshwater type (Equation 3). Results are shown in Table 1.</td>
</tr>
</tbody>
</table>

Figure 1
Figure 3