Supplementary Information

S.1 Existing Assumption

Bunn and Arthington (2002) proposed four guiding principles to substantiate the influence of flow alterations on stream biodiversity: 1) The physical habitat is primarily determined by the flow, which in turn determines the biotic composition, 2) Flow variations directly influenced the evolution of aquatic species, 3) Viability of an aquatic population is determined by the longitudinal and lateral connectivity of the river systems, and 4) Alteration of flow regimes facilitates invasive, exotic species. These four principles and other basin-scale evidence (Leigh and Datry, 2017; Mathers et al., 2019; Sarremejane et al., 2020; Zeiringer et al., 2018) suggest that freshwater biodiversity has an inverse relationship with EF violations. According to this assumption, as the EF violation increases, the associated freshwater biodiversity will decrease. Furthermore, the EF-biodiversity relationship is assumed to be scale-independent, meaning that its nature does not change with spatial scale. A graphical representation of this assumption is given in Fig. S1. When the assumption is valid, a curve fitted against the freshwater biodiversity and EF violation should yield a negative gradient (Fig. S1 a) and the median value of biodiversity (either relative value or absolute value) of all violated basins should be significantly lower than the non-violated counterpart (Fig. S1 b). If either of these conditions are not met, then the assumption could be considered invalid.

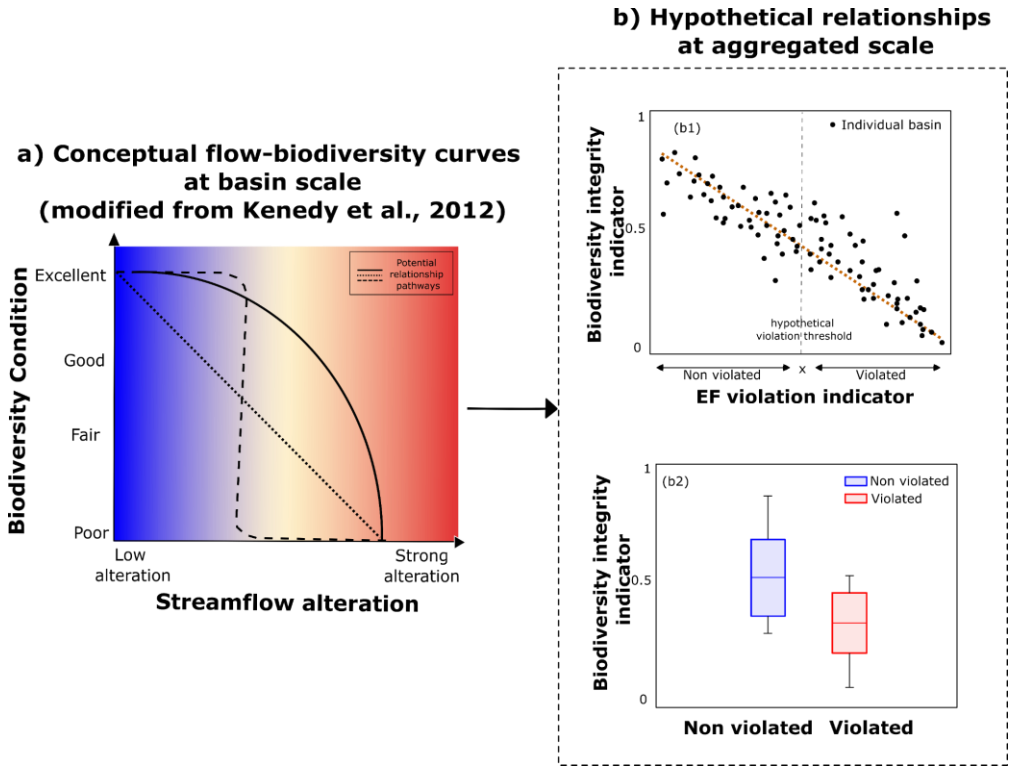


Fig. S1 a) Conceptual flow-biodiversity curves at basin scale modified from (Kendy et al., 2012) and b) hypothetical graphs of the simplest EF-biodiversity relationship (linear) at aggregated scale.

S.2 Data requirement

We use the following global datasets in this study: Streamflow data, Environmental Flow Envelopes, Freshwater fish richness data, Freshwater fish facets, Subbasin boundaries. Each of the datasets selected are described and justified in Supplementary Table S1

Table S1: Data sources, description, and summary of all data used in this study

Datasets	Description
Aquatic fish richness data	**Data Source:** Tedesco et al. (2017) **Temporal Resolution:** Temporal aggregate from data compiled from reports between 1960 and 2014 **Spatial Resolution (extend):** 30 arc second (3119 drainage basins; ~80% of Earth's land)

	Description: Fish richness data was compiled and processed from n 1436 published papers, books, grey literature and web-based sources published between 1960 and 2014.
Freshwater fish facets	**Data Source:** Su et al. (2021) **Temporal Resolution:** Representative of 2015 **Spatial Resolution (extend):** Basin scale (2465 drainage basins) **Description:** Each facet indicates the change in the corresponding biodiversity component compared to the 18th century (roughly pre-industrial era). The taxonomic facets measure the occurrence of fish in a riverine system. Whereas functional facets are calculated using the morphological characteristics of each species that are linked to the feeding and locomotive functions which in turn relates to larger ecosystem functions like food web controlling and nutrition transport. On the other hand, the phylogenetic facets measure the total length of branches linking all species from the assemblage on the phylogenetic tree. The richness component of the three categories calculate the diversity among the assemblage whereas the dissimilarity accounts for the difference between each pair of fish assemblage in the same realm.
EFE	**Data Source:** Virkki et al. (2022) **Temporal Resolution:** Monthly (Pre-industrial: 1801-1860) **Spatial Resolution (extend):** Aggregated to Level 5 HydroBASIN (global) **Description:**
Streamflow	**Data Source:** ISIMIP (2020) **Temporal Resolution:** Monthly (Pre-industrial: 1801-1860, Historical: 1976-2005) **Spatial Resolution (extend):** Aggregated to Level 5 HydroBASIN (global) **Description:** The streamflow data was obtained from Inter-Sectoral Impact Model Intercomparison Project (ISIMIP) simulation phase 2b outputs of global daily discharge (aggregated to monthly for this study); available at https://esg.pik-potsdam.de). ISIMIP is a community-driven climate-impact modelling initiative that offers a consistent framework for cross-sectoral, cross-scale modelling of the impacts of climate change. The streamflow was obtained for four Global Hydrological Models (GHM) (H08, Lpjml, PCR-GlobWB, WaterGap2) and four Global Circulation Models (GCM) (GFDL-EMS2M, HADGEM2-es, IPSL-CM5A-lr, MICROC5) .

Sub-basin boundaries	**Data Source:** HydroSHEDS (Lehner and Grill, 2013) **Temporal Range:** NA **Spatial Resolution (extend):** Level 5 HydroBASIN (global) **Description:** HydroBASINs are the watershed boundaries derived from the hydrographic information from HydroSHED Database, developed with a goal of providing a global coverage of hierarchically nested sub-basins at various scales. The HydroBASIN catchment nesting follows the Pfafstetter coding system (Verdin and Verdin, 1999) and offers 12 levels (level 1 - course and level 12 - detailed) of sub-basin classification globally

Table S2. Characteristics of biodiversity indicators

Biodiversity indicator	Relative measure	Gridded value	Biota data derived
Fish Richness (FiR)		X	X
Taxonomic Richness (TR)	X		X
Functional Richness (FR)	X		X
Phylogenetic Richness (PR)	X		X
Taxonomic Dissimilarity (TD)	X		X
Functional Dissimilarity (FD)	X		X
Phylogenetic Dissimilarity (PD)	X		X

S.3 Environmental flow estimation methods

This study uses five EF estimation methods

Table S3. Environmental flow estimation methods

EF method	Flow regime classification	EFR calculation	Other details
Smakhtin (Smakhtin et al., 2004)	Highly variable flow regimes (Q90 < 10% MAF) Intermediate variable flow (10% MAF Q90 < 20% MAF) Low variable flow (20% MAF Q90 < 30% MAF)	High: Q90+ 0.2 . MAF Intermediate: Q90+ 0.15. MAF Low: Q90+ 0.07. MAF	• Stable EFRs throughout the year • No inter annual variability
Tennant (Tennant, 1976)	High-flow season (MMF> MAF) Low-flow season (MMF≤ MAF)	High: 0.4 · MAF Low: 0.2 · MAF	
Q90-Q50 (Pastor et al., 2014)	High-flow season (MMF> MAF) Low-flow season (MMF≤ MAF)	High: Q50 Low: Q90	• Based on annual flow quantiles
Tessmann (Tessmann, 1979)	High (MMF > 40%MAF and 40% MMF > 40% MAF) Intermediate (MMF > 40%MAF and 40% MMF = 40% MAF) Low-flow months (MMF ≤ 40% of MAF)	High: 0.4 . MMF Intermediate: 0.4 . MMF Low-flow months: 1. MMF	• Consider inter annual variability
Variable Monthly Flow (Pastor et al., 2014)	High (MMF > 80% of MAF) Intermediate (MMF is 40–80% of MAF) Low-flow months (MMF <= 40% of MAF)	High: 0.3 . MMF Intermediate: 0.45 . MMF Low-flow months: 0.6 . MMF	• Consider inter annual variability • Can be aggregated and validated at basin and global scales

S.4 Catchment classification based on flow variability

The catchments were classified based on the criteria provided by Smakhtin et al. (2004) The categorization criteria are given in Table S3.

Table S4. Criteria for different flow variability regime category

Category	Criteria	Characteristics
Highly variable flow regimes	Q90<10%MAF	receive most of the annual flow as floods
Intermediate variable flow regimes	10%MAF<Q90<20%MAF	<Fill>
Low variable flow regimes	20%MAF<Q90<30%MAF	<Fill>
Stable catchments with high base flows	Q90>30%MAF	year round steady high baseflow; relatively less increase in flow during wetter periods

Note: MAF = Mean Annual Flow

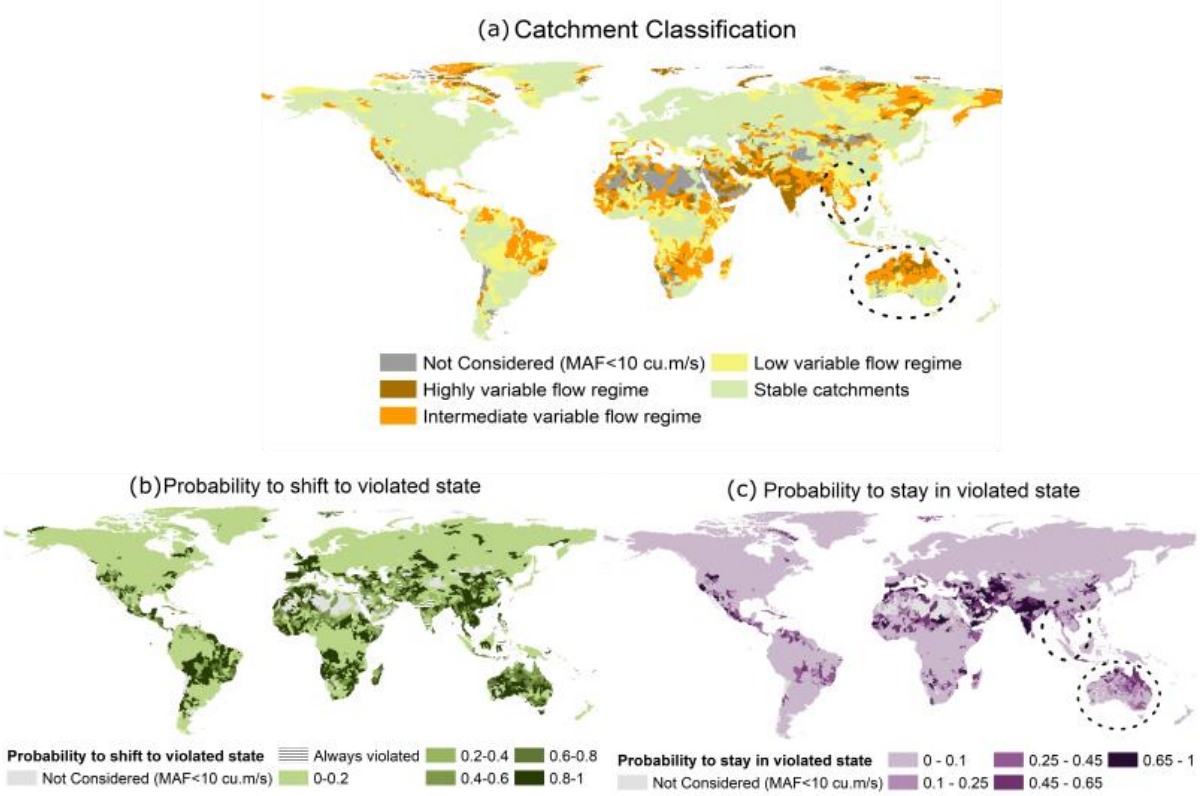


Fig. S2 (a) Catchment classification based on flow variability, EF violation maps for (b) P.shift and (c) P.stay with stable regions with high P.shift and low P.stay marked using dotted circle (eg. Australia)

S.5 Aggregation methods

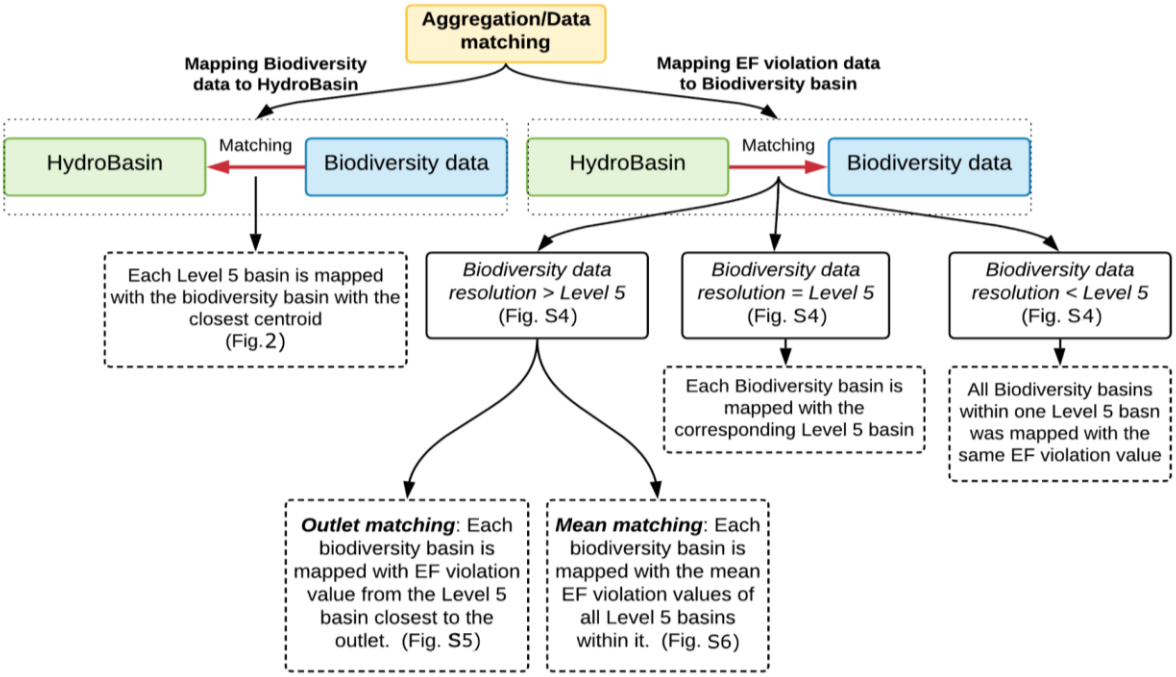


Fig. S3. Flow chart of various data mapping techniques used to match the spatial resolution of EF violation and biodiversity data.

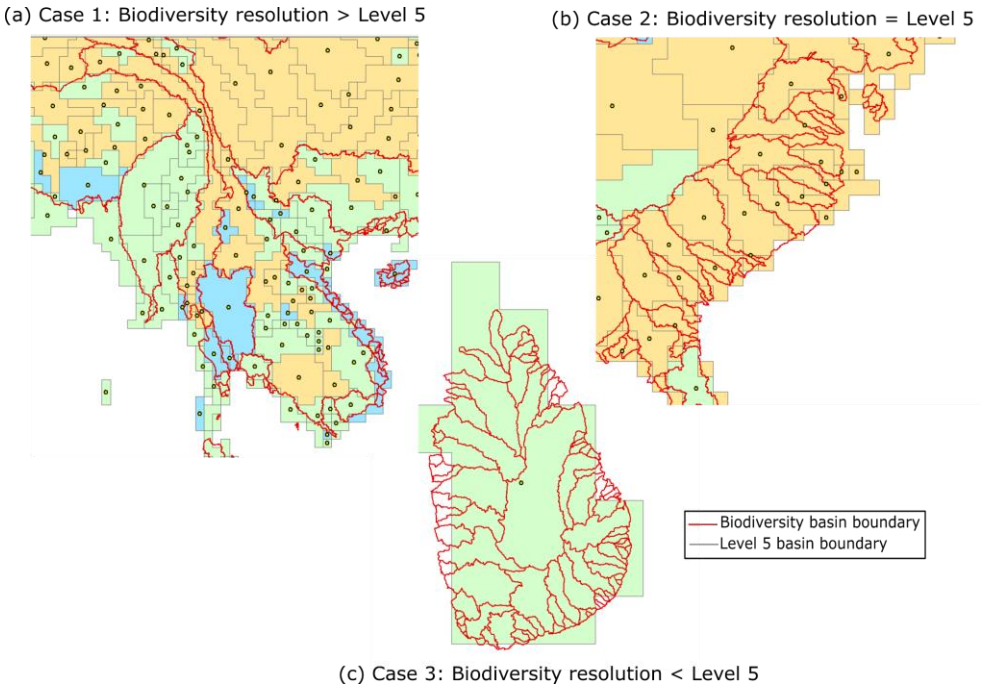


Fig. S4 (a-c) Spatial scale discrepancies between Level 5 HydroBASIN (EF violation) and biodiversity basin (Fish facets data)

Fig. S5 R value of relationship between EF violation indices and Biodiversity indicators spatially matched using Outlet matching method

Fig. S6 R value of relationship between EF violation indices and Biodiversity indicators spatially matched using Mean matching method

S.6 G200 biome classification

WWF's Global 200 project analyzed global patterns of biodiversity to identify a set of the Earth's terrestrial, freshwater, and marine ecoregions that harbor exceptional biodiversity and are representative of its ecosystems. This process yielded 238 ecoregions--the Global 200--comprising 142 terrestrial, 53 freshwater, and 43 marine priority ecoregions. In this study we used 7 main freshwater ecoregion categories which is a coarse aggregation of the 53 freshwater ecoregions identified in Global 200 project.

S.7 Freshwater Major Habitat Types (MHT) classification

Freshwater Ecoregions of the World (FEOW), provides a new global biogeographic regionalization of the Earth's freshwater biodiversity, virtually all freshwater habitats on Earth (https://databasin.org/datasets/0b6963be65074bca9306b1b6f05149d2/). The FEOW are categorised based on the Major Habitat Type (MHT) to study the aquatic behavior in similar types of habitat (Fig. S7) (Abell et al., 2008).

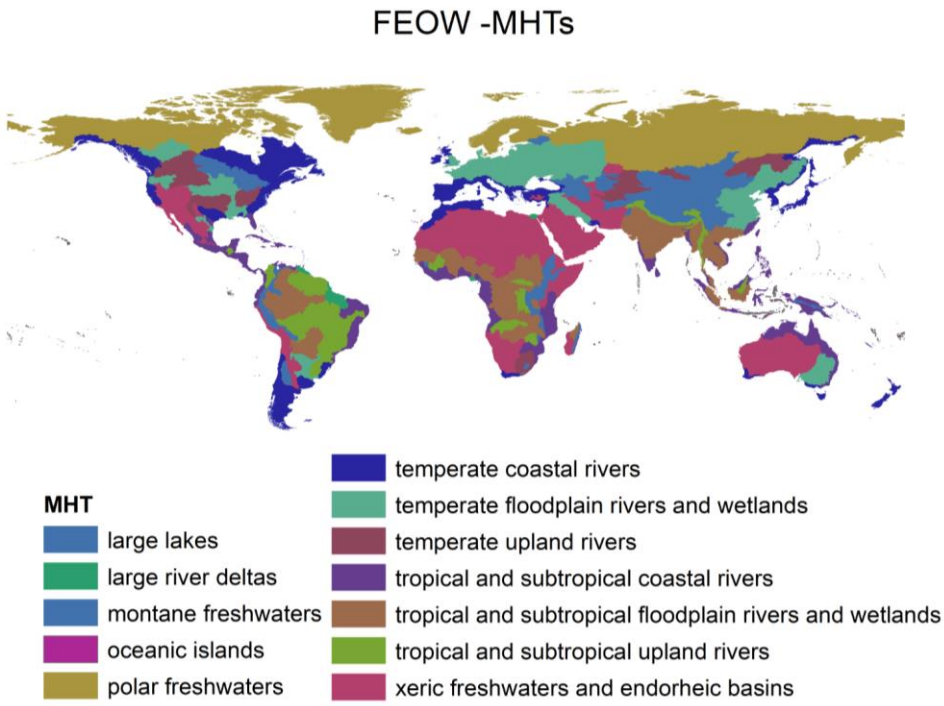
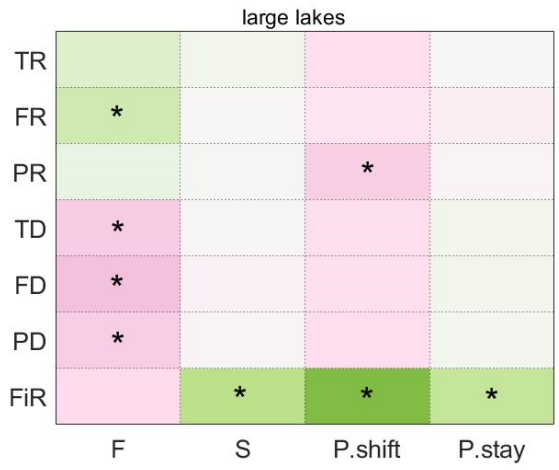
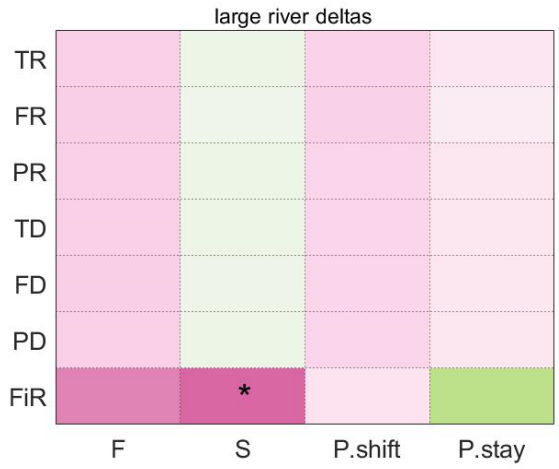
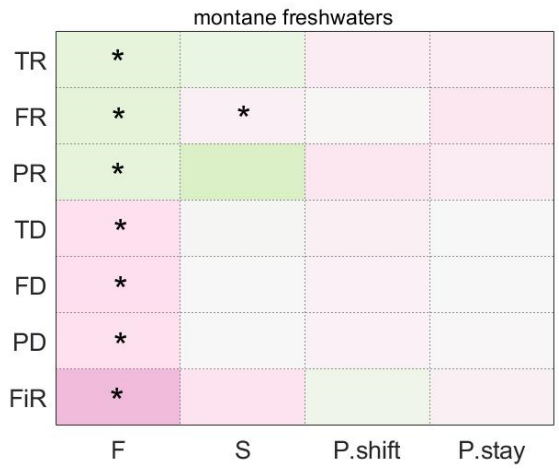
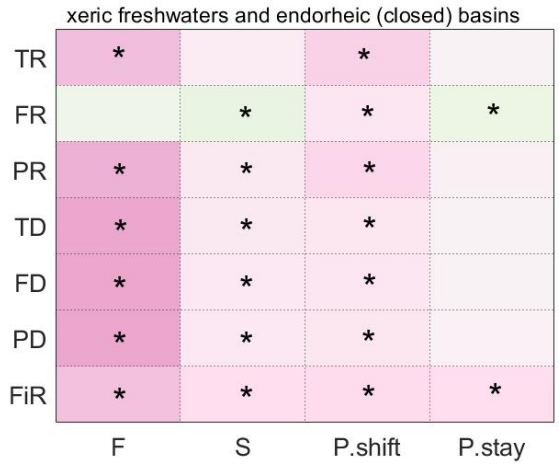
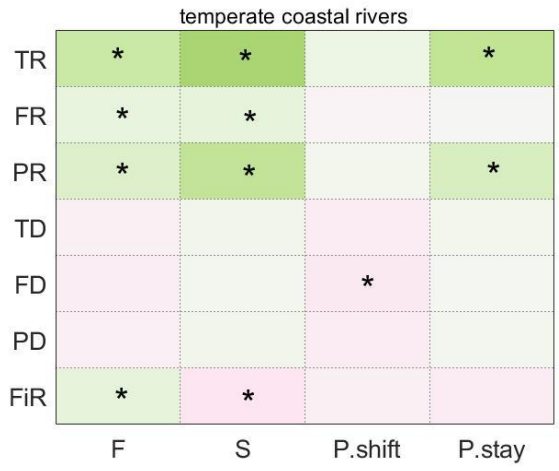
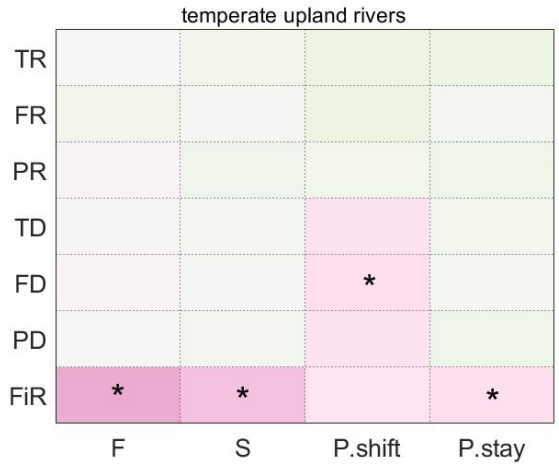
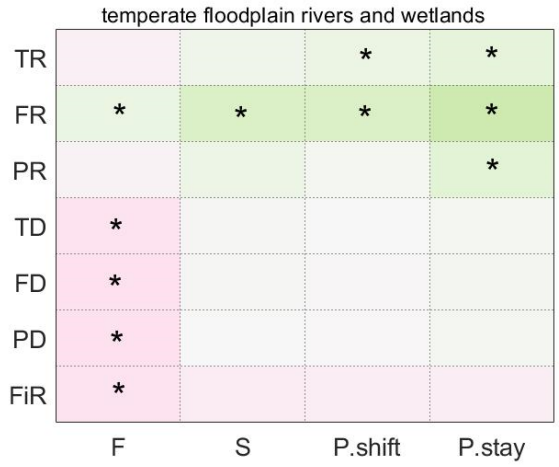


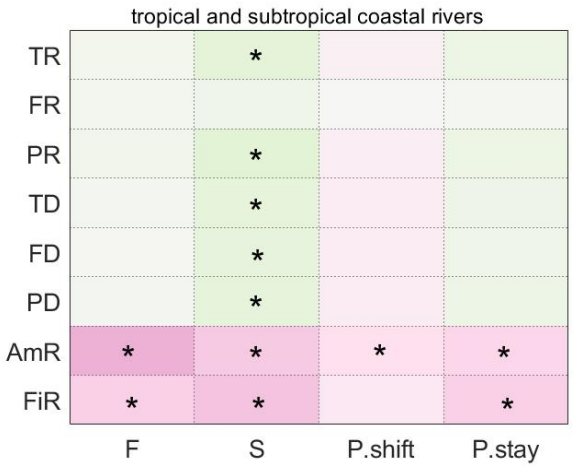
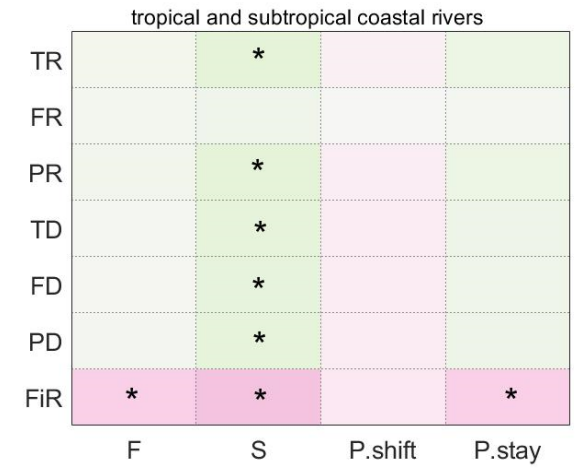
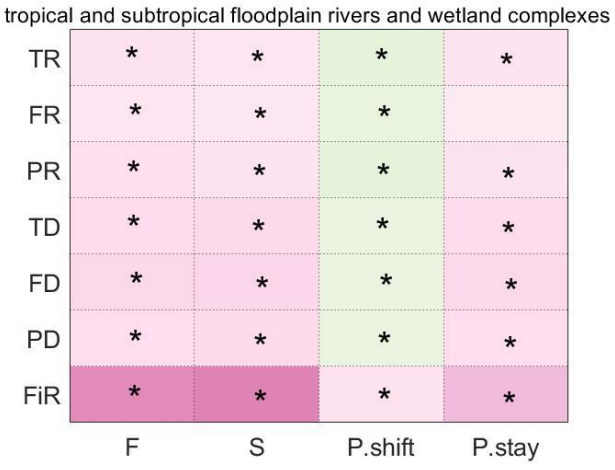
Fig. S7 Map of Major Habitat Types (freshwater)

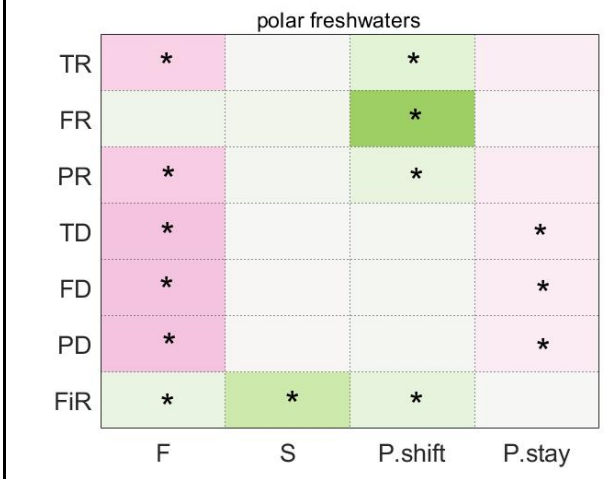
Table S5. correlation between EF violation indices and freshwater biodiversity indicators for different freshwater MHT (N.B. * marks the statistically significant relationships (p <0.05)

Freshwater Major Habitat Type (MHT)	Correlation matrix	Total basin in MHT
Large lakes		109

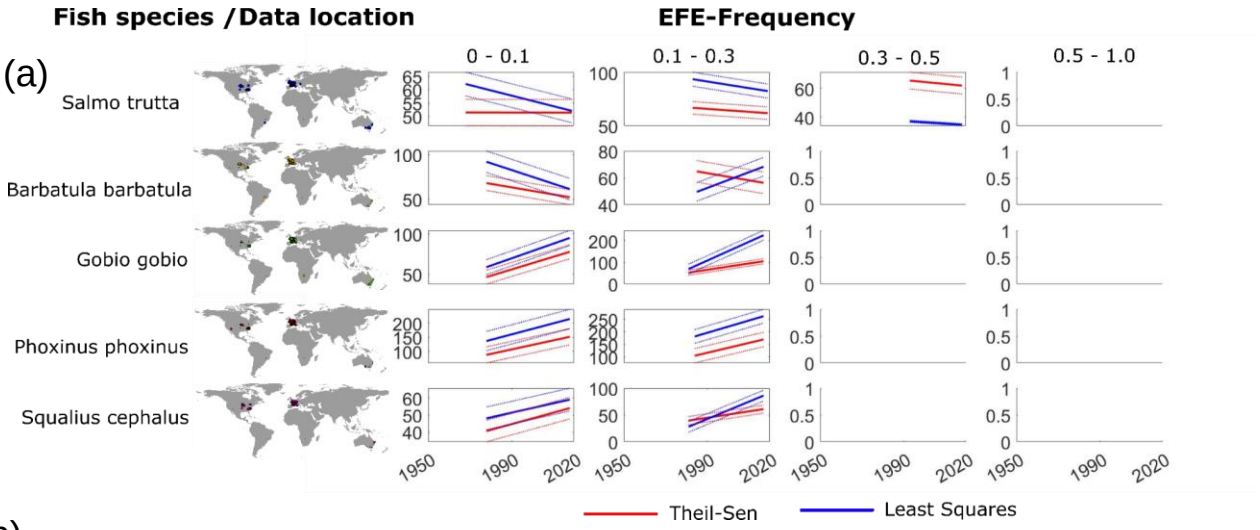
Large river delta		28
Montane freshwater		264
Xeric freshwater and endorheic basin		864

Temperate coastal rivers		483
Temperate upland rivers		180
Temperate floodplain river and wetlands		538

Tropical and subtropical coastal rivers		428
Tropical and subtropical upland rivers		223
Tropical and subtropical floodplain rivers and wetlands		462

Polar freshwaters		784

S.8 Analysis using RivFishTIME dataset



(b)

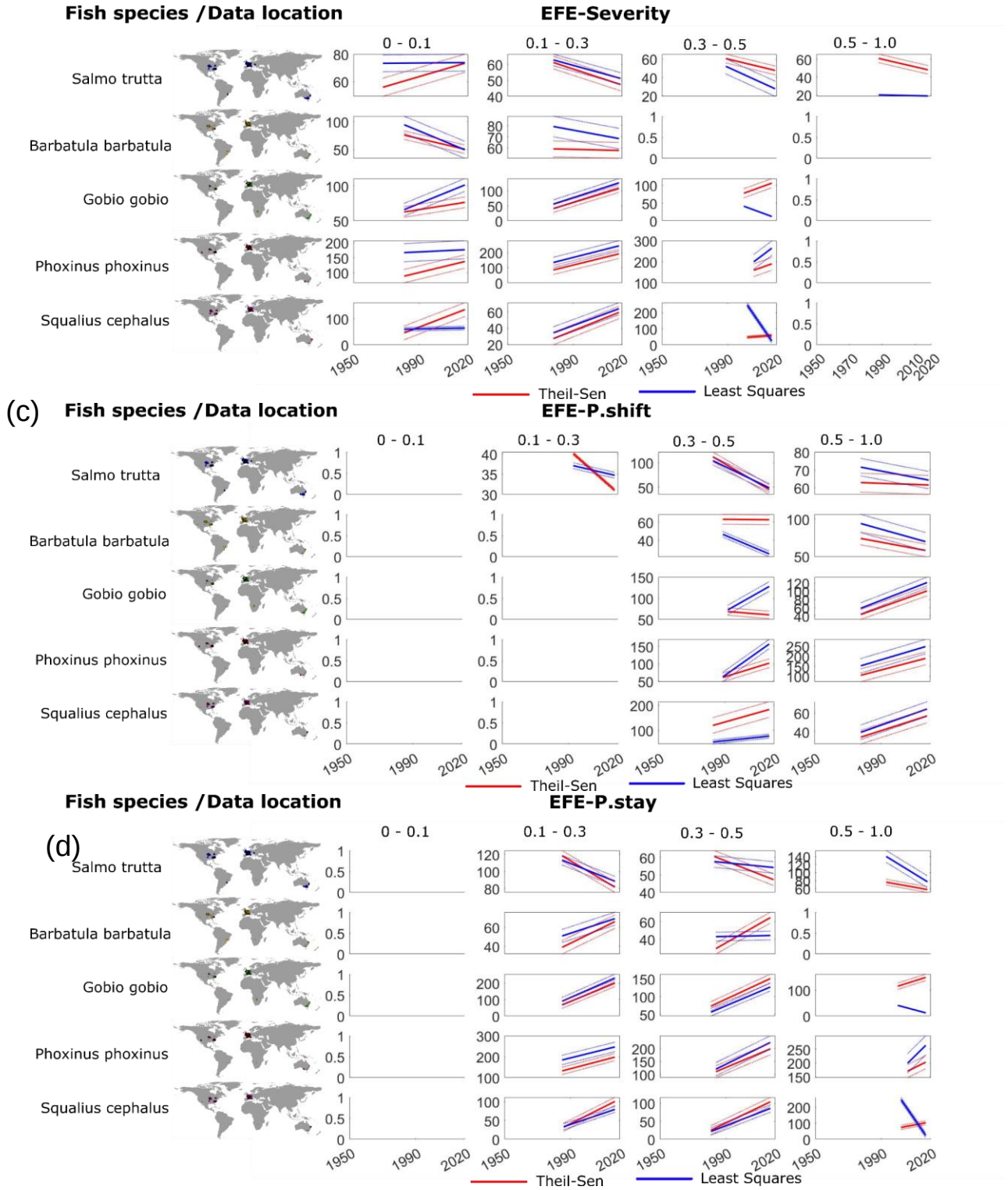


Fig. S8 Directional change in the abundance in 5 freshwater species categorized by different levels of EF violation indices; (a) F, (b) S, (c) P.shift and (d) P.stay.

Note: The dotted lines around the solid line represent the spread in different basins in each category

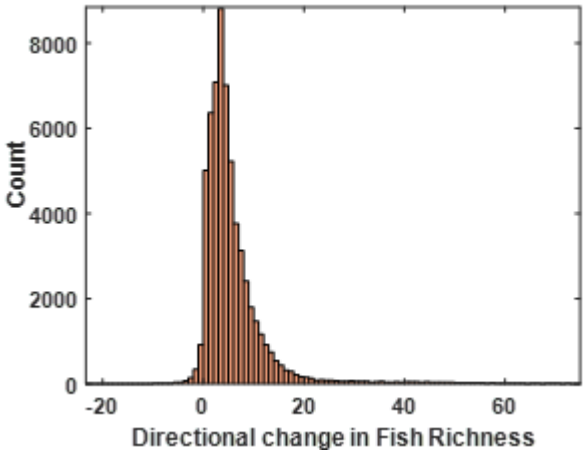


Fig. S9 Histogram of directional change in all the time series in RivFishTIME dataset. 97% of the fish time series are showing an increasing trend over time

RivFishTIME dataset was compiled from long-term riverine fish surveys from 46 regional and national monitoring programmes and from individual academic research efforts (Comte et al., 2021). The database includes 646,270 species-specific abundance time series covering 704 fish species. The data were collected from 11386 sites spanning over 19 countries (Fig. S9)

Fig. S10 Data locations of RivFishTIME dataset

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