



Year: 2023

Homogeneity in terrestrial land cover is reflected in fish diversity patterns in a Chinese river system

Kirschner, Dominik ; Zhang, Yan ; Zhong, Wenjun ; Zhang, Xiaowei ; Pellissier, Loic ; Altermatt, Florian

Abstract: In river systems worldwide, land cover changes have been identified as major drivers of biodiversity change. Quantifying how terrestrial land cover impacts riverine diversity requires local biodiversity assessments. In this study, we investigated the association of terrestrial land cover and the corresponding riverine fish species communities using eDNA-metabarcoding in the Chinese Shaying River basin. This basin is home to about 37 million people and is largely dominated by a mix of intense agriculture and urban areas, creating a relatively homogeneous, intensely used landscape. We investigated whether the homogeneous landscape is mirrored in the composition and structure of fish communities in the river network. We sampled eDNA in spring and fall of 2018, amplified it with a primer designed for local fish species and used operational taxonomic units (OTU) assigned to fish as proxy for diversity. Furthermore, we used redundancy analysis, general linear models, and distance decay curves to assess the effects of land cover on fish communities. We found that the Shaying River showed relatively high basin-wide richness (63 OTU) and seasonal differences in local richness, but limited community differentiation. Variations in alpha- and beta-diversity, measured as local OTU richness and pairwise distance-decay across the basin were low. Redundancy analysis showed only a weak association between observed aquatic communities and their terrestrial surroundings in a 10 km buffer upstream. The lack of community differentiation assessed by eDNA metabarcoding reflects the homogeneous and intense land-use in this basin.

DOI: <https://doi.org/10.1002/edn3.480>

Posted at the Zurich Open Repository and Archive, University of Zurich

ZORA URL: <https://doi.org/10.5167/uzh-252748>

Journal Article

Published Version



The following work is licensed under a Creative Commons: Attribution-NonCommercial 4.0 International (CC BY-NC 4.0) License.

Originally published at:

Kirschner, Dominik; Zhang, Yan; Zhong, Wenjun; Zhang, Xiaowei; Pellissier, Loic; Altermatt, Florian (2023). Homogeneity in terrestrial land cover is reflected in fish diversity patterns in a Chinese river system. *Environmental DNA*, 5(6):1679-1690.

DOI: <https://doi.org/10.1002/edn3.480>

Homogeneity in terrestrial land cover is reflected in fish diversity patterns in a Chinese river system

Dominik Kirschner^{1,2,3}  | Yan Zhang⁴  | Wenjun Zhong⁴  | Xiaowei Zhang⁴  |
Loïc Pellissier^{1,3}  | Florian Altermatt^{2,5} 

¹Department of Environmental Systems Science, ETH Zürich, Ecosystems and Landscape Evolution, Institute of Terrestrial Ecosystems, Zürich, Switzerland

²Department of Aquatic Ecology, Swiss Federal Institute of Aquatic Science and Technology (Eawag), Dübendorf, Switzerland

³Department of Landscape Dynamics & Ecology, Swiss Federal Research Institute WSL, Birmensdorf, Switzerland

⁴State Key Laboratory of Pollution Control & Resource Reuse, School of the Environment, Nanjing University, Nanjing, China

⁵Department of Evolutionary Biology and Environmental Studies, University of Zurich, Zürich, Switzerland

Correspondence

Dominik Kirschner, Department of Environmental Systems Science, ETH Zürich, Ecosystems and Landscape Evolution, Institute of Terrestrial Ecosystems, Zürich CH-8092, Switzerland. Email: dominik.kirschner@eawag.ch and dominik.kirschner@usys.ethz.ch

Funding information

Eidgenössische Technische Hochschule Zürich; Schweizerischer Nationalfonds zur Förderung der Wissenschaftlichen Forschung, Grant/Award Number: 31003A_173074

Abstract

In river systems worldwide, land cover changes have been identified as major drivers of biodiversity change. Quantifying how terrestrial land cover impacts riverine diversity requires local biodiversity assessments. In this study, we investigated the association of terrestrial land cover and the corresponding riverine fish species communities using eDNA-metabarcoding in the Chinese Shaying River basin. This basin is home to about 37 million people and is largely dominated by a mix of intense agriculture and urban areas, creating a relatively homogeneous, intensely used landscape. We investigated whether the homogeneous landscape is mirrored in the composition and structure of fish communities in the river network. We sampled eDNA in spring and fall of 2018, amplified it with a primer designed for local fish species and used operational taxonomic units (OTU) assigned to fish as proxy for diversity. Furthermore, we used redundancy analysis, general linear models, and distance decay curves to assess the effects of land cover on fish communities. We found that the Shaying River showed relatively high basin-wide richness (63 OTU) and seasonal differences in local richness, but limited community differentiation. Variations in alpha- and beta-diversity, measured as local OTU richness and pairwise distance-decay across the basin were low. Redundancy analysis showed only a weak association between observed aquatic communities and their terrestrial surroundings in a 10 km buffer upstream. The lack of community differentiation assessed by eDNA metabarcoding reflects the homogeneous and intense land-use in this basin.

KEYWORDS

eDNA, fish diversity patterns, land cover, metabarcoding, OTU, riverine diversity

1 | INTRODUCTION

Anthropogenic changes represent major threats to biodiversity in large river systems worldwide (Dudgeon et al., 2006; Newbold et al., 2015; Vörösmarty et al., 2010). Freshwater systems have

lost a large fraction of their aquatic species following anthropogenic exploitation of resources, pollution, and habitat degradation, such as conversion and alteration of natural riverine corridors (Allan, 2004; Carpenter et al., 1998; IPBES, 2019). Anthropogenic changes such as river channelization or damming

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial](https://creativecommons.org/licenses/by-nc/4.0/) License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

© 2023 The Authors. *Environmental DNA* published by John Wiley & Sons Ltd.

alter the hydrological regime within a river directly, yet the effect of land cover changes is complex (Allan, 2004; Su et al., 2021; Vörösmarty et al., 2010). Different land cover types can have multiple, even contrasting, effects within the same system. The terrestrial system's influence on the aquatic fauna is not always straightforward or can display scale dependence (Ho et al., 2022; McFadden et al., 2023). For instance, agricultural land cover effects on river systems can have various results, ranging from water pollution and habitat fragmentation to changes within the riverbed structure and hydrological regimes (Dala-Corte et al., 2016; Leitão et al., 2018).

Fish are the richest group of aquatic vertebrates globally, and are particularly sensitive to habitat degradation, pollution, and over-exploitation (Su et al., 2021). Like most aquatic organisms, fish species in river systems are not uniformly distributed, and their occurrence follows certain biogeographic (Oberdorff et al., 1995) as well as regional patterns (Altermatt, 2013; Colleen et al., 2014). For instance, in most big river systems, species richness (a metric of alpha diversity) increases along the spatial gradient from the headwaters to the downstream river sites, resulting in increasing local richness with increasing catchment size (Oberdorff et al., 2011). Furthermore, dissimilarity in community composition (beta-diversity) generally increases with increasing pairwise distance between sites (Blackman et al., 2021; Muneepeerakul et al., 2008; Soininen et al., 2007). These almost universal patterns of diversity in natural river systems are determined by the hydrological, chemical, and spatial gradients within rivers (Oberdorff et al., 1995; Vannote et al., 1980) as well as the basins' history (Tedesco et al., 2005; Williams & Johnson, 2022). In anthropogenically altered and disturbed riverine systems, numerous additional factors can modify fish diversity. For instance, Dala-Corte et al. (2016) showed that agricultural runoff leads to the siltation of riverbeds, resulting in compositional changes in both local and basin-wide fish communities. On a larger scale, there is evidence of decreases in aquatic and terrestrial species richness and changes in communities in Amazonian tropical streams linked to increased deforestation in the upstream basins (Cantera et al., 2022). Poorer and more stochastic assemblages of organisms generally occur under high human pressure (McFadden et al., 2023; Vellend et al., 2007, 2014; Zeni et al., 2020). However, assemblage information across large river systems is not readily available, leading to a lack of data on species distribution in certain global regions (Davison et al., 2021) and within the river systems.

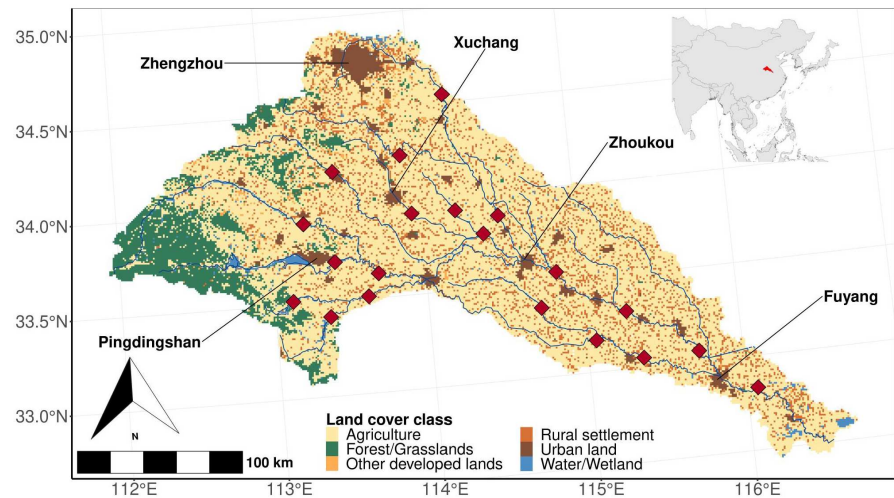
Environmental DNA (eDNA) metabarcoding is a powerful monitoring method to uncover riverine fish assemblages (Blackman et al., 2021; Deiner et al., 2017; Keck et al., 2022; Thomsen & Willerslev, 2015; Valentini et al., 2016) that has the potential to increase our understanding of associations between land-use/land cover and fish diversity. Environmental DNA-based methods build on the collection and identification of DNA extracted from environmental samples without obvious presence of the target organism (Pawlowski et al., 2020; Thomsen et al., 2012). The process usually includes the filtration of small to large volumes of water, followed by

amplification of eDNA using universal or specific primer sets and finally visualizing the results by electrophoresis, qPCR, or sequencing using next-generation high-throughput sequencing (Metabarcoding, Miya et al., 2015; Valentini et al., 2016). Environmental DNA-based methods applied in river systems therefore allow rapid diversity assessment of entire river basins, across multiple taxonomic groups and spatial scales (Deiner et al., 2016; Li et al., 2020; Pont et al., 2018). Communities identified by conventional techniques are local snapshots. By contrast, eDNA gets transported along the river system and therefore incorporates information across larger temporal scales (e.g., the time lag between DNA shedding and detection) and spatial scales (up to several km upstream) (Carraro et al., 2023; Deiner & Altermatt, 2014; Pont et al., 2018; Van Driessche et al., 2023). Landscape-level integration of eDNA methods may allow the identification of riverine biodiversity on local- to catchment-wide scales and better understand the spatial scale at which the aquatic matrix connects to the surrounding terrestrial matrix (Cantera et al., 2022; Zhang et al., 2023).

Here, we studied the association between fish diversity and land cover composition in the Shaying River basin in China using extensive eDNA sampling across the whole catchment. With its basin covering around 42,000 km², the Shaying River is the biggest tributary of the Huai River (Figure 1). It is dominated by intense agricultural land cover and several large cities, harboring a total population of ~37 million people (Li et al., 2020). Like many river systems in China, it is under intense anthropogenic pressure, with highly altered channels and polluted waters (Shi et al., 2016; Xiao-nan et al., 2015). With the implementation of water pollution prevention and control in the past 10 years, the deterioration of the water ecosystem in the Huai River basin has been effectively curbed. However, many anthropogenic stressors, such as water withdrawal, damming, and agriculture chemical pollution, persist. Li et al. (2020) focused on the "human fingerprint" on different aquatic groups in the Shaying River and showed that anthropogenic land cover influences and shapes the observed community composition, but the study did not cover fish. Thus, the Shaying River basin, as the largest tributary of the greater Huai River basin, provides opportunities for an in-situ study to identify the results of anthropogenically influenced terrestrial processes on patterns of fish diversity. We hypothesized that the fish communities would follow the lack of gradients and patterns observed in the terrestrial matrix. Therefore, we sampled eDNA at 20 sites in two seasons (spring and autumn) to answer the following research questions:

1. Is eDNA-metabarcoding able to uncover fish diversity in large rivers and do the species recovered vary across sampling seasons?
2. Do the observed fish assemblages in this system follow known biodiversity patterns in riverine systems (e.g., more dissimilar communities with increasing distance and a headwater to downstream gradient in richness)?
3. How is diversity associated with land-use/land cover types and possible further environmental variables?

FIGURE 1 Map of the Shaying River basin and its position in China. The major river tributaries are given as dark blue lines. Red diamonds depict sampling sites, major settlements in the basin are marked and named. Land cover classes are color-coded according to the classification used in the study.



2 | METHODS

2.1 | Study site and eDNA sample collection

The Shaying River basin is located in eastern China, shared between the provinces of Anhui and Henan (two of the most populated provinces in China). The land cover in this area is significantly shaped by human activities, particularly intense agriculture, industry, and urban settlements (Li et al., 2020). To capture a wide range of aquatic diversity in the basin, we selected sampling sites at the confluences of major tributaries, following recommendations for eDNA sampling in large river systems (Altermatt et al., 2020). To cover large spatial gradients, sites are spread out across the whole system with an average pairwise distance of 237.37 km (min: 27.89 km, max: 502 km, SD: 110.27 km, Supp_S7). In 2018, we collected aquatic environmental DNA samples at 20 sites in April and October (Figure 1) according to the methodology described in Li et al. (2018). Briefly, at each selected site along the stream channel, we sampled a cross-stream transect using six 1-L surface water samples (3 sampling points \times 2 water samples). These samples were cooled to temperatures between 0 and 4°C and transported to the laboratory. Within 6 hours, they were divided into 400–500 mL subsamples and filtered through a 0.45 μ m hydrophilic nylon membrane (Merck Millipore). The resulting filter discs were placed in 5 mL reaction tubes and frozen at –20°C. To minimize the risk of contamination, we followed best practices for eDNA fieldwork (Bruce et al., 2021; Pawlowski et al., 2020). To assess potential sampling contamination, we included one field blank per site, which consisted of deionized autoclaved water and was treated similarly to the samples. Some deviations from this protocol were made: in April, sites S03 and S20 were inaccessible and therefore not sampled; as part of internal tests, site S11 included 11 subsamples across the river instead of three, and in October, site S18 included five subsamples across the channel instead of three. In addition, samples in October were pooled, resulting in only three samples per transect.

2.2 | Chemical measurements

We utilized the same set of 16 abiotic chemical parameters described by Li et al. (2021, Table S2). In summary, we measured dissolved oxygen (DO) in mg/L, electrical conductivity (EC) in μ S/cm, water temperature (WT) in °C, and pH on-site using an AP-2000 Multiparameter Water quality instrument (Aquaread, UK). Chemical oxygen demand (COD) in mg/L, ammonia nitrogen (NH₃) in mg/L, total phosphorus (TP) in mg/L, and total nitrogen (TN) in mg/L were measured in the laboratory from a 1 L surface water sample following national standards (NEPB, 2002). For the analysis of arsenic (As) in mg/L, cadmium (Cd) in mg/L, chromium (Cr) in mg/L, copper (Cu) in mg/L, iron (Fe) in mg/L, manganese (Mn) in mg/L, nickel (Ni) in mg/L, and zinc (Zn) in mg/L, we filtered a 1 L surface water sample and conducted analyses on the filter using inductively coupled plasma mass spectrometry (Thermo Fisher).

2.3 | Hydrological and land cover data

We extracted distance measurements and basic river basin properties (e.g., distance of reach to source, upstream basin size) across the entire drainage basin using the HYBAS database for Asian waterbodies (Lehner & Grill, 2013). To calculate pairwise topological distance (“as the fish swims”), we utilized the “rivdist” package for R (Tyers, 2022). The 2018 Chinese land cover data at a 1000 m resolution for the river system were then extracted and the land cover classes summarized into broader categories such as “Agriculture,” “Forest/Grassland,” “Water/Wetlands,” “Urban land,” “Rural settlement,” and “other developed lands” (Table S3). We assumed that land cover within a 10 km radius upstream would have an influence on the system, so we calculated the percentage of land cover classes within this radius for each sampling site. For all subsequent geographic analyses, we utilized the “sf” package (Pebesma, 2018) and the “raster” package (Hijmans et al., 2023) in R.

2.4 | Laboratory protocol

We extracted eDNA from the filters using the Dneasy Power Water kit (QIAGEN) as described in Li et al. (2020) and Li et al. (2021). Next, we amplified the extracted DNA using a fish-specific primer set adapted to Chinese fish fauna, targeting a ~170 BP fragment of the 12S rRNA region (Forward-TCGTGCCAGCCACCGCGGTTA; Reverse- ATAGTGGGGTATCTAATCCCAG, Yang et al., 2023). To distinguish samples, we added a unique 12-bp nucleotide fragment (barcode) to the 5'-ends of the forward or reverse primers (Shanghai Generay Biotech Co., Ltd). For each sample, we conducted the PCR three times, with a 30 μ L reaction mixture consisting of 19.1 μ L of ddH₂O, 6 μ L of 5 Phusion Green HF Buffer, 0.6 μ L of 10 mM dNTPs, 1 μ L of forward and reverse primers (10 μ M), 2 μ L of template, and 0.3 μ L of Phusion Green Hot Start II High-Fidelity DNA Polymerase (Thermo Fisher Scientific). The amplification protocol consisted of an initial denaturation at 98°C for 30 s, followed by 30 cycles of denaturing at 98°C for 5 s, annealing at 62.5°C for 30 s, and extending at 72°C for 15 s. We concluded the amplification with a final extension at 72°C for 5 min. Afterward, we cooled all PCR assays to 4°C until removal. Subsequently, we pooled the PCR replicates for each site for sequencing. To ensure quality control, we included PCR negative controls using nuclease-free water as the DNA template for all assays. Finally, we prepared libraries using the Ion AmpliSeq™ Library Kit (ThermoFisher) following the manufacturers protocol and sequenced the products using the Ion Proton p300 sequencer (Thermo Fisher Scientific).

2.5 | Metabarcoding to final operational taxonomic unit (OTU) table

We processed the raw reads using Cutadapt (Martin, 2011), VSEARCH (Rognes et al., 2016), and SWARM (Mahé et al., 2014, 2015, 2021). Species assignment was done using IDTAXA within the DECIPHER package (Murali et al., 2018; Wright, 2015) trained with a subset of the MIDORI sRNA database for vertebrates (Leray et al., 2022, accessed 11.2022). To subset the global reference, we retrieved a list of Chinese fish species from Fishbase and used the perl script provided on the Midori website ("filter_RDP.pl") to extract sequences from Chinese fish only. Barcoding tags and primers were trimmed using Cutadapt (minimum length 140 BP, minimum F/R primer fit $\geq 2/3$ of primer length), followed by dereplication and cleaning using VSEARCH, resulting in FASTA files for all sites. Those FASTA sequences were clustered into OTUs using the SWARM algorithm ($d=1$), denoised using LULU at 75% minimum frequencies (Frøslev et al., 2017) and in the last step assigned to a taxonomic level using DECIPHER (intermediate strictness at 50% probability to keep assignments). Further analysis excluded all OTUs flagged as chimeras; OTUs with an occurrence of fewer than 10 sequences overall; OTUs with an occurrence in fewer than three replicates; and OTUs not assigned to "Actinopterygii." Field blanks were measured using a Qubit fluorometer and were negative for DNA. Contamination in

PCR controls was observable, but after filtering steps, only a few frequent and highly abundant OTUs remained as potential contaminants. Based on this, we decided not to remove these OTUs from the dataset, as this would exclude the most abundant species (in occurrence and read numbers).

2.6 | Richness-based analyzes

All analyses were carried out in R (version 4.2, <https://cran.r-project.org/>). In the first step, we analyzed the overlap of our species assignments with two available checklists for the Shaying River system from 1980 and 2013 (Weixian (舒卫先) & Cuizhen (韦翠珍), 2015). To ensure taxonomic agreement between the datasets, we used the "rfishbase" package (Boettiger et al., 2021) to clean the taxonomies. We calculated the overlap between both checklists and our species assignment and visualized it with Venn diagrams using the "ggvenn" package (Yan, 2023). Because of low species level overlap, all further calculations were based on taxonomically filtered OTU, as they are usually good surrogates for real species richness (Marques et al., 2020). Before analyzing our richness data further, we calculated species accumulation curves for the combined seasons using the "specaccum" function (method="random", permutations=500) in "vegan" (Oksanen et al., 2022) and found sufficient replication to saturate OTU richness (Figure S1). We calculated OTU richness per site and season and assessed the level of seasonality within the basin using non-metric-dimensional scaling (NMDS) to identify clusters using the function "metaMDS" in "vegan" and Jaccard's dissimilarity. To determine whether seasons are similar to each other, we used the "anosim"-function in vegan to assess differences in community presence/absence between seasons/sites. Based on the significant distinct clusters between seasons and the overall higher OTU richness in the combined data, we decided to continue with the combined data for the full year, as it offers a better depiction of communities within the system (alpha diversity=OTU richness per site). We excluded abiotic variables with a VIF > 10. Therefore, we only included DO, EC, WT, pH, TP, and TN in further analysis. We standardized the variables by scaling them to zero variance and mean using the "decostand" (method=standardize) function. To test the hypothesis of land cover as the main driver of OTU richness, we built one model with the spatial variables (sppr-land cover) and another global model including environmental and land cover variables.

2.7 | Assemblage dissimilarity analyses

To identify the spatial distribution of community dissimilarities within the Shaying River basin, we calculated beta diversity components, namely turnover, nestedness, and total beta diversity. First, we calculated pairwise dissimilarities using the "beta.pair" function in the "betapart" package (Baselga et al., 2022). By combining these matrices with the topological distances (Table S7), we calculated distance decay models for each component using the "dist.

decay" function in the same package (method="exp"). As richness alone does not cover the full spectrum of diversity along a stream, we further identified drivers of community composition within this system using redundancy analysis (RDA). We created a presence/absence-based species/site matrix for the whole system and applied a Hellinger transformation for the following redundancy analysis (Legendre & Gauthier, 2014). To quantify the explained variance of land cover on the observed communities, we built a model including "Agriculture," "Forest & Grasslands," "Other developed lands," and "Urban-lands" (community_matrix ~ Agriculture_10km + Rural_settlement_10km + Forest_Grasslands_10km + Other_developed_lands_10km), excluding "Water and Wetlands." The significance of each model was identified by 9999 permutations of the RDA using the "anova.cca" function in the package "vegan."

3 | RESULTS

3.1 | Abiotic factors and land cover

We found that the land cover classes in the basin were dominated by agricultural land (70%), followed by rural settlements (13%), forests & grasslands (10%), urban land (4%), water and wetlands (2%), and other developed lands (1%, Table S1). Within the 10km buffers upstream of the sampling sites, agricultural land predominates (mean=76%, sd=11%) followed by rural settlements (mean=18%, sd=10%) and urban land (mean=3%, sd=6%). Forested and grasslands, as indicators for natural environments, account for <1% (sd=2%) of the buffer area upstream of the sampling sites.

3.2 | OTU richness, species richness

We recovered a total of 15,732,383 reads from 14 sequencing pools. After filtering steps and denoising, a total of 7,043,300 reads remained, which were subsequently assigned to 63 Actinopterygii-taxa (Table S4), of which 42 could be assigned to species level. The species checklists from 1980 and 2013 reported 46 and 33 fish species in the study area, respectively, with a combined total of 62 distinct species (Table S8). After unifying and cleaning the taxonomy, the overlap between the most recent checklist from 2013 and eDNA was low at species level, with an overlap of 14 species (including generalists like *Hypophthalmichthys molitrix* and *Hypophthalmichthys nobilis*), while 29 species were only detected by eDNA and 19 species were only reported in the checklist from 2013 (Figure S2).

3.3 | Seasonality

We found on average higher OTU numbers per site in October compared to April (Wilcoxon paired rank test, $V=18.5$, $p=0.0065$; Figure 2). The range of OTUs observed per site was 25–59 in October and 24–41 in April. Combining the OTUs found per site resulted in OTU richness values between 31 and 59 (Figure 2a). The NMDS analysis showed that OTU richness is evenly distributed across the river system and that communities differ between seasons (Figure 2b, NMDS, stress=0.19, ANOSIM, p -value <0.05). Based on this seasonal variation and to account for the fact that land cover is not changing between seasons, we pooled samples from the two seasons for further analysis to get the community corresponding to the entire year at a site. We did not

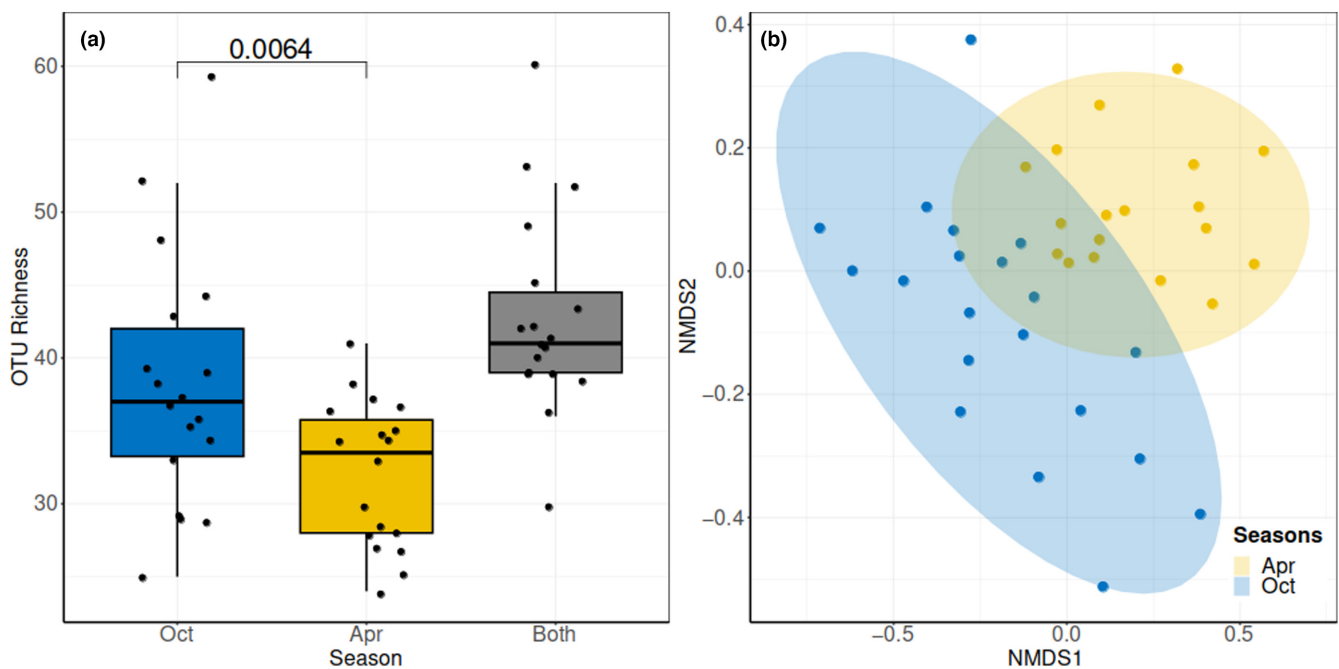


FIGURE 2 (a) Boxplot showing OTU richness within individual seasons and combined across seasons. The values between the two seasons are compared with a paired Wilcoxon test and the respective p -value is given. (b) NMDS depicting the community clusters in October (blue) and April (yellow).

find an increase in species richness with increasing upstream basin size (Figure 3). In the combined dataset, the highest richness was found at one of the uppermost sites S12 (60 OTUs), followed by S19 (53 OTUs), and only then the most downstream Site S01 (48 OTUs). The lowest number of OTUs were found in the middle reaches of the Shaying River basin, namely S07 (30 OTUs) and S08 (36 OTUs).

3.4 | Community composition

Differences in community composition were quantified using the components of Jaccard's beta diversity (nestedness, turnover, and overall beta diversity; Figure 4). Following our hypotheses of community homogenization within the system, turnover and nestedness were low, as was overall beta diversity (beta-diversity: mean=0.29, sd=0.07; turnover: mean=0.17, sd=0.1; nestedness: mean=0.12, sd=0.1). To statistically test for the beta-diversity decay along increasing pairwise stream distance, we fitted distance decay models to all three facets of beta diversity (Figure 4). There was no significant increase in dissimilarity with increasing distance ($\text{slope}_{\text{beta-diversity}}=0.0002$; $\text{slope}_{\text{turn}}=0.0002$; $\text{slope}_{\text{nest}}=-0.00008$) and none of the models were significant ($p\text{-value}_{\text{beta-diversity}}=0.12$; $p\text{-value}_{\text{turn}}=0.13$, $p\text{-value}_{\text{nest}}=0.7$). In addition, all the decay models showed an overall poor model fit ($\text{pseudo-}r^2_{\text{beta-diversity}}=0.03$, $\text{pseudo-}r^2_{\text{turn}}=0.04$, $\text{pseudo-}r^2_{\text{nest}}=0.004$).

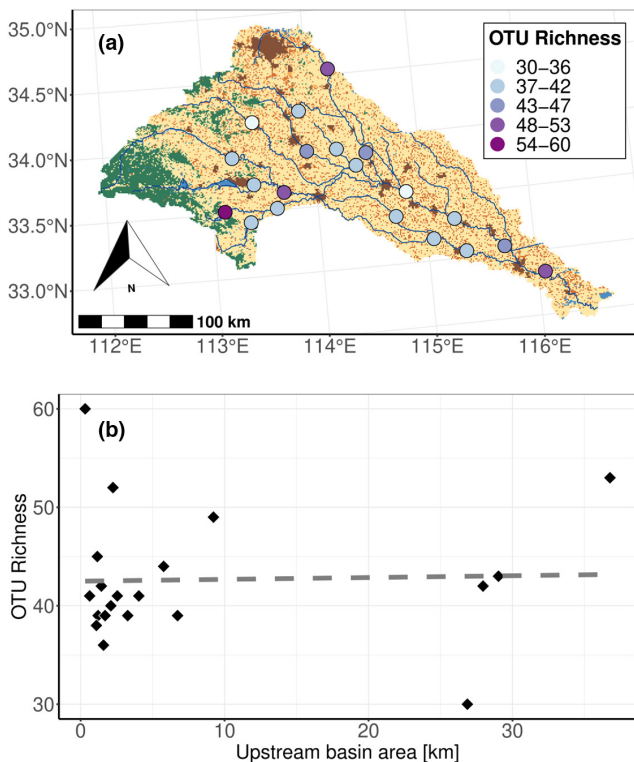


FIGURE 3 (a) Combined (across both seasons) OTU richness along the river basin. Color of circles depicts OTU richness. Land cover classes are the same as in Figure 1. (b) OTU richness as function of upstream basin size. Dashed line shows the non-significant linear model trend line.

3.5 | Drivers of richness

We investigated whether species richness is associated with land cover upstream of the sampling site. In the global model, none of the variables considered showed a significant effect on OTU richness. In the model considering only land cover, the land cover 10km upstream of the basin is also not significantly associated with OTU richness. Overall, both models show low model fit, suggesting a generally low association between richness and the variables considered (Full model: McFadden-pseudo- $R^2=0.078$, $p=0.678$; LC model: McFadden-pseudo- $R^2=0.055$, $p=0.212$; detailed output in Tables S5 and S6).

3.6 | Drivers of assemblage dissimilarity

To identify drivers of community composition, we applied redundancy analysis. We found that the RDA including land cover predictors showed significant associations (anova.cca: $p\text{-value}=0.04$; 9999 permutations), but the model explained low levels of variance in the data (Figure 5, explained variation: 16%, constrained: 5%, unconstrained 11%). The first two axes of the RDA explained 9.66% and 8.91% of the variance within the communities, respectively. Within the RDA, the amount of upstream "Agriculture" and "Forests & grasslands" contributed to overall explained variation at a low level (1.25% and 1.27%, respectively). These land cover classes were the only significant predictors of community variability in the system (0.04 and 0.04). The model fit was low, with adjusted R^2 at 0.07.

4 | DISCUSSION

In this study, we applied eDNA-based metabarcoding to investigate fish diversity patterns in the highly anthropogenically impacted Shaying River basin in eastern China. To understand species distribution and richness, we collected eDNA samples at 20 sites across two seasons and identified 63 Actinopterygii OTUs. Despite this system being highly anthropogenically altered, we found relatively high local and regional OTU richness (31–59). However, richness was not explained by spatial position or environmental factors, indicating that the large-scale homogenization of land cover is mirrored by relatively homogeneous and undifferentiated fish communities across thousands of km^2 . Because richness alone does not reflect spatial community composition, we also calculated the distance decay of community dissimilarity (beta diversity) between sites and found that the communities in this basin showed signs of spatial homogenization similar to the homogeneous land cover in the basin. However, neither land cover nor chemical parameters were associated with observed patterns, indicating a potential decoupling of fish assemblages from environmental drivers, a loss of variation due to species homogenization or insufficient sampling with regard to the basin size.

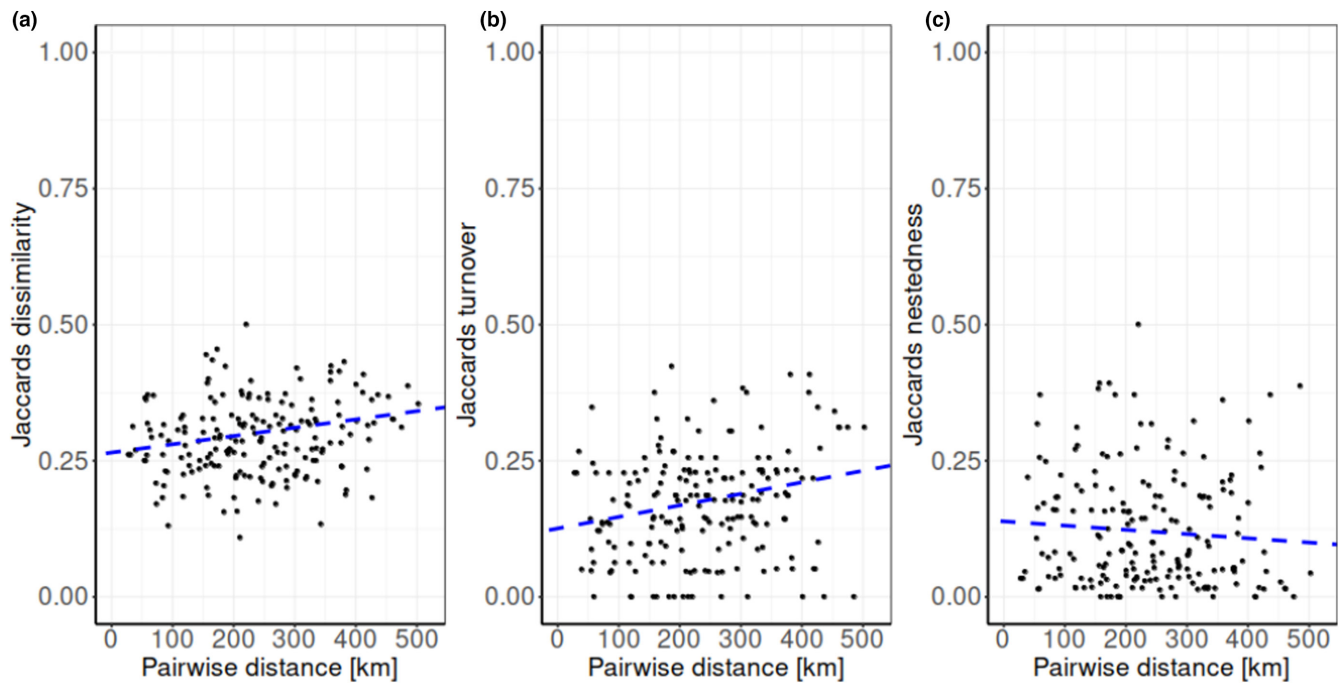


FIGURE 4 Distance decay models for (a) beta diversity, (b) nestedness, and (c) turnover between site-pairs within the Shaying River system. Dashed lines show predicted, yet non-significant slopes of the respective generalized linear distance-decay models.

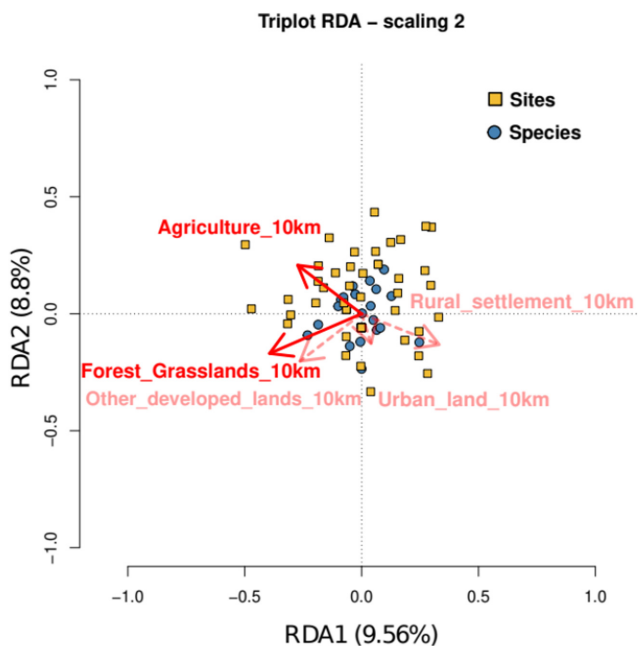


FIGURE 5 RDA-triplot showing the influence of land cover classes on the fish communities. Thick red arrows indicate significance of the variable. Blue circles correspond to species, yellow boxes correspond to sites.

4.1 | Observed richness patterns

When comparing species richness in the basin (gamma diversity) to the checklists of freshwater fish from 2013 and 1980, only 14 species overlapped between the checklist from 2013 and our eDNA

results from 2018. The imperfect overlap of checklists is a recurring issue in eDNA studies (Keck et al., 2022) and, at least in our case, can have multiple causes. First, it could be a lack of proper reference sequences in the databases for Chinese freshwater fish. In 2022, only 56% of all Chinese species had at least one sequence in the reference database (Li et al., 2022). Second, it is possible that the 2013 and 1980 checklists were incomplete. They showed a decrease from 46 to 33 species, indicating that either the collection was not complete in 2013 or there was a significant loss of species between those years. Although concrete evidence for the latter is absent, considering the extensive shift in species composition within the neighboring Yellow River system from 1960 to 2015 (Xie et al., 2018), the trend we observe for the Shaying River appears plausible. Still, given the data we have, we think it is rather a change in abundance and/or sampling effort between the years. Furthermore, in contrast to 33 and 43 species on the checklists, we found 63 Actinopterygii OTUs and therefore overestimated known richness. Considering the identity of some species in the eDNA data, we found signals for small and/or rare species (e.g., the different *Acheilognathus* species, *Oryzias sinensis*, *Carassius gibelio*); benthic and hidden living species (e.g., *Microphysogobio tungtingensis*, *Sinobdella sinensis*); and species related to widespread human introduction (e.g., *Gambusia affinis*) or consumption (*Silurus soldatovi*, *Channa maculata*). This is not surprising, as eDNA can be more sensitive to rare, small and/or benthic living species that are frequently missed by conventional methods (Cilleros et al., 2019; Olds et al., 2016; Sigsgaard et al., 2015). Still, the OTU approach can reliably recover diversity patterns in different environments. For instance, Marques et al. (2020) showed that the use of (M) OTUs is a viable proxy for species in biodiversity analysis in marine environments, but tends to overestimate local richness. In

freshwater, Blackman et al. (2021) successfully identified fish diversity across the Chao Phraya catchment in Thailand, showing the feasibility of partly taxonomy-free eDNA-based biodiversity studies to recover not only richness but also community patterns across river basins with similar or even slightly lower sampling efforts (with only one season covered, and sampling volumes being lower). However, the Chao Phraya basin has much more pronounced land-use and land cover heterogeneity compared to the Shaying River.

When identifying site-specific species richness (alpha diversity), we found that it differed across seasons (April, October) and the overall highest richness was measured when seasons were combined. There can be multiple reasons for this, the first being a real biological signal of migration or spawning, such that the richness or abundance of fish at sites varies across seasons. Fish species are known to migrate not only over long distances, but also show local and regional migration patterns on different temporal scales, according to their phenology (Brönmark et al., 2014; Schlosser, 1991) which potentially affects eDNA detection. For instance, spawning was shown to impact eDNA recovery for multiple taxa, and is often coupled with migratory behavior (Thalinger et al., 2019; Tillotson et al., 2018). Especially for cyprinids, within-stream migration behavior has been documented, and would therefore be a possible explanation of changing diversity patterns in this cyprinid-heavy study system (Brönmark et al., 2014). While these migration patterns are probably limited in anthropogenically altered systems (Perkin & Gido, 2012), we speculate that they may be still present and detectable at low levels for some species in the Shaying River. Yet, we also acknowledge that the spatial homogeneity of this effect is large, and it remains unclear if fish richness across such large ranges varies consistently between seasons. Second, it is possible that we detected eDNA-specific variation rather than community variation. Studies on the seasonality of eDNA showed that extremes of river flow (Thalinger et al., 2021) can lead to lower eDNA yield through greater dilution of DNA. In this study, there was higher discharge due to bad weather and snowmelt runoff during the spring sampling period, which could have led to more diluted DNA signals and incomplete sampling. Yet, in our study the species accumulation curves nearly reached a plateau for OTU richness in both seasons, indicating sufficient sampling and probably a good depiction of local richness. Still, by combining both seasons we ensured that possible effects of seasonality on the richness and species composition at each site are time-averaged for comparisons in the entire basin and across spatial gradients in the river network.

4.2 | Community patterns

By investigating the distance decay of beta diversity patterns along spatial gradients within the Shaying River basin, we identified only a small and statistically insignificant species turnover. This is surprising, as an increase in turnover with spatial distance is a nearly universal pattern and observable for many organism groups (Muneepeerakul et al., 2008; Soininen et al., 2007), again indicating

that the system studied is either insufficiently sampled or that the basin-wide homogenized land cover is removing otherwise biogeographically well-documented patterns. As described above, we are confident that insufficient sampling is not likely, given the saturated species accumulation curves. Given the anthropogenic pressures on the Shaying River basin, including dispersal barriers through dams, pollution, fishing, and strong channel alteration (Shi et al., 2016), it is likely that these communities are indeed highly similar; the current fish community is a depauperated subset of a formerly more diverse fish community; and sensitive and/or rare fish have already disappeared or been replaced by less sensitive species. It is known that river systems under anthropogenic pressure are more prone to the invasion of species, changing community composition and therefore altering (i.e., homogenizing) dissimilarity patterns on biogeographical scales (Leprieur et al., 2008; Su et al., 2021). In line with this, we found relatively stable signals of fish species associated with human consumption and aquaculture, such as the large Asian carps (*Hypophthalmichthys molitrix/nobilis*, *Ctenopharyngodon idella*) or the Snakehead (*Channa argus*), which are native to river systems from the Amur-river to Hainan, China, but are widely farmed or stocked and therefore invasive in some river systems, altering natural biodiversity patterns (Ju et al., 2020). Furthermore, some fish species are rare and/or highly specialized and therefore more sensitive to changes and pollution in their natural environment. They often disappear first, leading to communities dominated by widespread generalist species (Dala-Corte et al., 2016; Davies et al., 2004). When comparing the loss of species between 1980 and 2013 and 2018, the loss of some small, benthic catfish species (Genus *Pseudobagrus*) could indicate the loss or turnover of benthic species. This would be in line with a study in Brazilian grasslands, where Dala-Corte et al. (2016) showed how agricultural runoff changes the structure of a river bed through siltation and subsequent processes like primary production, leading to taxonomic loss and functional turnover of fish species. It has also been shown that the effects of agricultural land cover only affect riverine fish assemblages after a tipping point of more than 50% coverage within the river system (reviewed by Allan, 2004). These last points could be possible explanations of why, in the RDA, agriculture had some, relatively low, effects on fish species composition.

When comparing our findings about the fish communities to other organism groups assessed in the Shaying River basin in previous studies, some conclusions about the links between land cover and biodiversity are different. Li et al. (2020) showed strong effects of the surrounding land cover on multiple organism groups detected by eDNA metabarcoding, particularly on invertebrates. Specifically, they showed that dissimilarity of communities increases with human impact; across organism groups, there is a significant distance decay of communities; and land cover affects observable community compositions. However, they focused on small organisms, like invertebrates, protozoans, bacteria, algae, and fungi. Because of different body size, more active dispersal, and different sensitivity to pollution, fish and macro-invertebrates are expected to be associated with different drivers, and thus patterns, of diversity.

5 | CONCLUSION

In conclusion, we showed that eDNA metabarcoding identified biodiversity patterns in a highly altered river system, which did not correlate with land cover. Our result suggest that the anthropogenic alteration of rivers might homogenize both the landscape and riverine biodiversity, linking to weaker associations compared to natural river systems (Vellend et al., 2007; Zeni et al., 2020). Furthermore, our study suggests that the weak explanatory power of land cover and other environmental variables on fish diversity and communities is probably linked to a lack of environmental gradients in this homogenized basin. Even though land cover and environmental predictors do not seem to be strongly coupled to fish communities, they are strong enough to result in losing expected distance-decay patterns, richness gradients, and homogenization of overall community composition along the river network. Therefore, our findings underscore that homogenized land cover in a basin can potentially lead to riverine community homogenization. Environmental DNA-based methods enable us to investigate these impacts across multiple degrees of anthropogenic influence, ecosystems, scales, and biogeographic regions, improving our understanding of how we as humans alter not only the landscape, but also the river-scape and its communities.

AUTHOR CONTRIBUTIONS

DK, FA, LP, and XZ carried out project conceptualization. DK carried out data analysis and writing. YZ and WZ were involved in laboratory and fieldwork. XZ, LP, and FA were involved in supervision and project lead.

ACKNOWLEDGMENTS

We thank the Editor and two anonymous Reviewers for their input to improve the quality of this manuscript and Gabrielle Vance for final proofreading.

FUNDING INFORMATION

Funding for DK was provided by the ETH research commission and the project "BECCY." FA is further supported by the University of Zürich Research Priority Program on Global Change and Biodiversity (URPP GCB) as well as the Swiss National Science Foundation, grant No. 31003A_173074. X.Z. was supported by the Fundamental Research Funds for the Central Universities.

CONFLICT OF INTEREST STATEMENT

No conflict of interest is declared by the authors.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available in the supporting information. Raw sequencing data (Fastq) are available from the corresponding author upon reasonable request. The code to reproduce the figures and analyses in this manuscript is available in the supplementary (Appendix S1) alongside the data. The data and scripts are also accessible on DRYAD: [10.5061/dryad.5x69p8d9p](https://doi.org/10.5061/dryad.5x69p8d9p).

ORCID

Dominik Kirschner  <https://orcid.org/0000-0001-5592-0494>
 Yan Zhang  <https://orcid.org/0000-0003-0688-5359>
 Wenjun Zhong  <https://orcid.org/0000-0003-3583-6695>
 Xiaowei Zhang  <https://orcid.org/0000-0001-8974-9963>
 Loïc Pellissier  <https://orcid.org/0000-0002-2289-8259>
 Florian Altermatt  <https://orcid.org/0000-0002-4831-6958>

REFERENCES

- Allan, J. D. (2004). Landscapes and riverscapes: The influence of land use on stream ecosystems. *Annual Review of Ecology, Evolution, and Systematics*, 35(1), 257–284. <https://doi.org/10.1146/annurev.ecolsys.35.1.20202.110122>
- Altermatt, F. (2013). Diversity in riverine metacommunities: A network perspective. *Aquatic Ecology*, 47(3), 365–377. <https://doi.org/10.1007/s10452-013-9450-3>
- Altermatt, F., Little, C. J., Mächler, E., Wang, S., Zhang, X., & Blackman, R. C. (2020). Uncovering the complete biodiversity structure in spatial networks: The example of riverine systems. *Oikos*, 129(5), 607–618. <https://doi.org/10.1111/oik.06806>
- Baselga, A., Orme, D., Villeger, S., Bortoli, J. D., Leprieur, F., & Logez, M. (2022). Betapart: Partitioning Beta Diversity into Turnover and Nestedness Components. Available from: <https://CRAN.R-project.org/package=betapart>
- Blackman, R. C., Osathanunkul, M., Brantschen, J., Di Muri, C., Harper, L. R., Mächler, E., Hänfling, B., & Altermatt, F. (2021). Mapping biodiversity hotspots of fish communities in subtropical streams through environmental DNA. *Scientific Reports*, 11(1), 10375. <https://doi.org/10.1038/s41598-021-89942-6>
- Boettiger, C., Chamberlain, S., Lang, D. T., Wainwright, P., & Cazelles, K. (2021). Rfishbase: R Interface to "FishBase". <https://CRAN.R-project.org/package=rfishbase>
- Brönmark, C., Hulthén, K., Nilsson, P. A., Skov, C., Hansson, L.-A., Brodersen, J., & Chapman, B. B. (2014). There and back again: Migration in freshwater fishes. *Canadian Journal of Zoology*, 92(6), 467–479. <https://doi.org/10.1139/cjz-2012-0277>
- Bruce, K., Blackman, R., Bourlat, S. J., Hellström, A. M., Bakker, J., Bista, I., Bohmann, K., Bouchez, A., Brys, R., Clark, K., Elbrecht, V., Fazi, S., Fonseca, V., Hänfling, B., Leese, F., Mächler, E., Mahon, A. R., Meissner, K., Panksep, K., ... Deiner, K. (2021). A practical guide to DNA-based methods for biodiversity assessment. *Advanced Books*, 1, e68634. <https://doi.org/10.3897/ab.e68634>
- Cantera, I., Coutant, O., Jézéquel, C., Decotte, J.-B., Dejean, T., Iribar, A., Vigouroux, R., Valentini, A., Muriene, J., & Brosse, S. (2022). Low level of anthropization linked to harsh vertebrate biodiversity declines in Amazonia. *Nature Communications*, 13(1), 3290. <https://doi.org/10.1038/s41467-022-30842-2>
- Carpenter, S. R., Caraco, N. F., Correll, D. L., Howarth, R. W., Sharpley, A. N., & Smith, V. H. (1998). Nonpoint pollution of surface waters with phosphorus and nitrogen. *Ecological Applications*, 8(3), 559–568. <https://doi.org/10.2307/2641247>
- Carraro, L., Blackman, R. C., & Altermatt, F. (2023). Modelling environmental DNA transport in rivers reveals highly resolved spatio-temporal biodiversity patterns. *Scientific Reports*, 13(1), 8854. <https://doi.org/10.1038/s41598-023-35614-6>
- Cilleros, K., Valentini, A., Allard, L., Dejean, T., Etienne, R., Grenouillet, G., Iribar, A., Taberlet, P., Vigouroux, R., & Brosse, S. (2019). Unlocking biodiversity and conservation studies in high-diversity environments using environmental DNA (eDNA): A test with Guianese freshwater fishes. *Molecular Ecology Resources*, 19(1), 27–46. <https://doi.org/10.1111/1755-0998.12900>
- Collen, B., Whitton, F., Dyer, E. E., Baillie, J. E. M., Cumberlidge, N., Darwall, W. R. T., Pollock, C., Richman, N. I., Soulsby, A.-M., &

- Böhm, M. (2014). Global patterns of freshwater species diversity, threat and endemism. *Global Ecology and Biogeography*, 23(1), 40–51. <https://doi.org/10.1111/geb.12096>
- Dala-Corte, R. B., Giam, X., Olden, J. D., Becker, F. G., Guimarães, T. F., & Melo, A. S. (2016). Revealing the pathways by which agricultural land-use affects stream fish communities in south Brazilian grasslands. *Freshwater Biology*, 61(11), 1921–1934. <https://doi.org/10.1111/fwb.12825>
- Davies, K. F., Margules, C. R., & Lawrence, J. F. (2004). A synergistic effect puts rare, specialized species at greater risk of extinction. *Ecology*, 85(1), 265–271. <https://doi.org/10.1890/03-0110>
- Davison, C. W., Rahbek, C., & Morueta-Holme, N. (2021). Land-use change and biodiversity: Challenges for assembling evidence on the greatest threat to nature. *Global Change Biology*, 27(21), 5414–5429. <https://doi.org/10.1111/gcb.15846>
- Deiner, K., & Altermatt, F. (2014). Transport distance of invertebrate environmental DNA in a Natural River. *PLoS One*, 9(2), e88786. <https://doi.org/10.1371/journal.pone.0088786>
- Deiner, K., Bik, H. M., Mächler, E., Seymour, M., Lacoursière-Roussel, A., Altermatt, F., Creer, S., Bista, I., Lodge, D. M., de Vere, N., Pfrender, M. E., & Bernatchez, L. (2017). Environmental DNA metabarcoding: Transforming how we survey animal and plant communities. *Molecular Ecology*, 26(21), 5872–5895. <https://doi.org/10.1111/mec.14350>
- Deiner, K., Fronhofer, E. A., Mächler, E., Walser, J.-C., & Altermatt, F. (2016). Environmental DNA reveals that rivers are conveyor belts of biodiversity information. *Nature Communications*, 7(1), 12544. <https://doi.org/10.1038/ncomms12544>
- Dudgeon, D., Arthington, A. H., Gessner, M. O., Kawabata, Z.-I., Knowler, D. J., Lévêque, C., Naiman, R. J., Prieur-Richard, A.-H., Soto, D., Stiassny, M. L. J., & Sullivan, C. A. (2006). Freshwater biodiversity: Importance, threats, status and conservation challenges. *Biological Reviews*, 81(2), 163–182. <https://doi.org/10.1017/S1464793105006950>
- Frøslev, T. G., Kjøller, R., Bruun, H. H., Ejrnæs, R., Brunbjerg, A. K., Pietroni, C., & Hansen, A. J. (2017). Algorithm for post-clustering curation of DNA amplicon data yields reliable biodiversity estimates. *Nature Communications*, 8(1), 1188. <https://doi.org/10.1038/s41467-017-01312-x>
- Hijmans, R. J., van Etten, J., Sumner, M., Cheng, J., Baston, D., Bevan, A., Bivand, R., Busetto, L., Canty, M., Fasoli, B., Forrest, D., Ghosh, A., Golicer, D., Gray, J., Greenberg, J. A., Hiemstra, P., Hingee, K., Ilich, A., Geosciences, I., ... Wueest, R. (2023). Raster: Geographic data analysis and modeling (3.6-14) [Computer software]. <https://CRAN.R-project.org/package=raster>
- Ho, H.-C., Brodersen, J., Gossner, M. M., Graham, C. H., Kaeser, S., Reji Chacko, M., Seehausen, O., Zimmermann, N. E., Pellissier, L., & Altermatt, F. (2022). Blue and green food webs respond differently to elevation and land use. *Nature Communications*, 13(1), 6415. <https://doi.org/10.1038/s41467-022-34132-9>
- IPBES. (2019). Summary for policymakers of the global assessment report on biodiversity and ecosystem services. Zenodo. <https://doi.org/10.5281/zenodo.3553579>
- Ju, R.-T., Li, X., Jiang, J.-J., Wu, J., Liu, J., Strong, D. R., & Li, B. (2020). Emerging risks of non-native species escapes from aquaculture: Call for policy improvements in China and other developing countries. *Journal of Applied Ecology*, 57(1), 85–90. <https://doi.org/10.1111/1365-2664.13521>
- Keck, F., Blackman, R. C., Bossart, R., Brantschen, J., Couton, M., Hürlemann, S., Kirschner, D., Locher, N., Zhang, H., & Altermatt, F. (2022). Meta-analysis shows both congruence and complementarity of DNA and eDNA metabarcoding to traditional methods for biological community assessment. *Molecular Ecology*, 31(6), 1820–1835. <https://doi.org/10.1111/mec.16364>
- Legendre, P., & Gauthier, O. (2014). Statistical methods for temporal and space–time analysis of community composition data†. *Proceedings of the Royal Society B: Biological Sciences*, 281(1778), 20132728. <https://doi.org/10.1098/rspb.2013.2728>
- Lehner, B., & Grill, G. (2013). Global river hydrography and network routing: Baseline data and new approaches to study the world's large river systems. *Hydrological Processes*, 27(15), 2171–2186. <https://doi.org/10.1002/hyp.9740>
- Leitão, R. P., Zuanon, J., Mouillot, D., Leal, C. G., Hughes, R. M., Kaufmann, P. R., Villéger, S., Pompeu, P. S., Kasper, D., de Paula, F. R., Ferraz, S. F. B., & Gardner, T. A. (2018). Disentangling the pathways of land use impacts on the functional structure of fish assemblages in Amazon streams. *Ecography*, 41(1), 219–232. <https://doi.org/10.1111/ecog.02845>
- Leprieux, F., Beauchard, O., Blanchet, S., Oberdorff, T., & Brosse, S. (2008). Fish invasions in the World's river systems: When natural processes are blurred by human activities. *PLoS Biology*, 6(2), e28. <https://doi.org/10.1371/journal.pbio.0060028>
- Leray, M., Knowlton, N., & Machida, R. J. (2022). eMIDORI2: A collection of quality controlled, preformatted, and regularly updated reference databases for taxonomic assignment of eukaryotic mitochondrial sequences. *Environmental DNA*, 4(4), 894–907. <https://doi.org/10.1002/edn3.303>
- Li, F., Altermatt, F., Yang, J., An, S., Li, A., & Zhang, X. (2020). Human activities' fingerprint on multitrophic biodiversity and ecosystem functions across a major river catchment in China. *Global Change Biology*, 26(12), 6867–6879. <https://doi.org/10.1111/gcb.15357>
- Li, F., Peng, Y., Fang, W., Altermatt, F., Xie, Y., Yang, J., & Zhang, X. (2018). Application of environmental DNA metabarcoding for predicting anthropogenic pollution in rivers. *Environmental Science & Technology*, acs.est.8b03869. <https://doi.org/10.1021/acs.est.8b03869>
- Li, F., Zhang, Y., Altermatt, F., & Zhang, X. (2021). Consideration of multitrophic biodiversity and ecosystem functions improves indices on river ecological status. *Environmental Science & Technology*, 55(24), 16434–16444. <https://doi.org/10.1021/acs.est.1c05899>
- Li, F., Zhang, Y., Altermatt, F., Zhang, X., Cai, Y., & Yang, Z. (2022). Gap analysis for DNA-based biomonitoring of aquatic ecosystems in China. *Ecological Indicators*, 137, 108732. <https://doi.org/10.1016/j.ecolind.2022.108732>
- Mahé, F., Czech, L., Stamatakis, A., Quince, C., de Vargas, C., Dunthorn, M., & Rognes, T. (2021). Swarm v3: Towards tera-scale amplicon clustering. *Bioinformatics*, 38(1), 267–269. <https://doi.org/10.1093/bioinformatics/btab493>
- Mahé, F., Rognes, T., Quince, C., de Vargas, C., & Dunthorn, M. (2014). Swarm: Robust and fast clustering method for amplicon-based studies. *PeerJ*, 2, e593. <https://doi.org/10.7717/peerj.593>
- Mahé, F., Rognes, T., Quince, C., de Vargas, C., & Dunthorn, M. (2015). Swarm v2: Highly-scalable and high-resolution amplicon clustering. *PeerJ*, 3, e1420. <https://doi.org/10.7717/peerj.1420>
- Marques, V., Guérin, P.-É., Rocle, M., Valentini, A., Manel, S., Mouillot, D., & Dejean, T. (2020). Blind assessment of vertebrate taxonomic diversity across spatial scales by clustering environmental DNA metabarcoding sequences. *Ecography*, 43(12), 1779–1790.
- Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet Journal*, 17(1), 10. <https://doi.org/10.14806/ej.17.1.200>
- McFadden, I. R., Sendek, A., Brosse, M., Bach, P. M., Baity-Jesi, M., Bolliger, J., Bollmann, K., Brockerhoff, E. G., Donati, G., Gebert, F., Ghosh, S., Ho, H., Khaliq, I., Lever, J. J., Logar, I., Moor, H., Odermatt, D., Pellissier, L., de Queiroz, L. J., ... Narwani, A. (2023). Linking human impacts to community processes in terrestrial and freshwater ecosystems. *Ecology Letters*, 26(2), 203–218. <https://doi.org/10.1111/ele.14153>
- Miya, M., Sato, Y., Fukunaga, T., Sado, T., Poulsen, J. Y., Sato, K., Minamoto, T., Yamamoto, S., Yamanaka, H., Araki, H., Kondoh, M., & Iwasaki, W. (2015). MiFish, a set of universal PCR primers for metabarcoding environmental DNA from fishes: Detection of more than 230 subtropical marine species. *Royal Society Open Science*, 2(7), 150088. <https://doi.org/10.1098/rsos.150088>

- Muneepeerakul, R., Bertuzzo, E., Lynch, H. J., Fagan, W. F., Rinaldo, A., & Rodriguez-Iturbe, I. (2008). Neutral metacommunity models predict fish diversity patterns in Mississippi–Missouri basin. *Nature*, 453(7192), 220–222. <https://doi.org/10.1038/nature06813>
- Murali, A., Bhargava, A., & Wright, E. S. (2018). IDTAXA: A novel approach for accurate taxonomic classification of microbiome sequences. *Microbiome*, 6(1), 140. <https://doi.org/10.1186/s40168-018-0521-5>
- Newbold, T., Hudson, L. N., Hill, S. L. L., Contu, S., Lysenko, I., Senior, R. A., Börger, L., Bennett, D. J., Choimes, A., Collen, B., Day, J., De Palma, A., Díaz, S., Echeverria-Londoño, S., Edgar, M. J., Feldman, A., Garon, M., Harrison, M. L. K., Alhusseni, T., ... Purvis, A. (2015). Global effects of land use on local terrestrial biodiversity. *Nature*, 520(7545), 45–50. <https://doi.org/10.1038/nature14324>
- Oberdorff, T., Guégan, J.-F., & Huguény, B. (1995). Global scale patterns of fish species richness in rivers. *Ecography*, 18(4), 345–352. <https://doi.org/10.1111/j.1600-0587.1995.tb00137.x>
- Oberdorff, T., Tedesco, P. A., Huguény, B., Leprieux, F., Beauchard, O., Brosse, S., & Dürr, H. H. (2011). Global and regional patterns in riverine fish species richness: A review. *International Journal of Ecology*, 2011, e967631. <https://doi.org/10.1155/2011/967631>
- Oksanen, J., Simpson, G. L., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B., Solymos, P., Stevens, M. H. H., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B., Borcard, D., Carvalho, G., Chirico, M., Caceres, M. D., Durand, S., ... Weedon, J. (2022). Vegan: Community Ecology Package. <https://CRAN.R-project.org/package=vegan>.
- Olds, B. P., Jerde, C. L., Renshaw, M. A., Li, Y., Evans, N. T., Turner, C. R., Deiner, K., Mahon, A. R., Brueseke, M. A., Shirey, P. D., Pfrender, M. E., Lodge, D. M., & Lambert, G. A. (2016). Estimating species richness using environmental DNA. *Ecology and Evolution*, 6(12), 4214–4226. <https://doi.org/10.1002/ece3.2186>
- Pawlowski, J., Apothéloz-Perret-Gentil, L., & Altermatt, F. (2020). Environmental DNA: What's behind the term? Clarifying the terminology and recommendations for its future use in biomonitoring. *Molecular Ecology*, 29(22), 4258–4264. <https://doi.org/10.1111/mec.15643>
- Pebesma, E. (2018). Simple features for R: Standardized support for spatial vector data. *The R Journal*, 10(1), 439. <https://doi.org/10.32614/RJ-2018-009>
- Perkin, J. S., & Gido, K. B. (2012). Fragmentation alters stream fish community structure in dendritic ecological networks. *Ecological Applications*, 22(8), 2176–2187. <https://doi.org/10.1890/12-0318.1>
- Pont, D., Rocle, M., Valentini, A., Civade, R., Jean, P., Maire, A., Roset, N., Schabuss, M., Zornig, H., & Dejean, T. (2018). Environmental DNA reveals quantitative patterns of fish biodiversity in large rivers despite its downstream transportation. *Scientific Reports*, 8(1), 10361. <https://doi.org/10.1038/s41598-018-28424-8>
- Rognes, T., Flouri, T., Nichols, B., Quince, C., & Mahé, F. (2016). VSEARCH: A versatile open source tool for metagenomics. *PeerJ*, 4, e2584. <https://doi.org/10.7717/peerj.2584>
- Schlosser, I. J. (1991). Stream fish ecology: A landscape perspective. *Bioscience*, 41(10), 704–712. <https://doi.org/10.2307/1311765>
- Shi, W., Xia, J., & Zhang, X. (2016). Influences of anthropogenic activities and topography on water quality in the highly regulated Huai River basin, China. *Environmental Science and Pollution Research*, 23(21), 21460–21474. <https://doi.org/10.1007/s11356-016-7368-8>
- Sigsgaard, E. E., Carl, H., Møller, P. R., & Thomsen, P. F. (2015). Monitoring the near-extinct European weather loach in Denmark based on environmental DNA from water samples. *Biological Conservation*, 183, 46–52. <https://doi.org/10.1016/j.biocon.2014.11.023>
- Soininen, J., McDonald, R., & Hillebrand, H. (2007). The distance decay of similarity in ecological communities. *Ecography*, 30(1), 3–12. <https://doi.org/10.1111/j.0906-7590.2007.04817.x>
- Su, G., Logez, M., Xu, J., Tao, S., Villéger, S., & Brosse, S. (2021). Human impacts on global freshwater fish biodiversity. *Science*, 371(6531), 835–838. <https://doi.org/10.1126/science.abd3369>
- Tedesco, P. A., Oberdorff, T., Lasso, C. A., Zapata, M., & Huguény, B. (2005). Evidence of history in explaining diversity patterns in tropical riverine fish. *Journal of Biogeography*, 32(11), 1899–1907. <https://doi.org/10.1111/j.1365-2699.2005.01345.x>
- Thalinger, B., Kirschner, D., Pütz, Y., Moritz, C., Schwarzenberger, R., Wanzenböck, J., & Traugott, M. (2021). Lateral and longitudinal fish environmental DNA distribution in dynamic riverine habitats. *Environmental DNA*, 3(1), 305–318.
- Thalinger, B., Wolf, E., Traugott, M., & Wanzenböck, J. (2019). Monitoring spawning migrations of potamodromous fish species via eDNA. *Scientific Reports*, 9(1), 15388. <https://doi.org/10.1038/s41598-019-51398-0>
- Thomsen, P. F., Kielgast, J., Iversen, L. L., Møller, P. R., Rasmussen, M., & Willerslev, E. (2012). Detection of a diverse marine fish Fauna using environmental DNA from seawater samples. *Plos One*, 7(8), e41732. <https://doi.org/10.1371/journal.pone.0041732>
- Thomsen, P. F., & Willerslev, E. (2015). Environmental DNA – An emerging tool in conservation for monitoring past and present biodiversity. *Biological Conservation*, 183, 4–18. <https://doi.org/10.1016/j.biocon.2014.11.019>
- Tillotson, M. D., Kelly, R. P., Duda, J. J., Hoy, M., Kralj, J., & Quinn, T. P. (2018). Concentrations of environmental DNA (eDNA) reflect spawning salmon abundance at fine spatial and temporal scales. *Biological Conservation*, 220, 1–11. <https://doi.org/10.1016/j.biocon.2018.01.030>
- Tyers, M. (2022). Riverdist: River network distance computation and applications (0.15.5) [Computer software]. <https://CRAN.R-project.org/package=riverdist>
- Valentini, A., Taberlet, P., Miaud, C., Civade, R., Herder, J., Thomsen, P. F., Bellemain, E., Besnard, A., Coissac, E., Boyer, F., Gaboriaud, C., Jean, P., Poulet, N., Roset, N., Copp, G. H., Geniez, P., Pont, D., Argillier, C., Baudoin, J.-M., ... Dejean, T. (2016). Next-generation monitoring of aquatic biodiversity using environmental DNA metabarcoding. *Molecular Ecology*, 25(4), 929–942. <https://doi.org/10.1111/mec.13428>
- Van Driessche, C., Everts, T., Neyrinck, S., Halfmaerten, D., Haegeman, A., Ruttink, T., Bonte, D., & Brys, R. (2023). Using environmental DNA metabarcoding to monitor fish communities in small rivers and large brooks: Insights on the spatial scale of information. *Environmental Research*, 228, 115857. <https://doi.org/10.1016/j.envres.2023.115857>
- Vannote, R. L., Minshall, G. W., Cummins, K. W., Sedell, J. R., & Cushing, C. E. (1980). The river continuum concept. *Canadian Journal of Fisheries and Aquatic Sciences*, 37(1), 130–137. <https://doi.org/10.1139/f80-017>
- Vellend, M., Srivastava, D. S., Anderson, K. M., Brown, C. D., Jankowski, J. E., Kleynhans, E. J., Kraft, N. J. B., Letaw, A. D., Macdonald, A. A. M., Maclean, J. E., Myers-Smith, I. H., Norris, A. R., & Xue, X. (2014). Assessing the relative importance of neutral stochasticity in ecological communities. *Oikos*, 123(12), 1420–1430. <https://doi.org/10.1111/oik.01493>
- Vellend, M., Verheyen, K., Flinn, K. M., Jacquemyn, H., Kolb, A., Van Calster, H., Peterken, G., Graae, B. J., Bellemare, J., Honnay, O., Brunet, J., Wulf, M., Gerhardt, F., & Hermy, M. (2007). Homogenization of forest plant communities and weakening of species–environment relationships via agricultural land use. *Journal of Ecology*, 95(3), 565–573. <https://doi.org/10.1111/j.1365-2745.2007.01233.x>
- Vörösmarty, C. J., McIntyre, P. B., Gessner, M. O., Dudgeon, D., Prusevich, A., Green, P., Glidden, S., Bunn, S. E., Sullivan, C. A., Liermann, C. R., & Davies, P. M. (2010). Global threats to human water security and river biodiversity. *Nature*, 467(7315), 555–561. <https://doi.org/10.1038/nature09440>

- Weixian (舒卫先), S., & Cuizhen (韦翠珍), W. (2015). Analysis on the species composition and characteristics of fish in the Shaying River. *Governance of the Huaihe River*, (1), 27–28.
- Williams, T. J., & Johnson, J. B. (2022). History predicts contemporary community diversity within a biogeographic province of freshwater fish. *Journal of Biogeography*, 49(5), 809–821. <https://doi.org/10.1111/jbi.14316>
- Wright, E. S. (2015). DECIPHER: Harnessing local sequence context to improve protein multiple sequence alignment. *BMC Bioinformatics*, 16(1), 322. <https://doi.org/10.1186/s12859-015-0749-z>
- Xiao-nan, Z., Qiu-ping, G., Xiao-xue, S., Sheng-wen, Y., & Guo-yu, Q. (2015). Water quality, agriculture and food safety in China: Current situation, trends, interdependencies, and management. *Journal of Integrative Agriculture*, 14(11), 2365–2379. [https://doi.org/10.1016/S2095-3119\(15\)61128-5](https://doi.org/10.1016/S2095-3119(15)61128-5)
- Xie, J. Y., Tang, W. J., & Yang, Y. H. (2018). Fish assemblage changes over half a century in the Yellow River, China. *Ecology and Evolution*, 8(8), 4173–4182. <https://doi.org/10.1002/ece3.3890>
- Yan, L. (2023). ggvenn: Draw Venn Diagram by “ggplot2”. <https://cran.r-project.org/web/packages/ggvenn/index.html>
- Yang, J., Zhang, L., Mu, Y., & Zhang, X. (2023). Small changes make big progress: A more efficient eDNA monitoring method for freshwater fish. *Environmental DNA*. <https://doi.org/10.1002/edn3.387>
- Zeni, J. O., Hoehinghaus, D. J., Roa-Fuentes, C. A., & Casatti, L. (2020). Stochastic species loss and dispersal limitation drive patterns of spatial and temporal beta diversity of fish assemblages in tropical agroecosystem streams. *Hydrobiologia*, 847(18), 3829–3843. <https://doi.org/10.1007/s10750-020-04356-1>
- Zhang, H., Mächler, E., Morsdorf, F., Niklaus, P. A., Schaepman, M. E., & Altermatt, F. (2023). A spatial fingerprint of land-water linkage of biodiversity uncovered by remote sensing and environmental DNA. *Science of the Total Environment*, 867, 161365. <https://doi.org/10.1016/j.scitotenv.2022.161365>

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Kirschner, D., Zhang, Y., Zhong, W., Zhang, X., Pellissier, L., & Altermatt, F. (2023). Homogeneity in terrestrial land cover is reflected in fish diversity patterns in a Chinese river system. *Environmental DNA*, 00, 1–12. <https://doi.org/10.1002/edn3.480>