Effects of Eutrophication on Fish Functional Diversity in Freshwater Rivers Globally

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Content

Abstract

This study explores the impact of phosphorus on the functional diversity (FD) of freshwater fish globally. The FD metrics functional richness (FRic), functional evenness (FEve) and functional divergence (FDiv) are calculated using a compiled dataset of 36,868 total phosphorus (TP) stations and 4,478,635 fish occurrences, leading to a FD analysis for 2498 buffers.

Using linear mixed models and various potential covariates, the effects of TP and freshwater realm on FD was assessed. The best-performing model for FRic, selected based on ANOVA, marginal R^2 and conditional R^2, included ecoregions as a random factor and temperature as a covariate. This model explained 57.5% of the variance in FRic, but TP's effect was not significant. The models for FEve and FDiv are singular, therefore no response to TP has been found. Contrary to prior research suggesting a significant effect of phosphorus on aquatic ecosystems, these findings reveal no significant relationship between TP and the FD metrics. This result challenges the expectation of decreasing FRic based on findings of decrease in species richness, highlighting that species richness does not necessarily correlate with functional diversity.

These findings suggest that phosphorus alone may not be a reliable indicator of eutrophication's impact on functional diversity. The results underscore the importance of considering additional factors, such as the nitrogen-to-phosphorus (N/P) ratio, in assessing the ecological effects of eutrophication. Consequently, phosphorus should be reconsidered as the sole metric for evaluating freshwater eutrophication in life cycle assessments, especially when using FD as impact category.

Keywords: eutrophication, functional diversity, riverine ecosystems, total phosphorus

Introduction

One-third of vertebrate species live in freshwater ecosystems, even though freshwater bodies cover less than 1% of the world's surface. However, this ecosystem is also showing the greatest global decline in species populations. The major driving forces of this have been changes in land and sea use, climate change and pollution (WWF, 2022). Freshwater ecosystems also improve human well-being, as it is used for food production, energy production and recreation. Over 50% of the human population lives within 3 km of a freshwater body (WWF, 2022).

Since the Industrial Revolution, humans have been polluting freshwater ecosystems by using increasing amounts of nitrogen (N) and phosphorus (P). Especially the use of fertilizer in modern agriculture has been a leading cause of this (Rockström et al., 2009). Currently, N and P use cross the planetary boundary both regionally and globally (Richardson et al., 2023). The increasing amount of N and P leaking into the aquatic environment over the last years has put pressure on these ecosystems. In this process, called eutrophication, the additional nutrients can lead to harmful algae blooms or excessive growth of aquatic plants (Smith, 2003). These algae blooms deplete the water of oxygen which is detrimental for aquatic organisms or may be directly toxic to fish and shellfish (Glibert & Burford, 2017). Eutrophication is found to be one of the main causes of the decline of freshwater biodiversity (Cook et al., 2018).

Changes in the freshwater ecosystem can be measured by the functional diversity of fish. Fish are a good indicator of ecosystem health because of the services they provide like control of trophic networks and regulation of nutrient cycles (Villéger et al., 2017). FD can be used as a bridge between ecosystem functioning and species loss (De Souza et al., 2013). Diversity described by FD gives a better indication of habitat degradation than species diversity metrics (Mouchet et al., 2010; Villéger et al., 2010), as the use of traits for the FD metrics creates a stronger link to ecosystem functioning than the number of species would (Ahmed et al., 2019). For functional diversity the metrics functional richness, functional evenness and functional divergence presented in Villéger et al. (2008) are used. Functional richness represents the amount of functional space filled by the community. Functional evenness describes the evenness or regularity of abundance distribution in the functional trait space. Functional divergence relates to how abundance is distributed towards the extremities of the trait space. These metrics are calculated using four continuous traits: relative head length, relative body depth, trophic level and relative growth rate. These traits were selected by Scherer et al. (2023) when analysing the effect of climate change on FD.

Eutrophication is an impact category in many life cycle assessment (LCA) methodologies, but this method currently assesses the effect on species richness and has one global effect factor. Methodologies like RECIPE 2016, CML2001, TRACI, IMPACT world+ typically use either a single eutrophication indicator for both freshwater and marine ecosystems or operate under the assumption that phosphorus is limiting in freshwater and nitrogen in marine environments (Morelli et al., 2018). Loss of biodiversity is one of the crucial concerns in LCA studies (Muralikrishna & Manickam, 2017). However, the impacts on biodiversity have mainly been focused on species richness, instead of FD (de Visser et al., 2023; Dong et al., 2023; Zhou et al., 2023). Although there have been efforts to incorporate FD metrics as

impact categories within LCA studies (Ahmed et al., 2019), biodiversity responses to external pressures vary regionally. Zhou et al. (2023) found different effects on species richness from nitrogen emission between freshwater ecoregions developed by Abell et al. (2008). Scherer et al. (2023) found varying impacts from climate change on the FD of freshwater fish between freshwater realms.

Therefore, this study aims to assess the effect of eutrophication on the functional diversity of freshwater fish across the world, with a focus on the riverine ecosystem. The effects found in the study could aid in developing effect factors which in turn could be integrated into LCA studies. This topic is part of Work Package 2 “Quantify impacts on ecosystem functions” of the BAMBOO project (*BAMBOO-Horizon*, 2023). The residence time of N and P is different for lakes and rivers, the longer residence time in lakes increases the eutrophication potential (Islam & Mostafa, 2024). Thus within the scope of this research, a selection was necessary. As Barbarossa et al., Scherer et al., Zhou et al. (2021; 2023; 2024) looked at rivers, this study will do the same. The freshwater realms will be used to compare varying effects globally (Abell et al., 2008).

Methods

Eutrophication

The analysis will utilise a space-for-time substitution approach, assuming that spatial variability reflects temporal variability. Space-for-time substitution is increasingly being used to model climate-driven changes in species distribution and richness. Blois et al. (2013) found that space-for-time substitution was ~72% as accurate as time-for-time predictions. To increase the sample size of this study it was decided to use a space-for-time substitution to test the effect of eutrophication on FD.

Indicator

Eutrophication indicators can be nutrient, oxygen or algae-related (Deksne, 2022). Nutrient-related indicators are based on the principle of nutrient limitation, where an increase in the limiting nutrient within an ecosystem triggers harmful algae blooms. The subsequent decay of these algae causes oxygen depletion, posing threats to aquatic biodiversity (Smith, 2003). Oxygen-related indicator measure the oxygen depletion. Algae-related indicators quantify harmful algae blooms by assessing chlorophyll a concentration, a green pigment found in algae (*European Environment Agency*, 2004). LCA studies generally work under the assumption that phosphorus is the limiting nutrient in freshwater and nitrogen is limiting in marine ecosystems (Morelli et al., 2018).
Total phosphorus was selected as the eutrophication indicator for this study, as phosphorus is both well-represented in the water quality databases and the most commonly used indicator for freshwater eutrophication in LCA studies.

Databases

Multiple global databases that include TP are available. GemStat (*GEMStat Database of the Global Environment Monitoring System for Freshwater*, 2020), the GLObal River Chemistry database (GLORICH) (Hartmann et al., 2019), the Surface Water Chemistry SWatCH database (Rotteveel et al., 2022) and the Global River Water Quality Archive (GRQA) (Virro et al., 2021) are all relevant options. GemStat and GLORICH encompass data on 11,926 and 17,000 stations worldwide. Within GemStat the two indicators most measured are dissolved oxygen (8284) and TP (7019), within the GLORICH database TP (10.540) has the best coverage. SWatCH and GRQA include data from GemStat and GLORICH combined with other databases. This results in 10,363 river stations in SWatCH and 42,658 stations in GRQA. Other databases, like GLEON and NSF BCO-DMO, were unsuitable due to their limited geographic scope or focus solely on lakes (*Water Quality Database Inventory – AquaWatch*, 2017).

The GRQA includes most TP measuring stations, especially in Nearctic, Palearctic and Australasia compared to other databases. However, in Indo-Malay, some GemStat stations were not included within the GRQA, even though GemStat is included in the GRQA. No reason could be found why this was the case, therefore the GRQA and GemStat were combined into one dataset for this research.

To account for the yearly variability in TP measurements, the mean concentration over the most recent three years available was calculated. There is a high variability between the number and consistency of measurements over time between stations. Some have

measurements every month while others have measurements every day for some months and no measurements for other months. Therefore, taking the average over all measurements available for the last three years would potentially create a bias towards certain periods. Since seasons impact the fate of phosphorus (De Andrade et al., 2021) this could skew the TP measurements. Therefore, the three-year mean was first calculated for the dry and wet seasons separately, the dry season lasts from May to October and the wet season from November to April (De Andrade et al., 2021). After this, the mean of these two values was taken as the TP mean for the station. This way no season is overrepresented within the mean.
In these databases measurements between 1900 and 2023 are included, the first year with more than 10 measurements is 1968. Excluding data between 1900 and 1967 only excludes 8 stations, all from the Nearctic realm. Because this realm has the most stations and excluding these years reduces the overall time variability only measurements between 1968 and 2023 are included in the analysis.

Combining GRQA and GemStat led to some stations being less than 1 km apart. This boundary was set within the GRQA boundary for potential duplicate stations (Virro et al., 2021). Therefore, all station pairs within this boundary were detected using the R-package dbscan (Hahsler et al., 2024). The station with the lowest number of measurements in the most recent three years was removed. If both stations had the same number of measurements the first alphabetical station ID was kept. The number of stations for each realm taken from GRQA, GemStat and the total number of stations can be found in Table 1, a map of all stations and corresponding TP can be found in Appendix 1.

Table 1 Number TP stations from each database included in the TP dataset, the stations listed under GemStat are limited to those not included in the GRQA.

Realm	*GRQA*	*Additional from GemStat*	*Total*
Afrotropic	470	81	551
Australasia	930	0	930
Indo-Malay	191	412	603
Nearctic	25791	22	25813
Neotropic	2570	1106	3676
Oceania	51	0	51
Palearctic	4397	847	5244

Fish occurrences

The method for compiling the fish occurrence data is based on the approach of Barbarossa et al. (2020), to which some data-cleaning steps were added.

A comprehensive database of fish occurrences was created by combining occurrences of six databases. The database as compiled by Barbarossa et al. (2020) from FishNet2, Global Biodiversity Information Facility (GBIF), Portal da Biodiversidade, SpeciesLink and the Atlas of Living Australia (ALA) was utilised, with the addition of the Amazonfish database (Jézéquel et al., 2020).

Only occurrences between 1968 and 2023 were used to minimise temporal differences between fish occurrences and the TP measurements. Because the Amazonfish database did not include year data, occurrences with literature listed as the data source were excluded, as these are expected to be outside of the scope of 1968-2023.

In addition to the data cleaning by Barbarossa et al. (2020) only observations of species in the wild were included, consequently preserved specimens, fish living in aquaria and fossil specimen were excluded. As such, FishNet2, GBIF, ALA and SpeciesLink data were filtered to have HumanObservation or MachineObservation as the basis of record. For Portal da Biodiversidade the basis of record 'Solto/mantido na natureza' was included, thereby only including observations of wild specimens. The Amazonfish database lacks basis of records, therefore other filters were applied to retain only probable live, wild sightings and exclude fossils and aquarium fish. First, occurrences originating from the other five databases were removed, as there is a basis of record available in the original database and therefore the original database was preferred. Second, occurrences associated with museums, identified by including 'muse' in the institution name, were excluded as these were presumed to be preserved specimens.

The occurrences were selected to be freshwater fish included in the dataset of species and imputed traits created by Scherer et al. (2023). This allows for using these imputed traits in the functional diversity (FD) calculations. The dataset is expected to contain all freshwater fish species of the class Actinopterygii, which represents over 99% of all extant fish species. The species names database was expanded using the species name synonyms from Fishbase (Norén, 2023) and Tedesco et al. (2017). This resulted in 4,385,728 occurrences based on the dataset and an additional 135,138 occurrences because of name synonyms. These additional occurrences were added to the database using the names included in Scherer et al. (2023).

Duplicate occurrences were removed, resulting in a final dataset comprising of 4,478,635 occurrences and 5,966 species. All species have available trait data. Table 2 presents the number of records and species for each database, a map of all occurrences can be found in Appendix 2.

Table 2 Number of fish occurrences and species from each database included in the occurrence records

	Number of records	*Number of species*
ALA	423,774	1,010
Fishnet2	57,676	1,529
GBIF	3,962,308	4,551
Portal da Biodiversidade	8,386	1,274
SpeciesLink	230	97
Amazonfish	76,243	1,638
Merged records without synonyms	4,385,728	5,954
Additional records using synonyms	135,138	219
Total occurrence records	4,478,635	5,966

River buffer regions

To calculate FD a species presence-absence matrix was needed. To accomplish this, buffer regions around the measuring stations were created. The method was based on Deksne (2022). Each buffer region needs to have: a minimum of 3 species, this is essential to calculate FD (Villéger et al., 2008); a similar length of river; no overlap with other buffers and not too much length of river covered.
A uniform river length within the buffer region ensures a similar potential of occurrences within the buffer. Including a long length of river allows for larger deviations of the TP within the buffer compared to the measurement. Data on river length and location was taken from the hydroRIVERS database (Lehner & Grill, 2013).

To reduce the computational power required and shorten the run time, stations, rivers and occurrences were derived separately for each realm.
To include only stations where eutrophication is occurring a minimum TP of 0.01 mg/L was set. Extremely low stressor values can also negatively impact species presence (Niblick, 2019), however, this is not the effect of eutrophication but rather the absence of nutrients. A minimum TP of 0.01 mg/L was used as this was found to be the optimum phosphorus concentration for fish species richness in streams in both cold and temperate climates (Azevedo et al., 2013). Trends found below this optimum were expected to be due to nutrient absence, and anything above this trend was expected to be due to nutrient excess, therefore representing eutrophication. No optimum concentration for (sub)tropical regions was found, however, in lakes the optimum TP concentration was the same for both temperate and (sub)tropical regions. Therefore the same concentration is used for (sub)tropical and temperate regions (Azevedo et al., 2013). All stations with a TP concentration below 0.01 mg/L were removed.

To create the buffers the following steps were performed:
1) A buffer area was created around each station, starting with a 10 km radius, and increasing with 5 km steps until the buffer area included at least three species occurrences. The maximum buffer size was set at a radius of 30 km. Because of the small number of buffers found within Indo-Malay and Oceania the maximum radius was set to 50 km to increase the possibility of a station being included in the analysis. The buffer enlargements of 5 km are slightly different from Deksne (Deksne, 2022), who used 10 km increments. This reduced the runtime needed for step 3.
2) The river length in each buffer was calculated. The target river length within a buffer was set as the median of the river lengths within the buffers in the realm.
3) The buffer radii were increased or decreased with 50 m increments to match the target river length. After every increment, the river length covered was calculated to see whether it fell within 1% above or below the target river length. When the buffer area was decreased to get the target river length it was verified whether there were still 3 species occurrences within the buffer, and buffers without 3 species were removed. The buffer reductions are an addition to the methods of Deksne (Deksne, 2022), who only increased buffers to match the target river length.
4) Overlapping buffers had to be removed, to achieve this, overlapping buffers were clustered together. Within each cluster, the total overlap of a buffer was calculated and the buffer with the largest total overlap was removed. This was repeated until there were no overlapping buffers left. Some buffers were too close together to calculate the overlapping

area. In these clusters, the buffers were first decreased by 80%, then by 50% and lastly the original buffers were used. In each step, buffers were removed until there was no overlap anymore before going to the next step. Between every step it was checked whether the original buffers were still overlapping. The decrease steps of 80% and 50% still gave complications within the Nearctic realm, so the steps 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20% and 10% were used.

Around none of the stations in Oceania a buffer could be created that included at least three species, therefore this realm is excluded from the following analysis. The number of buffers that remain in the other realms after each step can be found in Table 3. For Indo-Malay, there are many stations for which not enough fish occurrences were found. However, no additional database with fish occurrences within this region was available to supplement the occurrence dataset.

Table 3 Number of buffers per step of creating the buffer regions

	Afrotropic	*Australasia*	*Indo-Malay*	*Nearctic*	*Neotropic*	*Palearctic*
Total measurement stations	551	930	603	25813	3676	5244
No. stations with TP above 0.01 mg/L	538	850	550	24489	3632	5064
No. buffers with minimum 3 species occurrences	179	837	41	14086	1500	3614
No. buffers with target river length	96	747	24	8445	964	3140
No. non-overlapping buffers	36	251	11	1074	315	835

The target river length and mean buffer can be found in Table 4. Indo-Malay has a target river length that is 10x bigger than some of the other lengths, this is due to Indo-Malay having a larger maximum buffer in step 2 of buffer creation. The mean buffer in Indo-Malay is also the largest, being nearly twice the size of the follow-up Afrotropic. Australasia, Nearctic, Neotropic and Palearctic all have a similar buffer size.

Table 4 Target river length and mean buffer size in the realms

Realm	*Target river length (m)*	*Mean buffer (m)*
Afrotropic	250773.0	20709.72
Australasia	142391.6	15079.32
Indo-Malay	1423021.7	39322.73
Nearctic	184824.5	17375.21
Neotropic	254900.9	17371.17
Palearctic	136670.7	16523.13

Functional diversity

The functional diversity metrics developed by Villéger et al. (2008) were calculated using the R-package FD (Laliberté et al., 2023). During the calculation, the option to standardise functional richness was activated, thereby scaling the functional richness to the global functional richness. This creates global functional richness values between 0 and 1. Because the scaling is done globally, the absolute functional richness values can better be compared between realms. Functional evenness and functional divergence are inherently between 0 and 1 and therefore do not need to be standardised.

These functional diversity metrics are calculated using the four continuous traits: relative head length, relative body depth, trophic level and relative growth rate. These traits were selected and imputed by Scherer et al. (2023) to have broad coverage of ecological functions and a weak correlation among them. The traits cover five ecological functions: food acquisition, locomotion, nutrient processing, reproduction and predator-prey interaction. The use of the imputed traits showed significant differences in the results as opposed to dropping species with missing trait values. Dropping the species with missing traits led to an overestimation of functional diversity loss (Scherer et al., 2023). Therefore, the imputed traits were used, thereby optimizing the number of species and spatial coverage included in the analysis.

To calculate the FD metrics a presence-absence matrix was used due to the lack of species abundance data. This presence-absence matrix was created using the buffers generated around the TP measurement stations. On average there are 16 species per buffer, with the median at 10 species per buffer.

Outliers

Outliers were identified using the Minimum Covariant Determinant (MCD) approach with a breakdown point of 0.25, as recommended by Leys et al. (2019). The variables used in the outlier detection were FRic, FEve, FDiv, and logTP. The outlier analysis was done using the R-package Routliers (Delacre & Klein, 2019), this led to the detection of 217 outliers. A visualisation of these outliers can be found in Appendix 2.

Linear Mixed Model

The total phosphorus values were log-transformed and the square root of functional richness was taken to normalise the distribution. The normal and log-transformed distribution of TP were visually analysed using the R package fitdistrplus (Delignette-Muller et al., 2024). The total phosphorus values were multiplied by 1000 to avoid negative log values, thereby transforming the total phosphorus from mg/L to g/L. The normal and square root distributions of FRic were visually analysed using the same R-package.

Various linear mixed models (LMMs) were analysed using an ANOVA test. All LMMs used the optimizer bobyqa, since this optimizer allowed for all the models to converge. The freshwater ecoregions from Abell et al. (2008) were included to account for the spatial variability. Different versions of the model were run: 1) with only realm as random intercept; 2) realm as a correlated random intercept; 3) realm as uncorrelated random intercept and slope; 4) ecoregion as correlated random intercept and slope; 5) realm and ecoregion as correlated random intercept and slope; 6) correlated random intercept and slope for realm

with ecoregion nested; and 7) realm as correlated random intercept and slope and ecoregion as random intercept. These models were conducted using the lme4 R package (Bates et al., 2017). These 7 models were compared using an ANOVA test, and model 7 was selected as the best model. This model had the lowest AIC/BIC scores and was significantly better than the simpler model 4, both with and without outliers. The output of the ANOVA test can be found in Table 6, the R^2 values in Appendix 6, the ANOVA output without outliers in Appendix 7, and the R^2 values without outliers in Appendix 8. Model 7 still had relatively low marginal R^2 and conditional R^2, namely 0.010 and 0.403 respectively. Therefore, it was tested whether the addition of more variables would improve the model further.

Time difference

Time difference between the TP measurements and the species occurrences could give a skewed representation of the FD. Therefore, the time difference was included as a possible fixed effect. The time difference between the occurrences and the TP measurements was calculated for each buffer. The differences between the most recent occurrence per species and the average year of the TP measurements were used to calculate the overall mean time difference for the buffer. There are 121 stations without year data for the occurrences, therefore no time difference could be calculated. These stations are lost when using the time difference as a fixed effect.

Number of species

With more than two traits, the number of species included in the FD calculations should be ideally $2^{(\text{number of traits})}$ in order to potentially fill the whole functional space (Villéger et al., 2008). This is not the case for 1708 stations, to mediate this the number of species within each buffer was included as a possible weighting factor within the LMMs.

Covariates

To minimise the effect of other environmental factors on FD, potential covariates were also included in the LMMs. The three covariates chosen are the water temperature, total dissolved solids (TDS) to represent salinity pollution, and fecal coliform (FC) as an indicator of pathogen pollution. This data was taken from the DynQual model database (Jones et al., 2023). This model has data for every month between 1980 and 2019. For each station, the mean year in which the TP measurements were taken was used as the year to extract the data from. If the average year was after 2019, covariate data from 2019 was used, when the year was before 1980 the covariate data from 1980 was used. For the indicator year the average temperature, TDS and FC were calculated and used as the covariate data.

Weights were calculated using the WeightIt package (Greifer, 2024). Propensity scores were computed for the environmental factors, and the balance of these factors was assessed using the Spearman correlation with a threshold of 0.1. This analysis indicated that temperature was balanced whereas TDS and FC were not. Because temperature was the only balanced covariate the weights were also calculated based only on temperature. Therefore, there are two potential weights, one based on only temperature and one based on a combination of temperature, TDS and FC. There were 23 stations without temperature data and 283 stations without data for temperature, TDS or FC.

The data distribution of temperature, TDS and FC was analysed. Temperature maintained a normal distribution, while TDS and FC showed a better distribution when transformed using the square root. A log transformation was not feasible since some values were zero. As separate model variations, these covariates were included in the model as potential fixed effects, considering both the inclusion of only temperature and the combination of temperature, sqrtTDS, and sqrtFC.

Model comparison

Different combinations of these potential fixed effects and weights were added to model 7, which led to 15 additional potential models. The inclusion of the different factors is summarised in Table 5. To perform an ANOVA test all models had to be run using the same dataset (Bates et al., 2003). Therefore, a dataset was created with only the stations that had values for all variables. This dataset included 2121 stations. Potential outliers identified using the complete dataset were also considered outliers within the ANOVA dataset. Based on the ANOVA test model 7.11 scored best on AIC, closely followed by 7.9 and 7.10. Model 7.10 scored best on BIC, closely followed by 7.11 and 7.8. The same was found for the analysis without outliers. Model 7.10 has the highest marginal R^2 value, followed by model 7.8. Model 7.8 has the highest conditional R^2 value, closely followed by 7.10. This led to model 7.10 (sqrtFRic ~ log_TP + (log_TP|realm) + (1|ecoregion_id) + temperature) being selected as the best model. This model was selected using sqrtFRic, the same model was used to analyse FEve and FDiv.

Table 5 Different options of improving the base model (sqrtFRic ~ log_TP + (log_TP|realm) + (1|ecoregion_id)). Time is the mean time difference between the last occurrence of species within a buffer and the mean year of TP measurements. TDS: Total Dissolved Solids, FC: fecal coliform, nbsp: number of species

	fixed effects				weights		
model	time difference	temperature	sqrtTDS	sqrtFC	nbsp	temp, TDS, FC	temperature
model7.1	x						
model7.2					x		
model7.3	x				x		
model7.4						x	
model7.5	x					x	
model7.6							x
model7.7	x						x
model7.8		x	x	x			
model7.9	x	x	x	x			
model7.10		x					
model7.11	x	x					
model7.12		x	x	x	x		
model7.13	x	x	x	x	x		
model7.14		x			x		
model7.15	x	x			x		

Results

Global distribution

There are 2498 buffers included in the final analysis, covering 6 realms and 71 ecoregions. In Figure 1 the buffers can be seen with the corresponding sqrtFRic. The maps of FDiv and FEve can be found in Appendix 4 and 5. There is a high density of stations in Southeast USA, Mexico, western Europe, New Zealand and the south-eastern coast of Australia, while in Eastern Europe, Asia and parts of Africa there are barely any stations. Most regions have a combination of high and low sqrtFRic, except for Brazil where most buffers have a higher sqrtFRic.

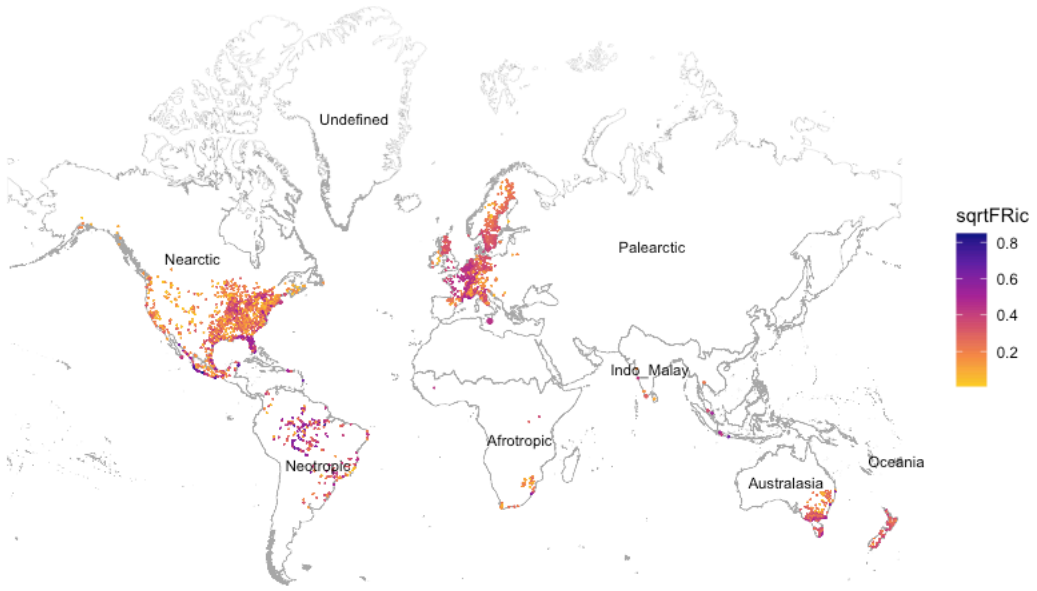

Figure 1 Global coverage of buffers and the square root of the functional richness

Model performance

Base model

The output of the ANOVA test on the 7 potential base models can be found in Table 6. Model 7 has the lowest AIC and BIC value, therefore the balance between complexity and explanatory power is optimised. The Pr(>Chisq) indicates that model 7 is significantly better than the simpler model 4. Model 5 and 6 are close in regards to the AIC and BIC values, but model 5 is not shown to be significantly better than model 7. The same remains true when the ANOVA test is run without outliers, these results can be found in Appendix 7. The marginal R^2 and conditional R^2 of the 7 base models with and without outliers can be found in Appendix 6 and Appendix 8 respectively.

Table 6 Results of ANOVA test of the 7 potential base models. The order is determined by the ANOVA function, the simplest models first and the most complex model last. The abbreviations are as follows: npar: number of parameters, AIC: Akaike Information Criterion, BIC: Bayesian Information Criterion, logLik: log-likelihood, Chisq: Chi-Squared statistic, DF: Degrees of Freedom, Pr(>Chisq): P-value of the Chi-squared test.

	npar	*AIC*	*BIC*	*logLik*	*deviance*	*Chisq*	*Df*	*Pr(>Chisq)*
model1	4	-2777.573	-2754.934	1392.786	-2785.573	NA	NA	NA
model3	5	-2794.724	-2766.426	1402.362	-2804.724	19.1516	1	1.21E-05
model2	6	-2802.938	-2768.980	1407.469	-2814.938	10.2135	1	1.39E-03
model4	6	-3046.332	-3012.374	1529.166	-3058.332	243.3939	0	NA
model7	7	-3076.537	-3036.919	1545.268	-3090.537	32.2049	1	1.39E-08
model5	9	-3074.938	-3024.002	1546.469	-3092.938	2.4017	2	3.01E-01
model6	9	-3074.938	-3024.002	1546.469	-3092.938	0	0	NA

Complete model

Model 7.10 was chosen based on the ANOVA results shown in Table 7 and the R^2 values shown in Table 8. There is not one model that has the best AIC and BIC scores, rather the top 3 models are different between the two scores. Regarding the AIC score the top three models are 7.11, 7.9 and 7.10, regarding the BIC score the top three models are 7.10, 7.11 and 7.8. Because models 7.10 and 7.11 score best for one of the scores and are in the top three of both scores, the R^2 values of these two models have been compared. Model 7.10 has the highest marginal R^2 and conditional R^2, therefore this is considered the best model. The marginal R^2 is 0.277, indicating that logTP and temperature combined explain 27.7% of the variance. The conditional R^2 is 0.575, showing that including the random factors realm and ecoregion considerably increases the explained variance to 57.5%.
The same model was found to be the best when the models were compared without potential outliers, these results can be found in Appendix 9 and Appendix 10.

Table 7 Results of ANOVA test of the 16 potential models. The order is determined by the ANOVA function, the simplest models first and the most complex model last. The abbreviations are as follows: npar: number of parameters, AIC: Akaike Information Criterion, BIC: Bayesian Information Criterion, logLik: log-likelihood, Chisq: Chi-Squared statistic, DF: Degrees of Freedom, Pr(>Chisq): P-value of the Chi-squared test.

	npar	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
model7	7	-3076.537	-3036.919	1545.268	-3090.537	NA	NA	NA
model7.2	7	-2672.818	-2633.201	1343.409	-2686.818	0	0	NA
model7.4	7	-3044.843	-3005.225	1529.421	-3058.843	372.024	0	NA
model7.6	7	-3049.531	-3009.913	1531.765	-3063.531	4.688	0	NA
model7.1	8	-3088.577	-3043.300	1552.289	-3104.577	41.047	1	1.49E-10
model7.3	8	-2723.765	-2678.488	1369.883	-2739.765	0	0	NA
model7.5	8	-3057.702	-3012.425	1536.851	-3073.702	333.937	0	NA
model7.7	8	-3062.889	-3017.612	1539.445	-3078.889	5.187	0	NA
model7.10	8	-3270.747	-3225.470	1643.374	-3286.747	207.858	0	NA
model7.14	8	-2984.915	-2939.638	1500.458	-3000.915	0	0	NA
model7.11	9	-3275.182	-3224.246	1646.591	-3293.182	292.267	1	1.59E-65
model7.15	9	-3012.521	-2961.584	1515.260	-3030.521	0	0	NA
model7.8	10	-3269.974	-3213.378	1644.987	-3289.974	259.454	1	2.26E-58
model7.12	10	-2989.669	-2933.073	1504.835	-3009.669	0	0	NA
model7.9	11	-3274.341	-3212.085	1648.170	-3296.341	286.671	1	2.64E-64
model7.13	11	-3017.739	-2955.483	1519.870	-3039.739	0	0	NA

Table 8 The Marginal R^2 and Conditional R^2 of the complete model and P-value of the logTP parameter in the 16 potential models

model	Marginal R^2	Conditional R^2	P-value logTP
model7	0.002	0.385	0.442
model7.1	0.007	0.401	0.522
model7.2	0.001	0.079	0.001
model7.3	0.004	0.108	0.009
model7.4	0	0.401	0.898
model7.5	0.005	0.439	0.968
model7.6	0.001	0.381	0.632
model7.7	0.006	0.417	0.823
model7.8	0.275	0.578	0.936
model7.9	0.235	0.56	0.918
model7.10	0.277	0.575	0.821
model7.11	0.24	0.554	0.883
model7.12	0.083	0.154	0.45
model7.13	0.075	0.159	0.587
model7.14	0.084	0.157	0.297
model7.15	0.077	0.16	0.419

Functional Diversity

Model 7.10 was applied to the complete dataset to predict the FD. The performance metrics of these models are detailed in Table 9. The models for FEve, FDiv, and all models without outliers are singular, therefore, limited conclusions can be drawn from them. Additionally, the p-values for sqrtFRic are not significant.

Table 9 Model performance metrics using model 7.10 for all three FD metrics with all data points and without potential outliers. The models for FEve, FDiv and all three models without outliers are singular.

	sqrtFRic	*FEve*	*FDiv*	*sqrtFRic without outliers*	*FEve without outliers*	*FDiv without outliers*
P-value logTP intercept	0.658	<0.001	<0.001	0.173	<0.001	<0.001
P-value overall logTP slope	0.821	0.747	0.433	0.005	0.004	0.111
Marginal R^2	0.277	0.001	0.002	0.252	0.135	0.012
Conditional R^2	0.575	-	-	-	-	-

The scatter plots in Figure 2 show the distribution of the FD metrics against logTP. No clear variation between realms can be discerned. The sqrtFRic values fall mainly between 0 and 0.5, the FEve between 0.75 and 0.9 and the FDiv between 0.7 and 0.8. Since the model did not show a significant effect of logTP on FD and the models are singular for FEve and FDiv no regression lines are shown. The slopes of the models with all measurements and without outliers can be found in Appendix 10 – Appendix 15.

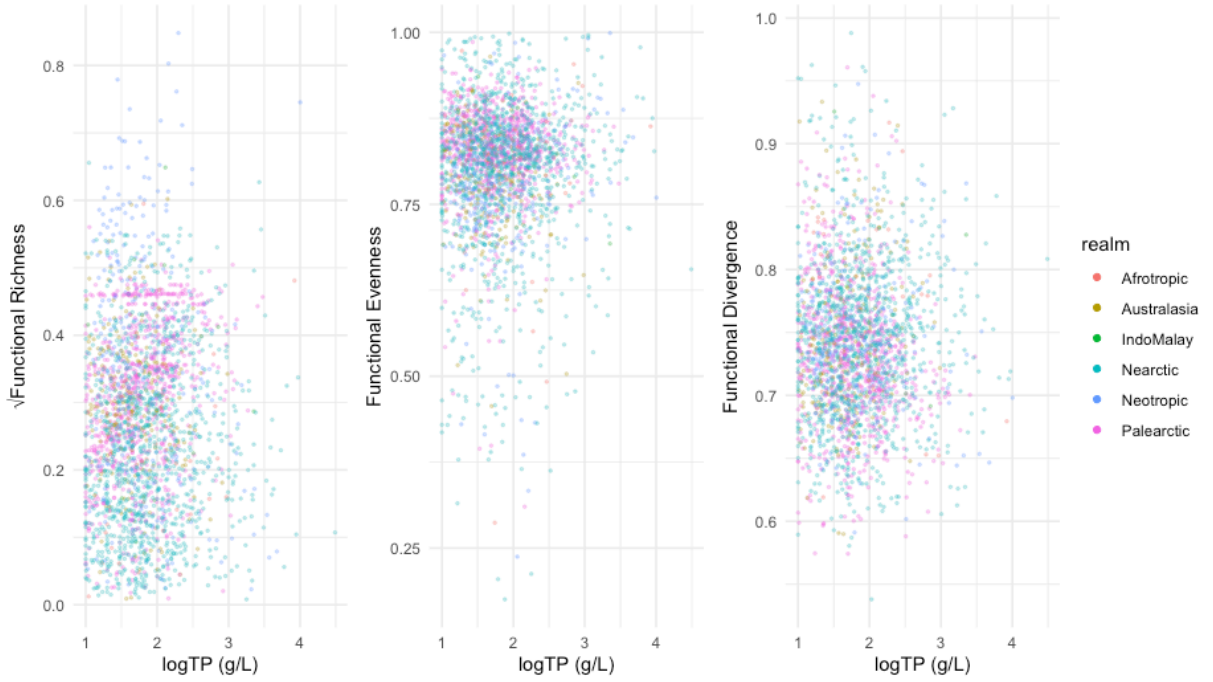

Figure 2 Scatterplot of FD metrics and logTP (g/L)

Discussion

The results show no effect of phosphorus on the functional diversity of freshwater fish globally. The effect from phosphorus was insignificant for functional richness, and the model is singular for functional evenness and functional divergence. Therefore, this model cannot give a representation of the effect of eutrophication. As the datasets are relatively large, starting with 36,868 TP stations and 4,478,635 fish occurrences worldwide and running the LMMs with 2498 stations these findings are deemed reliable. So even though an effect was expected based on the findings by Deksne (2022), most likely there is no generic effect of TP on FD. Potential reasons are listed below.

The N/P ratio could have more influence on eutrophication than phosphorus alone. Currently, it is assumed that phosphorus is the limiting nutrient in freshwater (Morelli et al., 2018), and therefore increasing phosphorus would increase eutrophication, which is why TP was chosen as the eutrophication indicator. However, the N/P ratio is also an important factor regarding eutrophication, and including this could express which nutrient is limiting, for example using the Redfield ratio (Yang et al., 2008). Studies in varying settings have already found the importance of the N/P ratio on eutrophication. Diatta et al. (2020) found that nitrogen was a more powerful eutrophication-regulating factor than phosphorus, especially at higher P levels. They put various N and P concentrations in distilled water, tap water and lacustrine water and monitored algal blooms to measure the eutrophication. In Rawapaning lake, Indonesia the development of algal biomass is also determined by the N/P ratio (Piranti et al., 2018). In this lake, the algal biomass development had no relationship with total nitrogen and a weak relationship with TP, but there was a significant relationship to N/P ratio. Even though in marine water nitrogen is assumed to be limiting (Morelli et al., 2018), the N/P ratio also plays a role in the growth of chlorophyll-a (Maslukah et al., 2023). These three studies show the importance of N/P ratio on eutrophication, besides the TP level.

Another reason could be that although species richness is affected by phosphorus (Azevedo et al., 2013; Deksne, 2022; Zhou et al., 2024), this cannot be directly generalised to functional richness and functional diversity as a whole. Using species richness could overestimate the effect of P on the ecosystem. This is supported by the findings of Deksne (2022), who found a greater effect of TP on species richness than on FRic, and FD as a whole. The inability to generalise this finding is also evident in Rodrigues-Filho et al. (2017), who found that the functional richness of fish in headwater streams is mainly influenced by environmental conditions, compared to taxonomic diversity. Around 30% of the variance in FRic could be explained by environmental factors, while 0% was explained by taxonomic diversity. Consequently, effects in species richness don't have to be reflected in effects in FRic.

There are some limitations to the methods which could be improved. First, the model selection was based solely on FRic, without integrating FEve and FDiv, which resulted in singular models for FEve and FDiv. This raises concern that the model is overfitted, and simplifying the model could avoid the singularity (Bates et al., 2003). Since most of the models 7-7.15 are singular for both FEve and FDiv, the problem likely originates in the base model 7, and this would be a good place to start simplifying. Second, the optimum TP

concentration of 0.01 mg/L was found for species richness (Azevedo et al., 2013), however this is not necessarily the same optimum for TP. Third, it could be checked whether increasing the minimum number of species per buffer or reducing the number of traits changes the result. Optimally, the number of species included in the FD calculation should be $2^{(\text{number of traits})}$ (Villéger et al., 2008). To follow this, either the minimum number of species should be increased to 16, or the number of traits needs to be decreased. However, both options result in information loss. Lastly, the current covariate analysis is opportunistic, only considering three covariates and one database. Including a more thorough analysis of potential covariates and databases, as well as a correlation test between the covariates enhances the quality of the covariate analysis.

Based on the results it should be reconsidered whether using phosphorus as the main indicator for freshwater eutrophication is justified, especially in LCA studies using FD metrics as impact categories. While it is recommended to use FD in the biodiversity metrics of LCA (Ahmed et al., 2019), no effect of TP on FD has been found. Therefore, creating effect factors based solely on TP does not reflect any impact on river ecosystem functions imposed by eutrophication. Further research could expand on the developed methods, by incorporating other eutrophication indicators and adapting the methods to be used for lake and marine ecosystems. Extending the analysis to these ecosystems would give a more holistic view of the effect of eutrophication.

Conclusion

This analysis finds no effect of phosphorus on functional diversity of fish in freshwater rivers. The model that best described the effect of TP on FRic per realm also included ecoregion as a random factor and temperature as a covariate. This model describes 57.5% of the variance in FRic but TP has no significant effect. The models for FEve and FDiv are singular. Because of the scale of the datasets, these results are deemed trustworthy. Therefore, to see the effect of eutrophication on the functional diversity of fish in freshwater rivers, other indicators, like the N/P ratio, need to be examined. In addition, it should be reconsidered whether using P as a eutrophication indicator in freshwater in LCA studies is justified.

Declaration use of AI

During this project the openAI software ChatGPT has been used. It has mainly been used to explain previously written R-code and debug my code. Additionally, small parts of code have been written by ChatGPT which have first been examined to work properly before incorporating it into the methods. While writing the report, ChatGPT has assisted by rewriting some unclear sentences.

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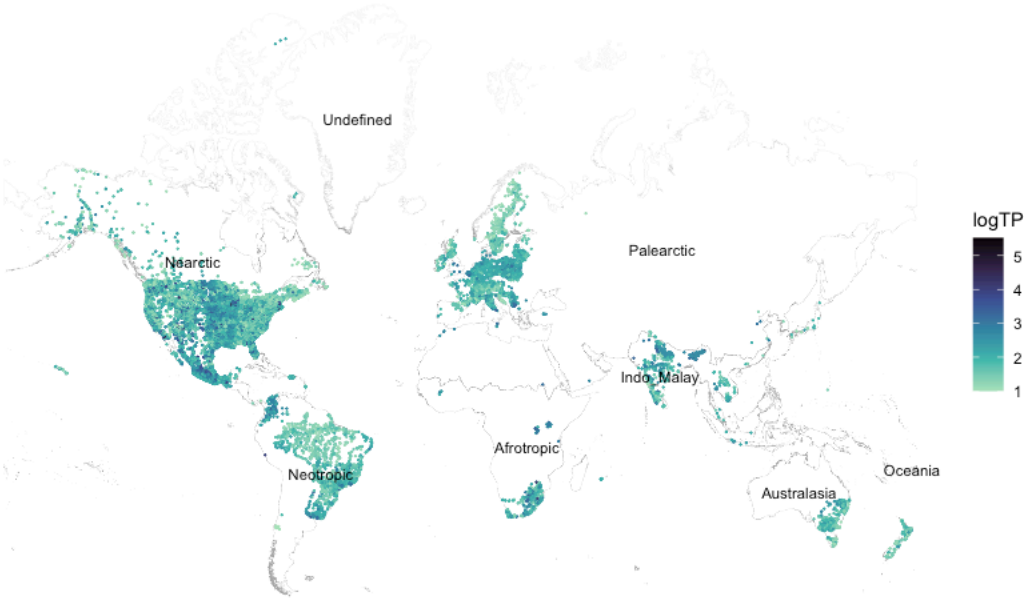
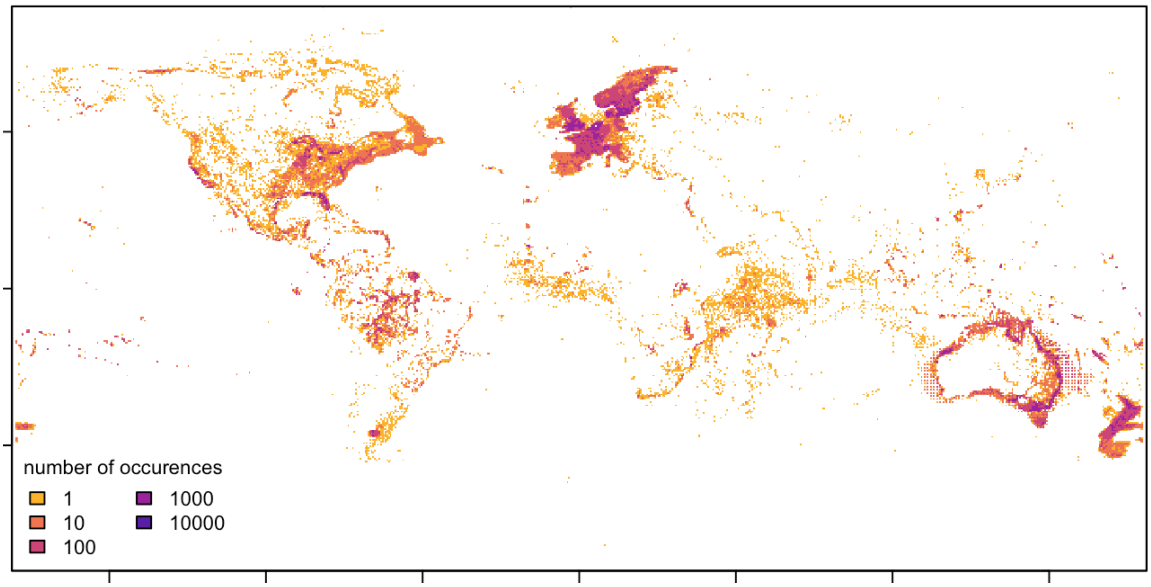
Appendix

TP stations



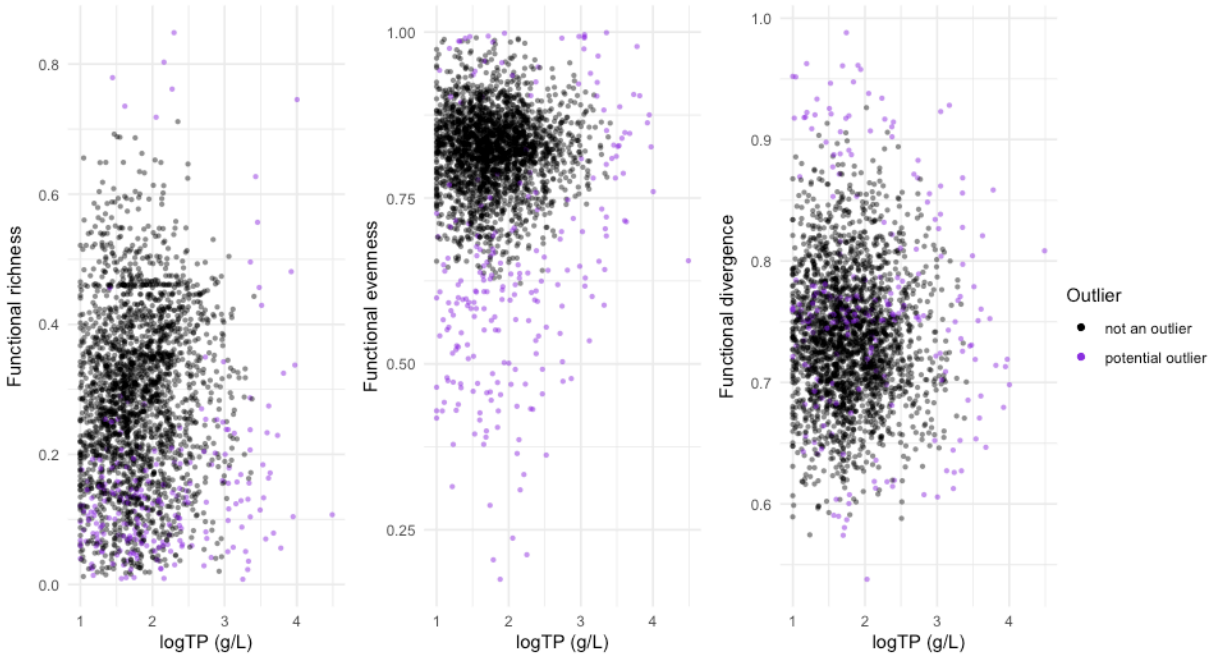
Appendix 1 All TP stations part of the dataset created by combining the GRQA and GemStat. The log of total phosphorus is g/L is shown.

Fish occurrences



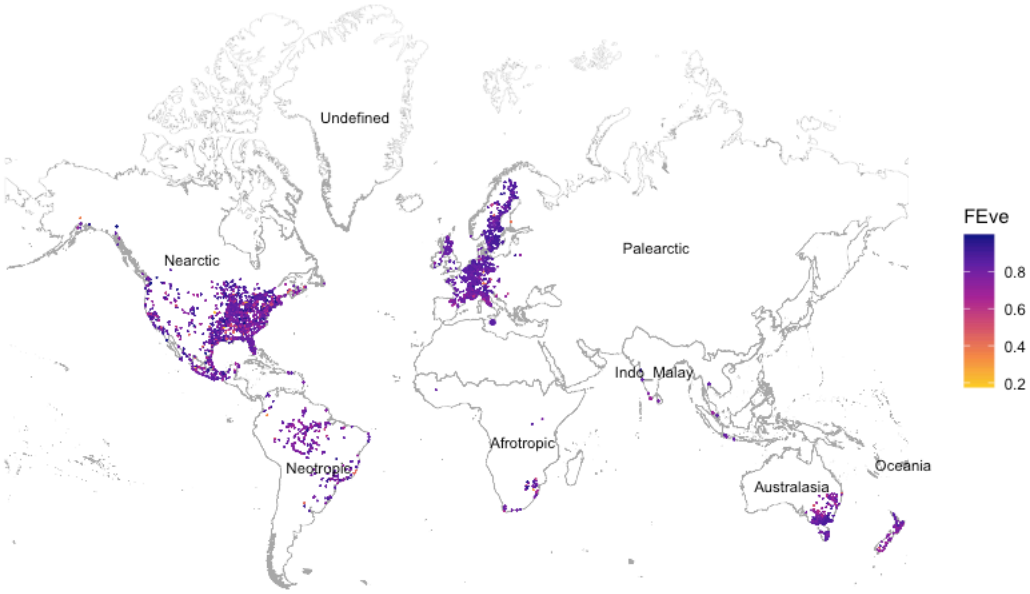
Appendix 2 All fish occurrences part of the dataset created by combining Fishnet2, GBIF, Portal da Biodiversidade, SpeciesLink and Amazonfish.

Outliers

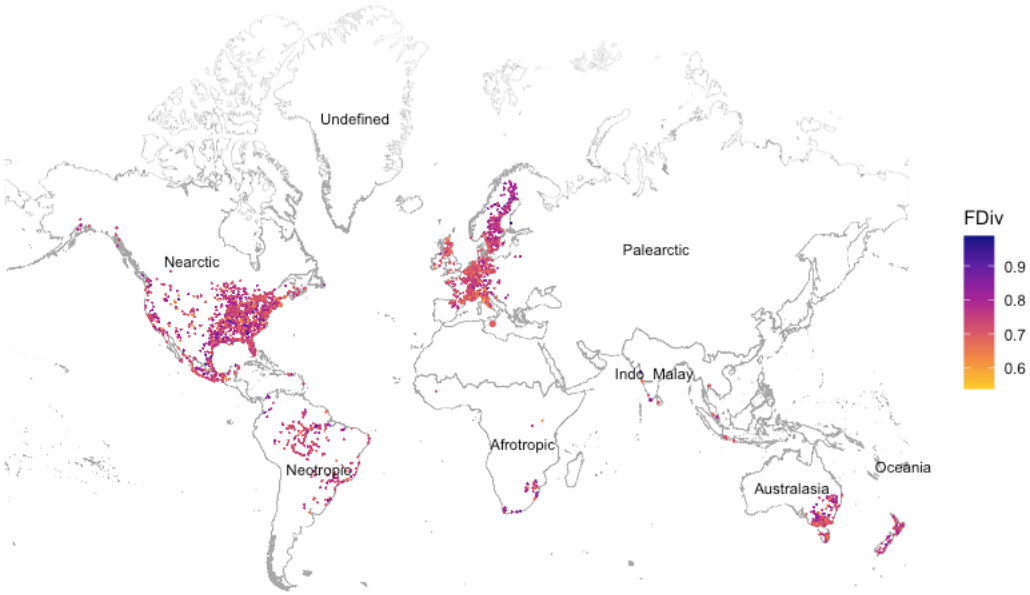


Appendix 3 Visualisation of the potential outliers found using the MCD approach with breakdown point 0.25

Global distribution FEve and FDiv



Appendix 4 Global distribution of buffers and the functional evenness



Appendix 5 Global distribution of buffers and the functional divergence

Model performance

Base model

Appendix 6 The Marginal R2 and Conditional R2 of the complete model and P-value of the logTP parameter in the base models.

model	*Marginal R^2*	*Conditional R^2*	*P-value logTP*
model1	0.006	0.154	<0.001
model2	0.001	0.159	0.679
model3	0.001	0.485	0.636
model4	0.002	0.406	0.041
model5	0.002	0.388	0.452
model6	0.002	0.388	0.452
model7	0.002	0.385	0.442

Appendix 7 Results of ANOVA test of the 7 potential base models without outliers. The order is determined by the ANOVA function, the simplest models first and the most complex model last. The abbreviations are as follows: npar: number of parameters, AIC: Akaike Information Criterion, BIC: Bayesian Information Criterion, logLik: log-likelihood, Chisq: Chi-Squared statistic, DF: Degrees of Freedom, Pr(>Chisq): P-value of the Chi-squared test. Model 5NO and model 6NO are singular

	npar	*AIC*	*BIC*	*logLik*	*deviance*	*Chisq*	*Df*	*Pr(>Chisq)*
model1NO	4	-2733.419	-2711.172	1370.709	-2741.419	NA	NA	NA
model3NO	5	-2745.464	-2717.655	1377.732	-2755.464	14.045	1	1.79E-04
model2NO	6	-2753.892	-2720.522	1382.946	-2765.892	10.428	1	1.24E-03
model4NO	6	-2964.155	-2930.785	1488.078	-2976.155	210.263	0	NA
model7NO	7	-2990.987	-2952.055	1502.493	-3004.987	28.832	1	7.89E-08
model5NO	9	-2988.805	-2938.750	1503.402	-3006.805	1.818	2	4.03E-01
model6NO	9	-2988.805	-2938.750	1503.402	-3006.805	0	0	NA

Appendix 8 The Marginal R2 and Conditional R2 of the complete model and P-value of the logTP parameter in the base models without outliers. Model 5NO and model 6NO are singular.

model	*Marginal R^2*	*Conditional R^2*	*P-value logTP*
model1NO	0.011	0.173	<0.001
model2NO	0.002	0.198	0.617
model3NO	0.001	0.479	0.531
model4NO	0.006	0.412	0.001
model5NO	0.005		0.294
model6NO	0.005		0.294
model7NO	0.003	0.4	0.273

Complete model

Appendix 9 Results of ANOVA test of the 16 potential models without outliers. The order is determined by the ANOVA function, the simplest models first and the most complex model last. The abbreviations are as follows: npar: number of parameters, AIC: Akaike Information Criterion, BIC: Bayesian Information Criterion, logLik: log-likelihood, Chisq: Chi-Squared statistic, DF: Degrees of Freedom, Pr(>Chisq): P-value of the Chi-squared test.

	npar	*AIC*	*BIC*	*logLik*	*deviance*	*Chisq*	*Df*	*Pr(>Chisq)*
model7NO	7	-2990.987	-2952.055	1502.493	-3004.987	NA	NA	NA
model7.2NO	7	-2820.687	-2781.755	1417.343	-2834.687	0	0	NA
model7.4NO	7	-2951.796	-2912.864	1482.898	-2965.796	131.109	0	NA
model7.6NO	7	-2954.495	-2915.563	1484.247	-2968.495	2.699	0	NA
model7.1NO	8	-3001.717	-2957.224	1508.859	-3017.717	49.223	1	2.29E-12
model7.3NO	8	-2854.745	-2810.252	1435.373	-2870.745	0	0	NA
model7.5NO	8	-2962.476	-2917.983	1489.238	-2978.476	107.731	0	NA
model7.7NO	8	-2965.718	-2921.225	1490.859	-2981.718	3.242	0	NA
model7.10NO	8	-3187.840	-3143.346	1601.920	-3203.840	222.121	0	NA
model7.14NO	8	-3115.403	-3070.910	1565.702	-3131.403	0	0	NA
model7.11NO	9	-3191.551	-3141.497	1604.776	-3209.551	78.148	1	9.56E-19
model7.15NO	9	-3131.131	-3081.076	1574.565	-3149.131	0	0	NA
model7.8NO	10	-3185.671	-3130.054	1602.835	-3205.671	56.540	1	5.51E-14
model7.12NO	10	-3120.100	-3064.484	1570.050	-3140.100	0	0	NA
model7.9NO	11	-3189.331	-3128.153	1605.665	-3211.331	71.230	1	3.18E-17
model7.13NO	11	-3135.985	-3074.807	1578.992	-3157.985	0	0	NA

Appendix 10 The Marginal R2 and Conditional R2 of the complete model and P-value of the logTP parameter in the 16 potential models without outliers.

model	*Marginal R^2*	*Conditional R^2*	*P-value logTP*
model7NO	0.003	0.4	0.273
model7.1NO	0.008	0.409	0.467
model7.2NO	0	0.073	0.011
model7.3NO	0.003	0.093	0.359
model7.4NO	0	0.416	0.626
model7.5NO	0.005	0.447	0.975
model7.6NO	0.002	0.401	0.289
model7.7NO	0.007	0.429	0.609
model7.8NO	0.298	0.6	0.935
model7.9NO	0.251	0.576	0.905
model7.10NO	0.299	0.601	0.608
model7.11NO	0.256	0.575	0.785
model7.12NO	0.078	0.144	0.268
model7.13NO	0.073	0.153	0.931
model7.14NO	0.078	0.145	0.15
model7.15NO	0.074	0.151	0.754

Slopes

Functional richness

Appendix 11 Slopes of the effect of logTP (g/L) on FRic

	Afrotropic	*Australasia*	*IndoMalay*	*Nearctic*	*Neotropic*	*Palearctic*	*Average*
model7	0.0376	-0.0014	-0.0023	0.0112	-0.0229	0.0508	0.0122
model7.1	0.0336	-0.0021	-0.0057	0.0106	-0.026	0.0512	0.0103
model7.2	0.0221	0.0255	0.0271	0.0123	0.0302	0.0286	0.0243
model7.3	0.0206	0.0206	0.0179	0.0118	0.0183	0.0316	0.0201
model7.4	0.0275	-0.0065	-0.0157	4.00E-04	-0.0317	0.0387	0.0021
model7.5	0.0267	-0.0081	-0.023	1.00E-04	-0.0398	0.0397	-0.0007
model7.6	0.0328	-0.0025	-0.0093	0.003	-0.0244	0.0458	0.0076
model7.7	0.0292	-0.0038	-0.0173	0.0028	-0.0349	0.0468	0.0038
model7.8	0.0181	-0.0254	1.00E-04	-0.0087	-0.0127	0.0222	-0.0011
model7.9	0.0184	-0.0264	-3.00E-04	-0.0086	-0.0149	0.0233	-0.0014
model7.10	0.028	-0.0266	0.0037	-0.0049	-0.0105	0.0292	0.0032
model7.11	0.0254	-0.0265	0.0022	-0.0047	-0.0142	0.0302	0.0021
model7.12	0.0244	-0.0172	0.0141	-0.0055	0.0306	0.0089	0.0092
model7.13	0.0141	-0.0125	0.0067	-0.0045	0.0172	0.01	0.0052
model7.14	0.0221	-0.0095	0.0151	-0.0031	0.0248	0.0143	0.0106
model7.15	0.0111	-0.0051	0.007	-0.0017	0.0122	0.0157	0.0065

Appendix 12 Slopes of the effect of logTP (g/L) on FRic without outliers

	Afrotropic	*Australasia*	*IndoMalay*	*Nearctic*	*Neotropic*	*Palearctic*	*Average*
model7NO	0.0339	-4.00E-04	-0.0012	0.0221	-0.0086	0.0486	0.0157
model7.1NO	0.0303	-0.0036	-0.0112	0.021	-0.0173	0.0494	0.0114
model7.2NO	0.0216	0.0186	0.0145	0.0182	0.0113	0.0289	0.0189
model7.3NO	0.0176	0.0127	-0.0076	0.0168	-0.0115	0.0326	0.0101
model7.4NO	0.021	-0.0071	-0.0147	0.018	-0.0121	0.035	0.0067
model7.5NO	0.0189	-0.0131	-0.0314	0.0168	-0.0248	0.0365	0.0005
model7.6NO	0.0321	2.00E-04	-0.0022	0.0167	-0.0047	0.0432	0.0142
model7.7NO	0.0293	-0.0045	-0.0171	0.0156	-0.02	0.0446	0.0080
model7.8NO	0.0142	-0.0361	0.0039	0.0039	-4.00E-04	0.0215	0.0012
model7.9NO	0.0101	-0.0379	-8.00E-04	0.0036	-0.0088	0.023	-0.0018
model7.10NO	0.0259	-0.0271	0.0085	0.0044	0.0028	0.0278	0.0071
model7.11NO	0.0208	-0.028	0.0034	0.0041	-0.0065	0.0293	0.0039
model7.12NO	0.0125	-0.0018	0.0091	0.0061	0.0124	0.0063	0.0074
model7.13NO	-0.002	-0.0031	2.00E-04	0.0018	-0.0026	0.0095	0.0006
model7.14NO	0.007	0.0079	0.0075	0.0065	0.0082	0.012	0.0082
model7.15NO	-0.0048	0.0049	-8.00E-04	0.0028	-0.0041	0.0157	0.0023

Functional evenness

Appendix 13 Slopes of the effect of logTP (g/L) on FEve

	Afrotropic	*Australasia*	*IndoMalay*	*Nearctic*	*Neotropic*	*Palearctic*	*Average*	*Singular*
model7	0.0051	-0.0156	-0.0032	0.0058	0.0135	0.0099	0.0026	Yes
model7.1	0.005	-0.0173	-0.0038	0.0061	0.017	0.0112	0.0030	Yes
model7.2	0.0048	-0.0147	-0.0027	0.0031	-0.0037	0.0113	-0.0003	No
model7.3	0.0059	-0.0154	-0.0032	0.0031	0.001	0.0114	0.0005	No
model7.4	0.0038	0.0041	0.0041	0.0034	0.003	0.0048	0.0039	Yes
model7.5	0.0048	0.0042	0.0047	0.0036	0.0051	0.0058	0.0047	Yes
model7.6	4.00E-04	-0.0121	-0.0023	0.0021	0.0055	0.0092	0.0005	Yes
model7.7	8.00E-04	-0.0134	-0.002	0.0024	0.0103	0.0105	0.0014	Yes
model7.8	0.0057	0.0057	0.0057	0.0057	0.0057	0.0057	0.0057	Yes
model7.9	0.0061	0.0061	0.0061	0.0061	0.0061	0.0061	0.0061	Yes
model7.10	0.0049	-0.0153	-0.0029	0.0066	0.0116	0.0109	0.0026	Yes
model7.11	0.0049	-0.0177	-0.0039	0.0064	0.0167	0.0116	0.0030	Yes
model7.12	0.0043	0.0016	0.0036	0.0029	-0.0039	0.0078	0.0027	Yes
model7.13	0.0045	0.0023	0.004	0.0034	-6.00E-04	0.0076	0.0035	Yes
model7.14	0.0034	5.00E-04	0.0038	0.0038	-0.0029	0.0077	0.0027	Yes
model7.15	0.0063	-0.0154	-0.003	0.0035	0.0012	0.0117	0.0007	No

Appendix 14 Slopes of the effect of logTP (g/L) on FEve without outlliers

	Afrotropic	*Australasia*	*IndoMalay*	*Nearctic*	*Neotropic*	*Palearctic*	*Average*	*Singular*
model7NO	0.0032	-0.0031	0.0022	0.0017	0.0032	0.0076	0.0025	No
model7.1NO	0.0039	-0.0014	0.0033	0.0016	0.005	0.0077	0.0034	Yes
model7.2NO	0.0021	-0.0095	3.00E-04	4.00E-04	0.0026	0.0112	0.0012	No
model7.3NO	0.0045	-0.0085	0.0019	0	0.016	0.0113	0.0042	Yes
model7.4NO	0.0047	0.0047	0.0046	0.0047	0.0046	0.0047	0.0047	Yes
model7.5NO	0.0051	0.0051	0.0051	0.0051	0.0051	0.0051	0.0051	Yes
model7.6NO	0.0026	-0.0028	0.0012	0.0016	9.00E-04	0.0073	0.0018	No
model7.7NO	0.0037	-7.00E-04	0.0032	0.0016	0.0048	0.0077	0.0034	Yes
model7.8NO	0.0051	0.0051	0.0051	0.0051	0.0051	0.0051	0.0051	Yes
model7.9NO	0.0051	0.0051	0.0051	0.0051	0.0051	0.0051	0.0051	Yes
model7.10NO	0.0041	0	0.0039	0.0026	0.0046	0.0087	0.0040	Yes
model7.11NO	0.0043	-0.0012	0.0035	0.0029	0.0044	0.0085	0.0037	Yes
model7.12NO	0.0051	4.00E-04	0.0036	0.0027	0.0037	0.0094	0.0042	Yes
model7.13NO	0.0075	-8.00E-04	0.0045	0.0028	0.0106	0.0104	0.0058	Yes
model7.14NO	0.003	-0.0076	0.0012	0.0015	0.003	0.012	0.0022	No
model7.15NO	0.0046	-0.0087	0.0018	0.0013	0.0128	0.0123	0.0040	Yes

Functional divergence

Appendix 15 Slopes of the effect of logTP (g/L) on FDiv

	Afrotropic	*Australasia*	*IndoMalay*	*Nearctic*	*Neotropic*	*Palearctic*	*Average*	*Singular*
model7	0.0047	0.005	0.0018	0.004	0.0022	-0.0057	0.0020	Yes
model7.1	0.0046	0.0051	0.0019	0.0038	0.0032	-0.0054	0.0022	Yes
model7.2	-0.0052	0.0072	-0.0014	0.0012	-0.0055	-0.0087	-0.0021	Yes
model7.3	-0.0031	0.0073	-2.00E-04	0.001	4.00E-04	-0.0083	-0.0005	Yes
model7.4	0.0062	0.0079	0.002	0.0052	0.0011	-0.0087	0.0023	Yes
model7.5	0.0062	0.0086	0.0023	0.005	0.0028	-0.0084	0.0028	Yes
model7.6	0.006	0.004	0.0018	0.0039	0.0023	-0.0047	0.0022	Yes
model7.7	0.0065	0.0064	0.0024	0.0044	0.0039	-0.0062	0.0029	Yes
model7.8	0.0073	0.006	0.0047	0.005	0.0046	-0.0062	0.0036	Yes
model7.9	0.0071	0.0068	0.0047	0.0049	0.0053	-0.0061	0.0038	Yes
model7.10	0.0057	0.0043	0.0027	0.004	0.0035	-0.0043	0.0027	Yes
model7.11	0.0054	0.0054	0.0027	0.0042	0.004	-0.0049	0.0028	Yes
model7.12	-0.0045	0.013	0.0028	0.0028	-0.0062	-0.0093	-0.0002	No
model7.13	0	0.0029	0.0015	0.0013	0.0021	-0.0068	0.0002	Yes
model7.14	-0.0081	0.01	-7.00E-04	0.0022	-0.0067	-0.0077	-0.0018	No
model7.15	-0.0016	0.0065	5.00E-04	0.0014	0.002	-0.0081	0.0001	Yes

Appendix 16 Slopes of the effect of logTP (g/L) on FDiv without outliers

	Afrotropic	*Australasia*	*IndoMalay*	*Nearctic*	*Neotropic*	*Palearctic*	*Average*	*Singular*
model7NO	0.0111	0.0097	-0.002	0.0071	0.0041	-0.0067	0.0039	Yes
model7.1NO	0.0119	0.0106	-0.0012	0.0074	0.0069	-0.0062	0.0049	Yes
model7.2NO	-0.0022	0.0075	-0.0033	0.0018	-0.0051	-0.0098	-0.0019	Yes
model7.3NO	9.00E-04	0.009	-0.0012	0.0021	0.0046	-0.0092	0.0010	Yes
model7.4NO	0.01	0.0099	-0.0015	0.0067	2.00E-04	-0.0093	0.0027	Yes
model7.5NO	0.0112	0.0109	-3.00E-04	0.0071	0.005	-0.0086	0.0042	Yes
model7.6NO	0.0109	0.0093	-0.0019	0.0066	0.0022	-0.0075	0.0033	Yes
model7.7NO	0.0119	0.0104	-6.00E-04	0.0072	0.0058	-0.0067	0.0047	Yes
model7.8NO	0.0121	0.009	6.00E-04	0.0065	0.0061	-0.007	0.0046	Yes
model7.9NO	0.0118	0.0077	0.0021	0.0063	0.0083	-0.0039	0.0054	Yes
model7.10NO	0.0117	0.0096	-0.0014	0.0072	0.0047	-0.0062	0.0043	Yes
model7.11NO	0.0127	0.0108	-3.00E-04	0.0077	0.0076	-0.0057	0.0055	Yes
model7.12NO	-0.001	0.0112	-7.00E-04	0.002	-0.0061	-0.011	-0.0009	No
model7.13NO	0.0022	0.0117	0.0017	0.0025	0.0055	-0.0104	0.0022	Yes
model7.14NO	-0.0029	0.0084	-0.0029	0.002	-0.0059	-0.0093	-0.0018	No
model7.15NO	0.0016	0.0085	-0.001	0.0024	0.0052	-0.0091	0.0013	Yes