

### Assessing global regionalized impacts of eutrophication on freshwater fish biodiversity Zhou, J.

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## **Chapter 5**

# Global regionalized characterization factors for phosphorus and nitrogen impacts on freshwater fish biodiversity

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#### Abstract

Inefficient global nutrient (i.e., phosphorus (P) and nitrogen (N)) management leads to an increase in nutrient delivery to freshwater and coastal ecosystems and induces eutrophication in these aquatic environments. This process threatens the various species inhabiting these ecosystems. In this study, we developed regionalized characterization factors (CFs) for freshwater eutrophication at  $0.5\times0.5$ -degree resolution, considering different fates for direct emissions to freshwater, diffuse emissions, and excessive erosion due to land use. The CFs were provided for global and regional species loss. CFs for global species loss were quantified by integrating global extinction probabilities. Results showed that the CFs for P and N impacts on freshwater fish are higher in densely populated regions that encompass either large lakes or the headwater of large rivers. Focusing on nutrient-limited areas increases country-level CFs for P in 51.9% of the countries and 49.5% of the countries for N compared to not considering nutrient limitation. This study highlights the relevance of considering freshwater eutrophication impacts via both P and N emissions and identifying the limiting nutrient when performing life cycle impact assessments.

#### 5.1 Introduction

Humans have altered the phosphorus (P) and nitrogen (N) cycle by harvesting P and N from the geosphere and the atmosphere, respectively, and using them to boost agricultural production (Jenny et al., 2016). Mineral and synthetic fertilizers have contributed to a five-fold increase in global food production, boosting and supporting the 2.5-fold population growth during the past five decades. The long-term use of fertilizer and phosphate mining has doubled phosphorus (P) and tripled nitrogen (N) inputs to the global environment (Bouwman et al., 2009; FAOSTAT, 2008; Zhang et al., 2021). This has led to problems such as eutrophication (Jenny et al., 2016; Schindler and Vallentyne, 2008) and species toxicity (Kroupova et al., 2018). These issues negatively affect aquatic ecosystems and can also be harmful to human health (Bryan

and van Grinsven, 2013; Vonlanthen et al., 2012). The rising global food demand and its concomitant fertilizer requirements indicate that eutrophication in freshwater systems is likely to continue increasing in the near future (Beusen et al., 2022; Mogollón et al., 2018a, 2018b; Nedelciu et al., 2020).

Life Cycle Assessment (LCA) is one approach used to evaluate the environmental impacts of eutrophication (Muralikrishna and Manickam, 2017). Ecosystem quality, represented by biodiversity, is one of the core areas of protection regarding which impacts are assessed. Characterization factors (CFs) link emissions to biodiversity loss (Payen et al., 2019). In freshwater ecosystems, fish biodiversity is considered a good indicator of ecosystem health (Villéger et al., 2017; Whitfield and Elliott, 2002) and this indicator has previously been used in LCA (de Visser et al., 2023.; Pierrat et al., 2022). Due to the various environmental conditions (including the prevalence of nutrient limitations) and species compositions, impacts of P and N emissions on fish species vary spatially, which requires regionalized CFs for impact assessment.

In 2017, the United Nations Environment Programme (UNEP) launched the third phase of the Global Life Cycle Impact Assessment Method (GLAM) project as a part of the "Life Cycle Initiative" to standardize and review life cycle methods globally (Payen et al., 2019). GLAM advocates for the use of spatially explicit models with global coverage to develop regionalized CFs. Specifically for eutrophication, an additional recommendation from an earlier GLAM phase was to improve the modeling of physical and biogeochemical processes (Payen et al., 2019). Moreover, GLAM recommended the consideration of global as opposed to just local or regional species loss (Verones et al., 2019), for which global extinction probabilities (GEPs) have just recently been developed (Verones et al., 2022).

Our study aims to follow the GLAM recommendations and develop regionalized CFs for freshwater eutrophication (with a 0.5×0.5-degree spatial resolution and yearly time step) by using the Integrated Model to Assess the Global Environment – Global Nutrient Model (IMAGE-GNM) (Beusen et al., 2015). We coupled the local nutrient

fate and concentrations from IMAGE-GNM to 41 years of freshwater fish species data (13,920 freshwater fish species) to estimate the impact at the local ecosystem level. Next, we employed GEPs (Verones et al., 2022) to extrapolate the regional species loss to global species loss. Our study provides practical indicators for economic actors to estimate the P and N impacts on the global freshwater ecosystem and serves as a roadmap for impact assessment of eutrophication in other ecosystems.

#### 5.2 Methods

#### 5.2.1 Characterization factors

In LCA, CFs connect the life cycle inventory (e.g., emissions) to impacts (e.g., on the ecosystem). Ideally, CFs are composed of multiple sub-factors following a cause-effect chain: fate, exposure, effect, and damage factors (Rosenbaum et al., 2015). In the case of eutrophication, fate factors (FFs) describe the fate of contaminants from emissions due to human activities eventually transported to the (aquatic) environment. In this study, we calculated FFs for 2010 by employing the approach of Zhou et al. (2022b), who distinguished emission routes into direct emissions to freshwater, diffuse emissions excluding erosion, and erosion caused by land use (their FFs were developed for the year 2000). While exposure factors (XFs) have sometimes been used in the past to represent impacts from eutrophication, such as hypoxia (Cosme and Hauschild, 2017), excessive nutrients also affect species in other ways, such as changes in the energy transfer in food webs (Wang et al., 2021), and noxious toxins produced by harmful algae (Chorus and Welker, 2021). Eutrophication-induced algae blooms also prevent predators from seeking prey by shading and diminishing light penetration (Lehtiniemi et al., 2005). Predators thus become more susceptible to nutrient increases compared with autotrophs, leading to decreases in cross-taxon congruence (Wang et al., 2021). These phenomena highlight the importance of considering the comprehensive effects of nutrients on biodiversity loss along with hypoxia when assessing the impacts of nutrient emissions. Therefore, we followed the widely used approach of deriving CFs directly from the fate to effect without considering exposure (e.g. LC-IMPACT (Azevedo et al., 2020), ReCiPe2016 (Huijbregts et al., 2017), and Jwaideh et al. (2022).

Damage to biodiversity is often expressed through the potentially disappeared fraction (PDF) of species. In contrast to previous studies (Rosenbaum et al., 2015) that assessed impacts through the potentially affected fraction, we directly linked the nutrient concentration levels  $(kg/m^3)$  to PDF by regressing species sensitivity distributions (SSDs) for P following the method of Zhou et al. (2023) to calculate EFs for P (PDF·m³/kg P) and N (PDF·m³/kg N).

By linking fate and effect factors, we derived CFs for impacts on regional species loss (Eq. 5.1). Additionally, we employed Global Extinction Probabilities (GEPs) (Verones et al., 2022) of freshwater fish for CFs that assess the global impacts of freshwater eutrophication (Eq. 5.2). GEPs denote a scaling factor for potential regional species loss with respect to potential global extinctions (Verones et al., 2022). GEPs are available for 20 species groups across marine, terrestrial, and freshwater ecosystems. We selected the GEPs for freshwater fish to match the species group used in the EFs of the regional CFs, as recommended by Verones et al. (2022).

We estimated eight types of CFs for the combination of different emission routes and the marginal vs. average methods in assessing EFs (i.e., marginal and average CFs for direct emissions to freshwater, diffuse emissions excluding erosion, erosion caused by land use transition from natural land to arable land, and that to pasture). Detailed descriptions of FFs, EFs, and GEPs can be found in Zhou et al. (2023, 2022b) and Verones et al.(2022).

$$CF_{regional,e\to i} = \sum_{i} FF_{e\to i\to j} \times \frac{EF_{j}}{V_{j}}$$
(5.1)

$$CF_{global,e\to i} = \sum_{i} FF_{e\to i\to j} \times \frac{EF_{j}}{V_{j}} \times GEP_{j}$$
 (5.2)

In Eq. 5.1 and Eq. 5.2,  $CF_{regional,e\rightarrow i}$  and  $CF_{global,e\rightarrow i}$  denote the cumulative CFs for regional species loss and global species loss in the source grid cell i (at a half-degree resolution) from an emission source e (erosion, diffuse sources excluding erosion, and

direct emissions to freshwater), respectively, and the subscript j indicates the downstream receptors of source cell i connected by advection.  $V_j$  is the water volume of the receptor j (m<sup>3</sup>). The unit of CFs for erosion is PDF·year/(m<sup>2</sup>·year), while that for diffuse sources excluding erosion and direct emissions to freshwater is PDF·year/kgX, where X represents P or N.

For the additional erosion caused by land use, we subtracted the initial CFs of arable land/pasture and natural land to express the eutrophication impact of human activities  $(CF_{erosion \rightarrow i, landuse}^*, PDF \cdot year/(m^2 \cdot year), Eq. 5.3)$ .

$$CF_{erosion \to i,landuse}^* = CF_{erosion \to i,landuse} - CF_{erosion \to i,natural\,land}$$
 (5.3)

#### 5.2.2 Model and data

SSDs were estimated by coupling the fish occurrence data to nutrient concentration levels within the same locations (i.e., in half-degree pixels) for each year (from 1970 to 2010). We compiled the occurrence data for freshwater fish species following the approaches of Barbarossa et al. (2021, 2020). In total, we acquired 13,920 freshwater fish species and 5,427,740 occurrence records from 1970 to 2010.

We used results from IMAGE-GNM (Beusen et al., 2022) from the year 2010 (the latest non-scenario year) for nutrient loadings, emissions, and concentrations for total P (TP) and total N (TN) to derive FFs, EFs, and emission-weighting data (see Eq. 5.4 below). IMAGE-GNM was designed to be spatially explicit and dynamic, with a resolution of  $0.5\times0.5$  degrees and a yearly time step (Beusen et al., 2016, 2015). This model uses a mechanistic approach to make predictions on nutrient transport processes, based on the hydrological cycle. The hydrological patterns were simulated by incorporating the PCRaster GLOBal Water Balance model (PCR-GLOBWB) (Sutanudjaja et al., 2018). For this study, we applied the hydrological and nutrient estimates of IMAGE-GNM from 1970 to 2010 for generating SSDs for P (SSDs for N were derived from Zhou et al. (2023).

#### 5.2.3 Freshwater nutrient limitation

In a specific water body, eutrophication can be limited by P or N, or co-limited by both nutrients, depending on the ratio of P and N content and their concentrations. Following McDowell et al.(2020), we assess the locations affected by nutrient over-enrichment and undesirable periphyton growth, which are directly tied to hypoxic "dead zones" and the loss of higher trophic species, such as fish (Wurtsbaugh et al., 2019).

According to McDowell et al. (2020), the nutrient limitation and the algal growth state are determined by a Redfield N:P ratio of 7:1 (by mass) and concentration thresholds for P and N in non-arid zones. Non-arid zones were defined as regions where discharge is nonzero. McDowell et al. (2020) differentiated limitations and the algal growth state into four types. We added a fifth type of acceptable periphyton growth without defining the limiting nutrient when both P and N concentrations are zero:

- If the TN:TP ratio ≥7 and TP concentration < 0.046 mg/L, or if TP concentration
  equals zero and TN is nonzero, the cell has acceptable periphyton growth and is
  P-limited (type 1).</li>
- 2) If the TN:TP ratio  $\geq 7$  and TP concentration  $\geq 0.046$  mg/L, the cell has undesirable periphyton growth and is P-limited (type 2).
- 3) If the TN:TP ratio <7 and TN concentration < 0.800 mg/L, or if TN concentration equals zero and TP is nonzero, the cell is considered to have acceptable periphyton growth and to be N-limited (type 3).</p>
- 4) If the TN:TP ratio <7 and TN concentration ≥ 0.800 mg/L, the cell has undesirable periphyton growth and is N-limited (type 4).
- 5) If TN and TP concentrations are both zero, the cell is assigned to have no periphyton growth (type 5).

By implementing this spatial information on nutrient limitation, more precise CFs can be derived to assess the impacts of freshwater eutrophication. That is, CFs for P can be applied where the cells are limited by P, and those for N can be used for regions limited by N.

#### 5.2.4 Aggregation of CFs

To match the freshwater eutrophication CFs with life cycle inventory data, the grid-level CFs can be aggregated to any scale through Eq. 5.4 and Eq. 5.5. We provided CFs aggregated to the country level, the common level of inventory data, using two methods: (1) considering only the P/N-limited regions; (2) considering the entire region.

$$CF_{r,e} = \frac{1}{\sum_{i} E_{e \to i \in r}} \cdot \sum_{i} CF_{e \to i \in r} \cdot E_{e \to i \in r}$$
 (5.4)

 $CF_{r,e}$  (PDF·year·kg<sup>-1</sup>) indicates the aggregation of nonzero CFs (i.e., we excluded CFs that are zero) for direct emissions to freshwater and diffuse emissions excluding erosion over a region r. In method 1, r only includes the P/N-limited regions within the country; in method 2, r indicates the whole country.  $E_{e \to i \in r}$  (kg year<sup>-1</sup>) is the emission-weighting data of the emission route e in the source cell i that belongs to the region r.

All nonzero  $CF_{i\in r,erosion,landuse}^*$  were summed over a region r weighted by the area of the land use type  $(A_{i\in r}, m^2)$  to provide  $CF_{r,erosion,landuse}^*$ .

$$CF_{r,erosion,landuse}^* = \frac{1}{\sum_i A_{i \in r}} \sum_i CF_{i \in r,erosion,landuse}^* \cdot A_{i \in r,landuse}$$
 (5.5)

We also calculated the proportion of emission-weighting data (direct emissions and diffuse sources) and area (arable land and pasture) for nutrient-limited regions across all countries. It equals the ratio of emission-weighting data or area between nutrient-limited regions and the whole country. If it remains unknown where in a country a nutrient is emitted and which nutrient is limiting, one could calculate the average of P and N impacts, weighted by such proportions. For ease of use, we also provide country-level CFs that already incorporate such weights.

#### 5.2.5 Global impact of eutrophication on freshwater fish species

We calculated the global freshwater fish species loss due to eutrophication from global nutrient emissions and agricultural land use in 2010 to test the application of the developed CFs. The impacts were obtained from the product of inventory data (direct or diffuse emissions, or land occupation area and time) and the respective CFs:

$$I_{emission} = \sum CF_{i,emission} \times E_i$$
 (5.6)

$$I_{erosion} = \sum CF_{i,erossion} \times A_i \times t$$
 (5.7)

where  $I_{emission}$  and  $I_{erosion}$  (PDF·year) represent the total impact on freshwater fish species, taking the sum of the impact of each source cell i;  $CF_{i,emission}$  (PDF·year·kgX<sup>-1</sup>, where X represents P or N) and  $CF_{i,erossion}$  (PDF·year/(m<sup>2</sup>·year)) indicate the CF for P and N emissions and for erosion, respectively, choosing the average CFs for global rather than regional species loss;  $E_i$  (kgX) denotes the emissions, for which we considered here the global emissions over a year;  $A_i$  (m<sup>2</sup>) is the land use area occupied, for which we considered here the total agricultural land use area; and t (year) is the occupation time, for which we considered one year. The emissions or land use areas were retrieved from IMAGE-GNM. The impact was aggregated over the world, considering the nutrient limitation.

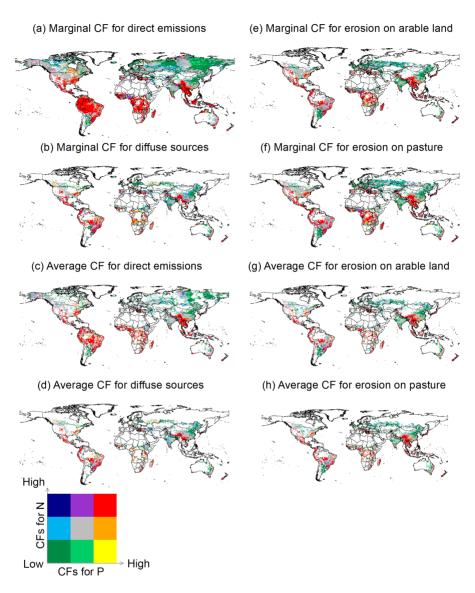
#### 5.2.6 Regionalized characterization factors

The gridded CFs for global freshwater species loss show similar hotspots for P and N (Figure 5.1, Figure S5.1 and S5.2 in Supporting Information). Results showcase only slight differences between using the average or marginal methods to assess effects. For the diffuse emissions and the direct emissions to freshwater, high CFs are located in densely populated regions that encompass either large lakes or the headwater of rivers, most of which are in tropical and temperate zones. For erosion, high CFs belong to those areas with intensive agriculture and animal husbandry. Compared with pastureland, more erosion per area occurs in arable land and thus leads to higher CFs.

High values for all the emission routes are found in the Andes Mountains (the upstream of the Amazon River), the Sierra Madre do Sul - Sierra Madre Occidental (the upstream of San Diego River and Marikina River), Great Salt Lake, Lake Tanganyika, and the Himalayas and Dangla Mountains (Yalu Cangbu River and Mekong River Basin).

Low CFs for P and N are found in high latitudes, such as the north of Canada, northern Europe, the south of Argentina, the east of Russia, and the northeast of China. These regions are sparsely populated and thus less affected by human activities, or they are located on the coasts where most contaminants disperse from freshwater to offshore environments.

Compared with CFs for regional species loss (Figure S5.3 and S5.4 in Supporting Information), CFs for global species loss show similar spatial patterns in tropical and temperate zones. Nevertheless, for direct emissions, CFs for global species loss seldom have hotspots in polar regions while CFs for regional species loss have hotspots in North Russia and North Canada. This difference results from considering or not considering GEP (Figure S5.13 in Supporting Information). In addition, CFs for global species loss show a larger variation between polar and temperate regions than CFs for regional species loss.



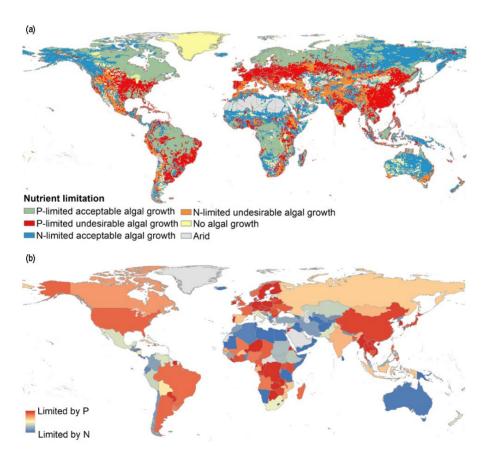
**Figure 5.1** Comparison of characterization factors between P and N at a half-degree resolution. Low CFs and high CFs indicate CFs < first quartile (Q25) and CFs > third quartile (Q75), respectively, and medium CFs are between low and high CFs.

#### 5.3 Results

#### 5.3.1 Nutrient limitation

Excluding the 7.8% of global arid regions without freshwater, the non-arid regions

limited by P and N occupy 52.3% and 36.5%. respectively, while 3.4% are covered by negligible P and N concentrations (Figure 5.2). Among these, 23.5% of the area limited by P and 11.7% of the area limited by N is susceptible to undesirable rates of periphyton growth. Undesirable eutrophication ties up with densely populated regions, while acceptable nutrient freshwater concentrations are distributed in sparsely populated areas such as the polar region and arid zones. The US, China, and countries in north and west Europe, east South America, and central Africa belong to P-limited regions with undesirable periphyton growth. These regions are affected by P contained in runoff, domestic and industrial wastewater from populated urbans and sludge in arable land. Australia, the countries in north Africa and west Asia are more affected by N limitation as they represent regions with historically relatively small proportions of arable land.

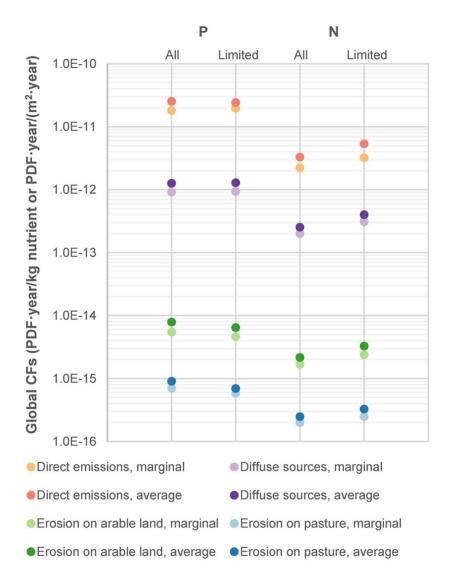


**Figure 5.2** Nutrient limitation for algal growth based on the Redfield ratio: (a) on the gridded scale, (b) on the country scale. Acceptable and undesirable algal growth in panel (a) was determined based on the current P and N concentrations. Panel (b) shows the proportion of P-N limitation that is weighted by direct emissions in those countries where average CFs for this emission route are available.

#### 5.3.2 Aggregation of characterization factors

The influence of considering nutrient limitation on country-level CFs varies across countries for all emission routes, while the hotspots (e.g. Cameroon) and low-CF countries (e.g. Libya) (Figures S5.5 - S5.12 in Supporting Information) maintain a similar pattern independent of nutrient limitation. Considering nutrient limitation leads to about half-half increase vs. decrease in country-level CFs. Take the average CFs for direct P emissions as an instance; the inclusion of nutrient limitation makes more countries have higher country-level CFs (56 countries higher vs. 52 countries lower than not considering nutrient limitation), whilst the opposite applies to direct N emissions (54 countries higher vs. 55 countries lower).

On a global scale, the inclusion of nutrient limitation leads to lower CFs for diffuse P emissions (by 2% to 3%) and higher CFs for other emission routes (by up to 30%). Yet, considering nutrient limitation makes CFs for N higher (by 25% to 62%) (Figure 5.3). The average CFs for all emission routes are higher than marginal CFs, independent of considering nutrient limitation.



**Figure 5.3** Globally aggregated characterization factors (CFs) for P and N impacts on global species richness. The unit of CFs for direct and diffuse emissions is PDF·year/kg nutrient, where nutrient represents P or N, while the unit of CFs for erosion is PDF·year/(m²·year). CFs consider either all regions (All) or only regions limited by the respective nutrient (Limited).

#### 5.3.3 Global impact of eutrophication on freshwater fish species

Considering nutrient limitation in CFs, we calculated the impact of global P and N emissions as well as erosion enhanced by agricultural land use during the year 2010 on freshwater fish species richness as 0.138 PDF·year (Table 5.1). Among all the emission routes, erosion on arable land contributes the most to the impacts, while diffuse emissions are the second strongest contributor. Direct emissions rank third and still contribute considerably, while the erosion on pastureland has the least influence. Regarding nutrients, P leads to more than double the species loss than N. The difference in impacts is particularly evident for erosion. In summary, P is the paramount nutrient for freshwater eutrophication (causing an impact of 0.098 PDF·year), but the impact of N should not be neglected (0.040 PDF·year).

**Table 5.1** Global species loss over the world considering the nutrient limitation. The unit is PDF·year.

	Impact of	Impact of	Impact of	Impact of	
	direct	diffuse	erosion on	erosion on	Sum
	emissions	emissions	arable land	pasture	
P	0.019	0.026	0.047	0.006	0.098
N	0.012	0.013	0.013	0.002	0.040

#### 5.4 Discussion

In this study, we used two methods for deriving EFs (average and marginal) and compiled the CFs for four emission routes at multiple spatial scales: global, country level, and half-degree grid level. These CFs can be used with emissions and land use areas from life cycle inventories to assess the nutrient-induced impacts on freshwater fish biodiversity. Our improvements include providing CFs that cover eutrophication more comprehensively than just hypoxia (Cosme and Hauschild, 2017) with a much finer resolution for both FFs and EFs than previous studies (Cosme et al., 2018; Payen et al., 2021; Verones et al., 2020). We note that our CFs for N also encompass the potential impact of N-induced toxicity (Kroupova et al., 2018), which is a different

impact category to eutrophication (Chislock et al., 2013; Dodds and Smith, 2016; Payen et al., 2019; Smith et al., 2006). However, N overloads have been deemed to predominantly affect the aquatic ecosystem through eutrophication (Chislock et al., 2013; Dodds and Smith, 2016; Wang et al., 2021), while direct toxicity contributes little to the influence because it only occurs at a very high concentration of certain forms of N, such as ammonia and nitrite (Jones et al., 2014; Kroupova et al., 2018; Thurston et al., 1981).

Compared with previous studies that only consider P-related freshwater eutrophication and N-related marine eutrophication in CFs, our study is the first to incorporate both P and N simultaneously and consider which of the two nutrients is limiting where. The information about nutrient limitation can guide users in the choice to assess the impacts of either P or N emissions. The CFs allow LCA practitioners to estimate the nutrient impact on freshwater fish species richness more accurately. This method can also serve as a prototype that may be adapted for eutrophication impact assessment related to marine or terrestrial ecosystems.

Based on the approach outlined above (considering nutrient limitation), the global impact of eutrophication on freshwater fish species is 0.138 PDF·year. The dominance of erosion as a contributor to the impacts of freshwater eutrophication is consistent with the findings of Scherer and Pfister (2015). Since the fate within the freshwater is relatively short (in the order of dozens of days) but the emissions and erosion are spread throughout the year, the exposure duration can be assumed to be roughly one year. This means that 13.8% of the fish species potentially disappear due to freshwater eutrophication. This result approximates 15.6% (= 24.8%×63%) of freshwater fish species threatened with extinction due to pollution, as estimated by Miranda et al. (2022). This agreement shows the validity of using our CFs to reproduce the influence of freshwater eutrophication on the global ecosystem.

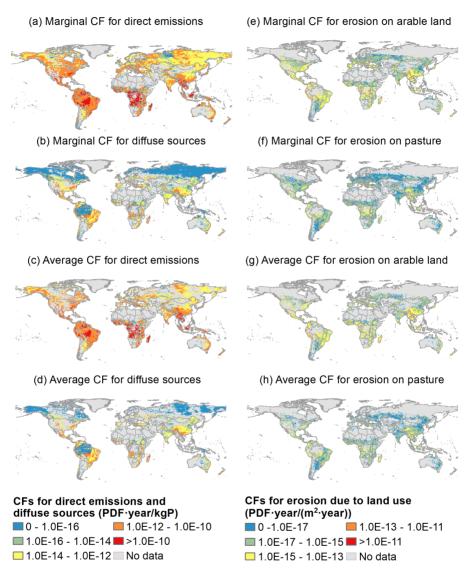
The model for nutrient transport and fate is crucial for determining CFs and can be a large source of uncertainty. For instance, 0.5% of the  $0.5\times0.5$  degree grid cells have a

diffuse N loading from surface runoff that exceeds diffuse emissions, since IMAGE-GNM does not isolate the influence of long-term retention of N in the soil surface from the short-term loads in surface water due to new emissions. The isolation of these processes might be possible by using a process-based mechanistic model such as the IMAGE-Dynamic Global Nutrient Model (DGNM) (Vilmin et al., 2020), which models water column and sediment dynamics, and the exchanges between them. A future version on a global scale can form a better basis for developing CFs (Vilmin et al., 2020).

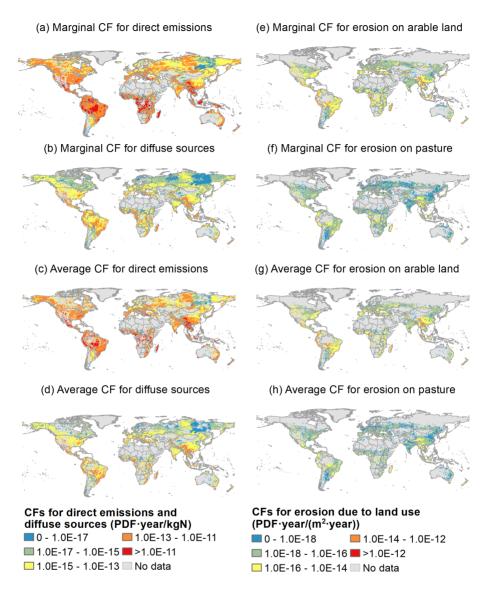
A large amount of data and high spatial resolution allowed distinguishing nutrient-species relationships across 425 ecoregions, which is substantially beyond the previous knowledge of only four geographical zones (Cosme et al., 2017). This resolution could even be improved for the effect factors by considering the background concentration at different locations within the ecoregions. More regionalized environmental indicators can help to better assess impacts at local scales. Our study showcases the advantages of finer spatial resolutions, and we recommend the continuation of this practice when developing new CFs in the future. Next to spatial differentiation, the temporal dynamics in emissions from human activities should be considered in future studies (Potting and Hauschild, 2006; Seppala et al., 2001).

In conclusion, we developed regionalized CFs for freshwater eutrophication at a fine spatial resolution and proposed a method to consider nutrient limitation in CFs. This work provides life cycle impact indicators and a roadmap for considering nutrient limitation to finetune CFs for multiple emission routes to assess the eutrophication impact on regional and global species richness for LCA practitioners. This roadmap and the consideration of comprehensive nutrient-species effects can be further used for developing regionalized CFs for eutrophication in other ecosystems.

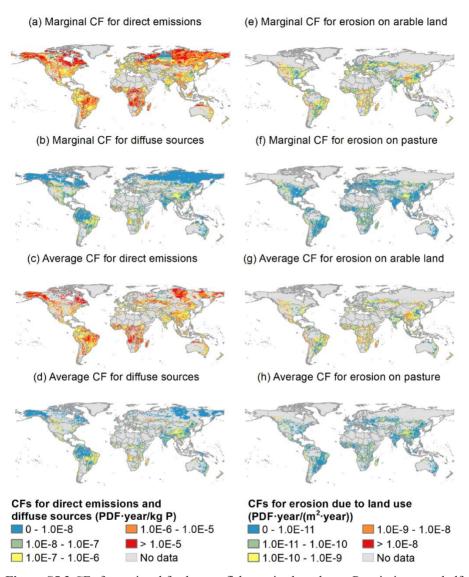
#### **Supporting Information**



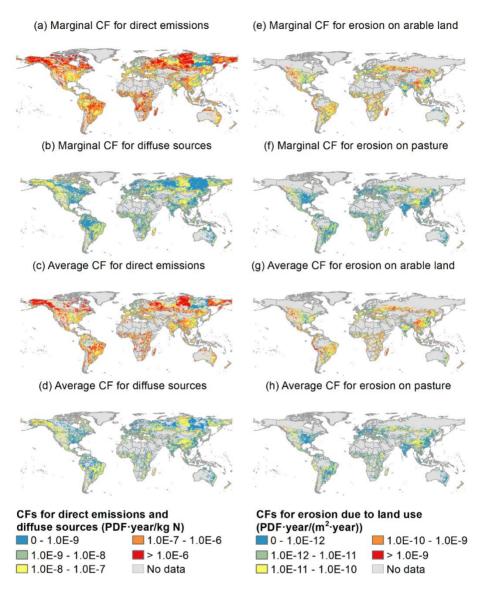
**Figure S5.1** Characterization factors for freshwater fish species due to P emissions at a half-degree resolution



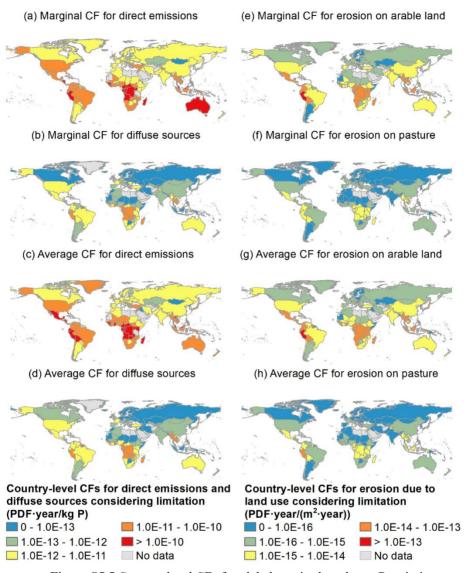
**Figure S5.2** Characterization factors for freshwater fish species due to N emissions at a half-degree resolution



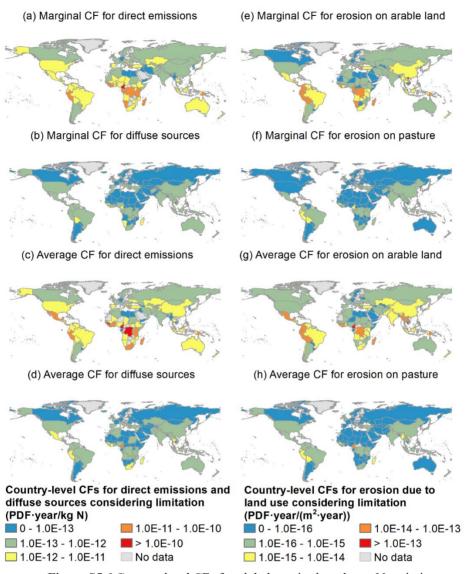
**Figure S5.3** CFs for regional freshwater fish species loss due to P emissions at a half-degree resolution



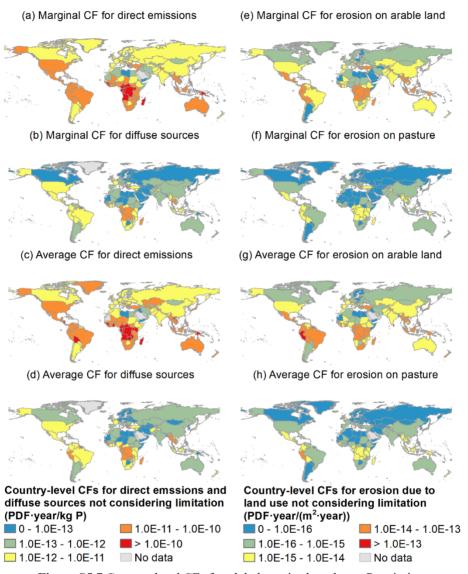
**Figure S5.4** CFs for regional freshwater fish species loss due to N emissions at a half-degree resolution



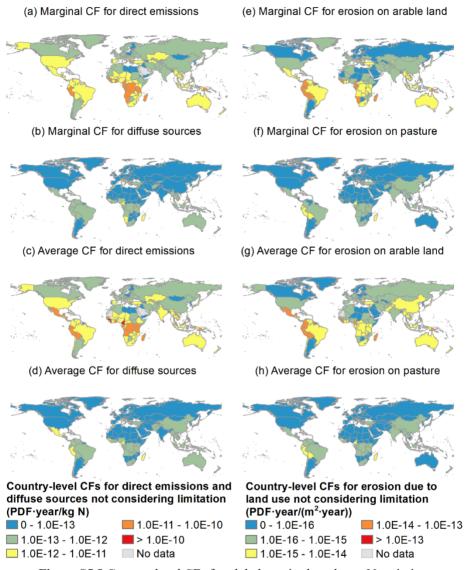
**Figure S5.5** Country-level CFs for global species loss due to P emissions considering P-limited regions only



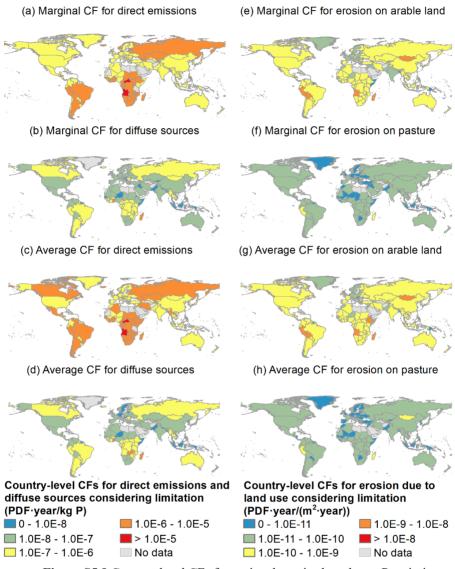
**Figure S5.6** Country-level CFs for global species loss due to N emissions considering N-limited regions only



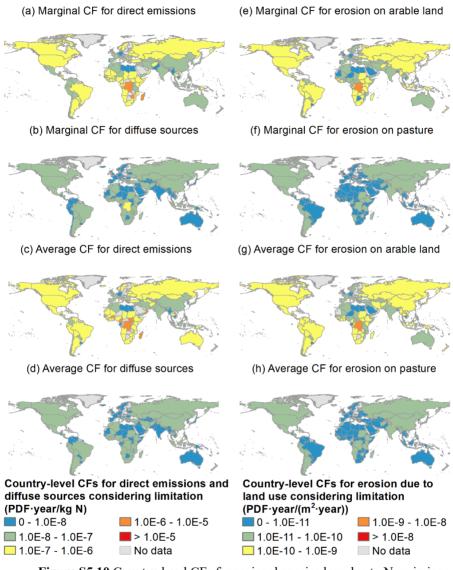
**Figure S5.7** Country-level CFs for global species loss due to P emissions not considering the nutrient limitation



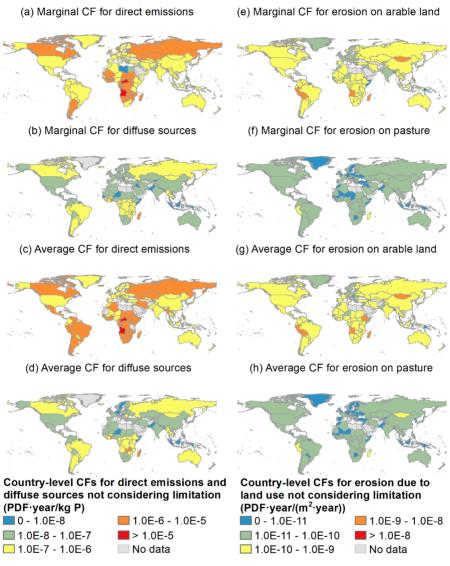
**Figure S5.8** Country-level CFs for global species loss due to N emissions not considering the nutrient limitation



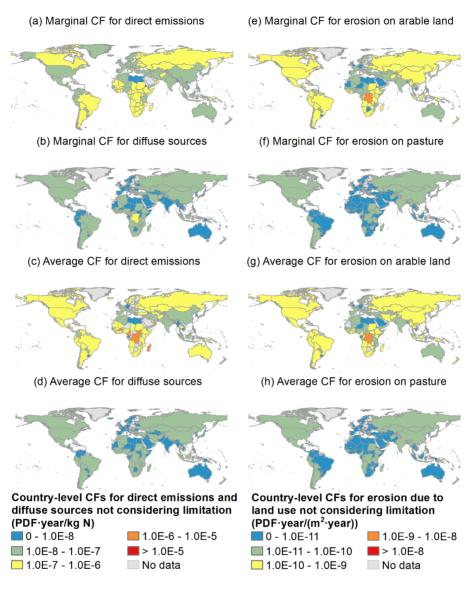
**Figure S5.9** Country-level CFs for regional species loss due to P emissions considering P-limited regions only



**Figure S5.10** Country-level CFs for regional species loss due to N emissions considering N-limited regions only



**Figure S5.11** Country-level CFs for global species loss due to P emissions not considering the nutrient limitation



**Figure S5.12** Country-level CFs for global species loss due to N emissions not considering the nutrient limitation

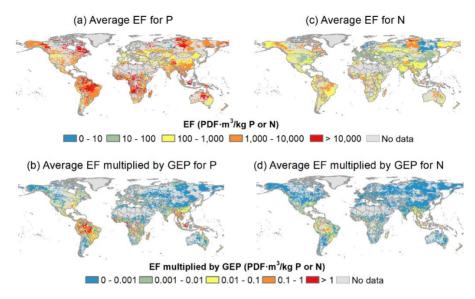


Figure S5.13 EF and EF multiplied by GEP for P and N