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Human activities' fingerprint on multitrophic biodiversity and ecosystem functions across a major river catchment in China

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Abstract

Human-induced global change dramatically alters individual aspects of river biodiversity, such as taxonomic, phylogenetic or functional diversity, and is predicted to lead to losses of associated ecosystem functions. Understanding these losses and dependencies are critical to human well-being. Until now, however, most studies have only looked either at individual organismal groups or single functions, and little is known on the effect of human activities on multitrophic biodiversity and on ecosystem multifunctionality in riverine ecosystem. Here we profiled biodiversity from bacteria to invertebrates based on environmental DNA (hereafter, 'eDNA') samples across a major river catchment in China, and analysed their dependencies with multiple ecosystem functions, especially linked to C/N/P-cycling. Firstly, we found a spatial cross-taxon congruence pattern of communities' structure in the network of the Shaying river, which was related to strong environmental filtering due to human land use. Secondly, human land use explained the decline of multitrophic and multifaceted biodiversity and ecosystem functions, but increased functional redundancy in the riverine ecosystem. Thirdly, biodiversity and ecosystem function relationships at an integrative level showed a concave-up (non-saturating) shape. Finally, structural equation modeling suggested that land use affects ecosystem functions through biodiversity-mediated pathways, including biodiversity loss and altered community interdependence in multitrophic groups. Our study highlights the value of a complete and inclusive assessment of biodiversity and ecosystem functions for an integrated land-use management of riverine ecosystems.

KEYWORDS

biodiversity, ecosystem functions, functional diversity, land use, phylogenetic diversity, river networks, taxonomic diversity

1 | INTRODUCTION

Rivers are an important ecosystem for diverse life forms on Earth and provide invaluable goods and services for mankind (Vörösmarty et al., 2010). Unfortunately, catastrophic declines in biodiversity caused by human activities have been observed, with decreases in species' populations over 80% since 1970 in freshwater (WWF, 2018). The declines have mainly been related to land-use change, pollution, damming and fragmentation (Best, 2019; Grill et al., 2019). Particularly, the marked changes in the global landscape led by expansion and intensification of land use (Gibbs et al., 2010; Hansen et al., 2013; Song et al., 2018) have had dramatic effects on riverine ecosystems (Best, 2019; Kominoski & Rosemond, 2012), including direct loss of species (Dudgeon et al., 2006). Furthermore, changes in riparian vegetation or alteration of natural flow regimes through damming affect the retention of organic matter in rivers, and subsequently alter ecosystem processes (e.g., transfer of matter and energy; Gounand et al., 2018; Grill et al., 2019; Kominoski & Rosemond, 2012). However, to date, we still lack a comprehensive perspective on how human land use impacts biodiversity and ecosystem functions in rivers.

Improving biodiversity data is among the most central steps to reveal the human-induced species loss and their respective roles in ecosystem processes (Altermatt et al., 2020). Although biodiversity variables include taxonomic, phylogenetic, and functional attributes, most studies have focused on generic taxonomic diversity measures (usually measured as species richness or abundance), but ignoring the evolutionary lineages connecting all species (phylogenetic diversity; Faith, 2006) and the growth forms and resource use strategies of species (functional diversity; Menezes et al., 2010). These three aspects of biodiversity do not necessarily relate to each other, may have contrasting effects on ecosystem functions (Le Bagousse-Pinguet et al., 2019), and may vary differently along spatial and environmental gradients (Jarzyna & Jetz, 2016). Furthermore, complete biodiversity assessments should include multidimensional perspectives, such as across taxonomic and functional groups, to better understand ecosystem functions (Eisenhauer et al., 2019). However, most studies only looked at one or a few groups (e.g., fish, algae, or invertebrates), which do not allow recognizing patterns of multitrophic biodiversity in response to human land use, and also hinder a complete understanding of the role of each group for specific ecosystem functions.

Ecosystem functions can be considered as endpoint consequences of ecological processes regulated by biodiversity (Cardinale, 2011; Cardinale et al., 2012). Biomass and changes in biomass are some of the most commonly measured ecosystem functions, especially in terrestrial plant communities. However, biomass does mostly capture productivity and not necessarily other metrics, such as decomposition or resource turnover (Gounand et al., 2020). Therefore, in riverine ecosystem other measures have been proposed, such as decomposition, assessed in leaf litter and cotton-strip assays (Chauvet et al., 2016; Jabiol et al., 2020). Furthermore, also enzyme activities have been used as metrics to describe ecosystem functions (Bodmer et al., 2016; Fuß et al., 2017; Schuldt et al., 2018). Enzyme activities not only provide the underlying mechanism for all ecosystem functions (as the enzymes are exhibiting the functions encoded in genes), but also reflect the role of microbiota in the transfer of matter and energy from low to high trophic level in ecosystems. However, it remains unclear how these multiple and key ecosystem functions are driven by human land use in rivers.

Biodiversity, ecosystem functions, and their links are all directly or indirectly regulated by specific-species interactions. Understanding and integrating of biodiversity within and across trophic levels is highly relevant in a biodiversity–ecosystem function (B-EF) perspective (Duffy et al., 2007; Eisenhauer et al., 2019; Wang & Brose, 2018). In recent decades, the study on B-EF relationships has increased substantially, but most work has focused on terrestrial ecosystems (Craven et al., 2018; Schuldt et al., 2018; Soliveres et al., 2016; Wang et al., 2019). These studies have consistently suggested that multitrophic and multifaceted biodiversity jointly drive ecosystem functions, but the direction and intensity of the B-EF relationships vary across ecosystems. By contrast, aquatic ecosystems have received less attention (Daam et al., 2019; Little et al., 2020; Vaughn, 2010), and most of the available studies were limited to micro- or mesocosms models (Cardinale et al., 2002; Jabiol et al., 2013; Pennekamp et al., 2018). Because effects of biodiversity loss themselves depend on the abiotic and biotic context and the spatial scale (Bond & Chase, 2002; Chase & Leibold, 2002; Daam et al., 2019; Vaughn, 2010), non-trivial dependencies are expected, especially in heterogeneous and highly spatially structured river networks (Altermatt, 2013; Shao et al., 2019).

Here we assessed biodiversity across major domains of life and ecosystem functions in a 40,000 km^2 region in the Shaying River basin (part of the Huai River) in China. Multitrophic biodiversity, including invertebrates, protozoa, fungi, algae, and bacteria, were obtained using environmental DNA (eDNA) technologies. eDNA technologies have rapidly advanced in their methodological development, with assessment of species' abundance or biomass being still more challenging than assessing species' presence/absence (Deiner et al., 2017). We take full advantage of the method to capture the complete biodiversity and to identify the spatial hierarchic structure of communities in rivers (Altermatt et al., 2020; Carraro et al., 2020; Deiner et al., 2016), so as to analyze the impact of human land use on multitrophic biodiversity and its dependencies with ecosystem functions (e.g., decomposition and enzyme activities). Our study hypothesizes that: (a) increased human land use reduces multitrophic and multifaceted biodiversity and ecosystem functions, yet the direction and intensity of their responses may be partially idiosyncratic at individual levels; (b) ecosystem functions are correlated with biodiversity, but their relationships may vary depending on the intensity of human land use.

2 | MATERIALS AND METHODS

2.1 | Study area and sampling

This study was conducted in the Shaying River basin, the largest tributary of the Huai River in China. This region has a high population

density (>26.4 million people) and intensity of human land use (agriculture, urbanization and industrialization). The whole region can be divided into three major groups (Figure 1; Tables S1 and S2): an upland area with a relatively dense riparian vegetation area and mild human imprint (*Mild Disturbance* region; 5 sites), and two further lowland areas that are characterized by intense agriculture and industry (*High Agriculture/Industry* region; 7 sites), and by intense agriculture (*High Agriculture* region; 7 sites), respectively. The sampling scheme adequately reflects the structure of river networks (Altermatt et al., 2020; Mächler et al., 2019) and the intensity of human land use. Finally, we set up 18 sites across the Shaying River basin. These sites are located in single tributaries and the further downstream reaches of their intersection, which can capture the hierarchic structure of diversity and ecosystem functions.

Field samples were collected across the Shaying River basin in April 2018. At each site, three one-liter samples of surface water were collected using sterile bottles (Thermo Fisher Scientific™), and immediately transferred to cryogenic incubators with several ice packs (ca. 0–4°C) until filtration treatment (within 6 hr; Li et al., 2018). We set up six field replicates (or subsamples) per site, for each of which 300–500 ml volume water (finally ca. 3 L of water at one site) was filtered using a Millipore 0.45 μm hydrophilic nylon membrane (Merck Millipore). All replicates of the eDNA membrane discs were individually placed in 5.0 ml centrifugal tubes, then immediately frozen and stored at −20°C until DNA extraction. At each site, blank controls were taken using autoclaved tap water (filtered 300 ml) to monitor possible contaminants.

2.2 | DNA extraction, PCR amplification, and sequencing

All the filter membrane discs were extracted using DNeasy PowerWater Kit (Qiagen). These eDNA extractions of six replicates and blank controls were subjected to all subsequent handling. PCR (polymerase chain reaction) assays were performed using three primer sets (Amaral-Zettler et al., 2009; Elbrecht & Leese, 2017; Klindworth et al., 2013; Muyzer et al., 1993; Table S3) for resolving communities of invertebrates, protozoa, fungi, algae, and bacteria. We added a unique 12 nt nucleotide tag at the 5'-ends of the forward or reverse primers. All PCRs were carried out in 30 μl reaction mixtures following the standard protocol (see Supplementary Note 1 for complete methods on protocol of PCR amplification and sequencing in the Supporting Information). All PCR products were quantified and pooled with equimolar quantities for subsequent sequencing. Depending on the PCR amplicon size, sequencing templates were sequenced in the Ion Proton sequencer (Life Technologies) and Illumina MiSeq PE300 platform (Illumina), respectively.

2.3 | Bioinformatic analysis

Raw reads generated by Ion Proton and Illumina sequencer were filtered through a series of quality control steps using QIIME toolkit (Caporaso et al., 2010; Table S4). First, read quality was assessed using fastx_toolkit. For sequencing reads generated by Illumina

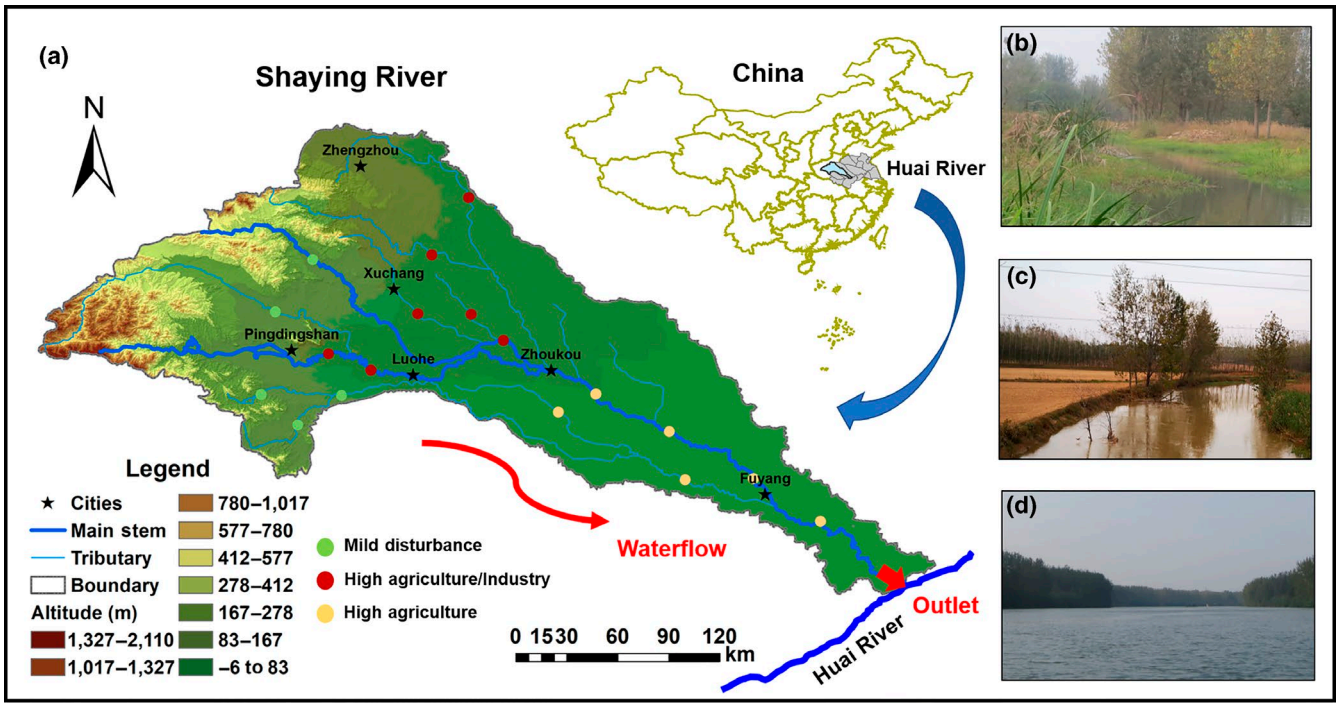

FIGURE 1 Overview and detailed map of the Shaying River basin showing the sampling sites, waterflow direction, and outlet location (a). Photographs are shown for rivers at different regions: stream (upland stream, *Mild Disturbance*, site Lihe; b), tributary (plain agricultural region, *High Agriculture/Industry*, site Qingliu; c) and main stem (plain agricultural region, *High Agriculture*, site Shaying; d). Site codes are as in Table S1 [Colour figure can be viewed at wileyonlinelibrary.com]

MiSeq PE300 platform, forward and reverse sequences were merged together using "*-fastq_mergepairs*" script with the default settings following USEARCH7 pipeline. Then, low-quality reads in both sequencers were discarded using "*split_libraries.py*" script with "*–w 50 –s 25 -l 100*" parameters. Next, sequences were de-replicated by removing exact duplicates, and discarded the singletons. PCR chimeras were identified using "*-uchime_denovo*" script with the default parameters following USEARCH7 pipeline. All cleaned sequences were clustered into OTU (Operational Taxonomic Unit) with 97% nucleotide similarity following the UPARSE pipeline. Taxonomic annotation of each OTU in protozoa, fungi, algae, and bacteria communities were assigned against the Greengenes database (DeSantis et al., 2006) and the Protist Ribosomal Reference database (Guillou et al., 2013) using "*align_seqs.py*" script, respectively. For each OTU in invertebrates community, the taxonomic annotation was assigned against a custom reference database (NCBI Genbank database and indigenous database) using BLASTN pipeline with ≥98% similarity cut-off. Indigenous database on invertebrates and the summary of results were presented in the Supplementary Note 2 for complete methods on barcode library for the Shaying River invertebrates in the Supporting Information. We kept only those taxa and OTU in invertebrates community that have been recorded in the Shaying River (Tables S5 and S6).

2.4 | Diversity measures

Any taxa and OTU detected in the blank controls were removed from all subsamples for subsequent analyses. Any OTU with relative abundances <0.001% and <10% detection frequency in all subsamples were discarded, these thresholds were used to clear all OTUs from the extraction and PCR negative controls. Next, OTU with detection frequency <50% among six subsamples in each site were discarded. Finally, the remaining OTU of subsamples were merged as reliable OTU detection in one site, the number of sequences in subsamples were averaged as the actually detected reads of each OTU in one site, and the relative abundance of each OTU was used for subsequent statistical analysis.

At each site, the taxonomic (species richness [Chao1 richness], Shannon's diversity index [*H′*], Pielou's evenness index [*J′*]), and phylogenetic (Faith's phylogenetic diversity index [*PD*]) diversity index of five trophic groups were calculated using *alpha_diversity.py* script in QIIME toolkit. Species traits of invertebrates, protozoa, and algae were summarized using different functional categories including body size, reproduction, respiration, food preference, and feeding habits (Abonyi et al., 2018; Bruno et al., 2019; Usseglio-Polatera et al., 2000; Yao et al., 2017). Three functional diversity indices were calculated to characterize all communities above (Bruno et al., 2019): functional richness (*FRic*), Rao's quadratic entropy (*FD*), and functional redundancy (*FRed*). *FRic* and *FD* indices were calculated using the *dbFD* function in the *FD* package (Casanoves et al., 2011). *FRed* was estimated as the difference between taxonomic diversity (using the Gini–Simpson diversity index) and *FD* index. Details on species traits and functional metrics calculation have been described (see Supplementary Note 3 for complete methods on functional traits in the Supporting Information, Tables S7–S9).

To obtain an integrated index reflecting taxonomic (MultiTaxa), phylogenetic (MultiPhyl), and functional (MultiFunc) diversity across multitrophic groups, we referred to previously published methods (Schuldt et al., 2018) by averaging the *z*-score of three kinds of diversity indices across five groups, respectively (see Supplementary Note 4 for complete methods on multitrophic metrics in the Supporting Information, Figure S1a–d).

2.5 | Ecosystem functions

We measured components of key ecosystem processes related to energy and nutrient flows across trophic levels in riverine ecosystems. Leaf litter (*Populus alba*) decomposition and cotton strip decomposition were measured, whereby we separated pure microbial decomposition from decomposition also including larger invertebrates (Chauvet et al., 2016). Microbial activities were surrogated by metrics of four functional enzyme activities that play important roles in decomposition processes and nutrient (C/N/P) cycling by degrading cellulose (β-glucosidase, β-xylosidase), chitin (chitinase), and polyphosphates (alkaline phosphatase; Schuldt et al., 2018; Tlili et al., 2017). All of these components were analyzed and linked to land-use drivers independently as well as in aggregated multifaceted way. Aggregated indices on ecosystem multifunctionality (EMF) of each sample were obtained following a previous study (Maestre et al., 2012). Briefly, the individually measured functions mentioned above were first standardized using the z-score transformation, and were then averaged to obtain a multifunctionality index. Details on sample collection, treatment, and formula involved in the measurement of ecosystem functions are described herewith (see Supplementary Note 5 for complete methods on ecosystem functions in the Supporting Information).

While the EMF index obtained by this simplified approach has some limitations (e.g., one function having high values compensating for a second function with low values), it provided a straightforward and easily interpretable measure of the ability of different communities to sustain multiple ecosystem functions. In addition, we also found that there were significant relations among the EMF index and single functional components (Figure S1e). For simplicity, we carried out the average approach in this study for obtaining the EMF index of each sample.

2.6 | Spatial analysis

River geospatial parameters, including spatial distance to outlet (to the main stem of Huai River) and topological distance between all sites, were calculated using the Hydrology Tool, Data Management, and Network Analysis tools in ArcGIS software (10.2 version). These two parameters reflect the inherent properties of river's dendritic

structure, and the flow distance and residence time of water in the river. Digital elevation model (DEM) data (250 m resolution) derived from SRTM (Shuttle Radar Topographic Mission) V4.1 dataset was used to delineate watershed boundaries and watercourse using the Hydrology tool in ArcGIS software. Human land-use raster maps (30 m resolution) interpreted from Landsat 8 remote-sensing image in 2018 were used to calculate parameters of land-use patterns. At each site, we focused on six buffer regions (at 500 m, 1, 5, 10, 25, and 50 km radius, see also [Seymour et al., 2016]) for the upstream of that site, and calculated the percentages of four land-use types (see Supplementary Note 6 for complete methods on land-use patterns in the Supporting Information, Figure S2; Table S2). This range describes a continuum of human activities from local to regional scales. Land-use parameters in the buffer created above were extracted through buffer tools in ArcGIS software. All remote-sensing images were downloaded or purchased from the resource and environment data cloud platform of the Chinese Academy of Sciences (http://www.resdc.cn/).

2.7 | Statistical analyses

All datasets, including diversity and functional metrics, were standardized by *z*-score transformation (mean of 0 and *SD* of 1), and were then used for all statistical analyses. To identify the difference in land-use types, diversity, and functional metrics between each region, one-way analysis of variance tests was conducted, followed by post hoc Bonferroni tests. To test the variation of communities' structure among three regions, non-metric multidimensional scaling (nMDS) ordination based on Jaccard (invertebrates) and Bray–Curtis (protozoa, fungi, algae, and bacteria) dissimilarity matrices were used (Anderson, 2001; Clarke, 1993), and the significant differences were tested by permutational multivariate analyses of variance (PERMANOVA) with the Monte Carlo 999 permutations test in PRIMER-e (version 7) with PERMANOVA+ add-on software (PRIMER-E Ltd).

To analyze the significant correlation between communities' dissimilarity and topological (along the river networks) or land-use distance (the difference between any two sites of land-use data), a Mantel test was performed using the "*Kendall tau*" correlation method, incorporating 999 permutations (Mantel, 1967). To explore bivariate relationships between each biodiversity and ecosystem functions, generalized linear mixed-effects models (GLMM) were separately fitted. We used the Akaike information criterion corrected (AIC_c) to select the most parsimonious models with the lowest value ($\Delta AIC_c \leq 2$) using the "*lme*" package (Pinheiro et al., 2020) in *R* platform (R Core Team, 2018). AIC_c is a second-order bias correction to Akaike's information criterion for small sample size.

Structural equation modeling (SEM) was performed with the "*lavaan*" package (Rosseel, 2012) in the *R* platform to infer possible direct or indirect effects of human land use on biodiversity and ecosystem functions. Before modeling, we did collinearity analyses of all parameters and excluded the redundant variables (Pearson's $r > .7$). A priori hypotheses were proposed based on the known effects and relationships among human land use, biodiversity, and ecosystem functions (Figure S3). The goodness of fit of the SEM was evaluated using following measures (Rodrigues et al., 2018): the root of mean square error of approximation (RMSEA, ≤0.05), the comparative fit index (CFI, ≥0.95), the standardized root-mean-squared residual (SRMR, ≤0.08), and low chi-square value.

3 | RESULTS

3.1 | Spatial patterns of multitrophic biodiversity and their response to human land use

A total of 624 invertebrates OTUs, 903 protozoa OTUs, 490 fungi OTUs, 1,008 algae OTUs, and 8,310 bacteria OTUs were obtained by eDNA method with multiple PCR assays, annotating to 55 phyla, 171 classes, 313 orders, 496 families, 785 genera, and 445 species, respectively (Table S10; Figure S4). Details on the species composition of each community were presented in Supplementary Note 7 for eDNA results in the Supporting Information.

Communities' structure across five taxonomic groups had similar patterns along the spatial distance (from headwater to the outlet; Figure 2a–e). The significant differences of communities' structure across five taxonomic groups among different regions were identified by PERMANOVA test (pseudo-$F_{invertebrates} = 2.237$, $p = .007$; pseudo-$F_{protozoa} = 1.751$, $p < .031$; pseudo-$F_{fungi} = 3.011$, $p = .007$; pseudo-$F_{algae} = 2.656$, $p < .011$; and pseudo-$F_{bacteria} = 1.356$, $p = .051$). The dissimilarity in communities' structure (β diversity) increased following the increase of river networks topological distance between sites (Figure 2f; Figure S5).

Most measures of taxonomic diversity (Chao1 richness, Shannon diversity, and Pielou's evenness), phylogenetic diversity, functional richness (*FRic*), and diversity (*FD*) were highest in *Mild Disturbance* region (Table S11). Functional redundancy (*FRed*) of invertebrates and protozoa was highest in *High Agriculture/Industry* region, which was on average 18% and 13% higher than other two, respectively. Although the individual aspects of diversity responses to human land use were partially idiosyncratic, their response at overall levels showed a clear and consistent pattern of a loss in biodiversity at an increasing land-use intensity (Figure 3a–f; Figure S6; only functional redundancy showed an opposite pattern). The impact of both cropland and impervious cover on biodiversity in the 5 km buffer region was higher than other buffer regions, followed by the 10 km buffer region. In addition, β diversity also increased with the increase of human land-use intensity (Figure 3g,h; Figure S7).

3.2 | Ecosystem functions and its dependence on biodiversity

Leaf litter and cotton strips decomposition were highest in *Mild Disturbance* regions and lowest in *High Agriculture/Industry* regions

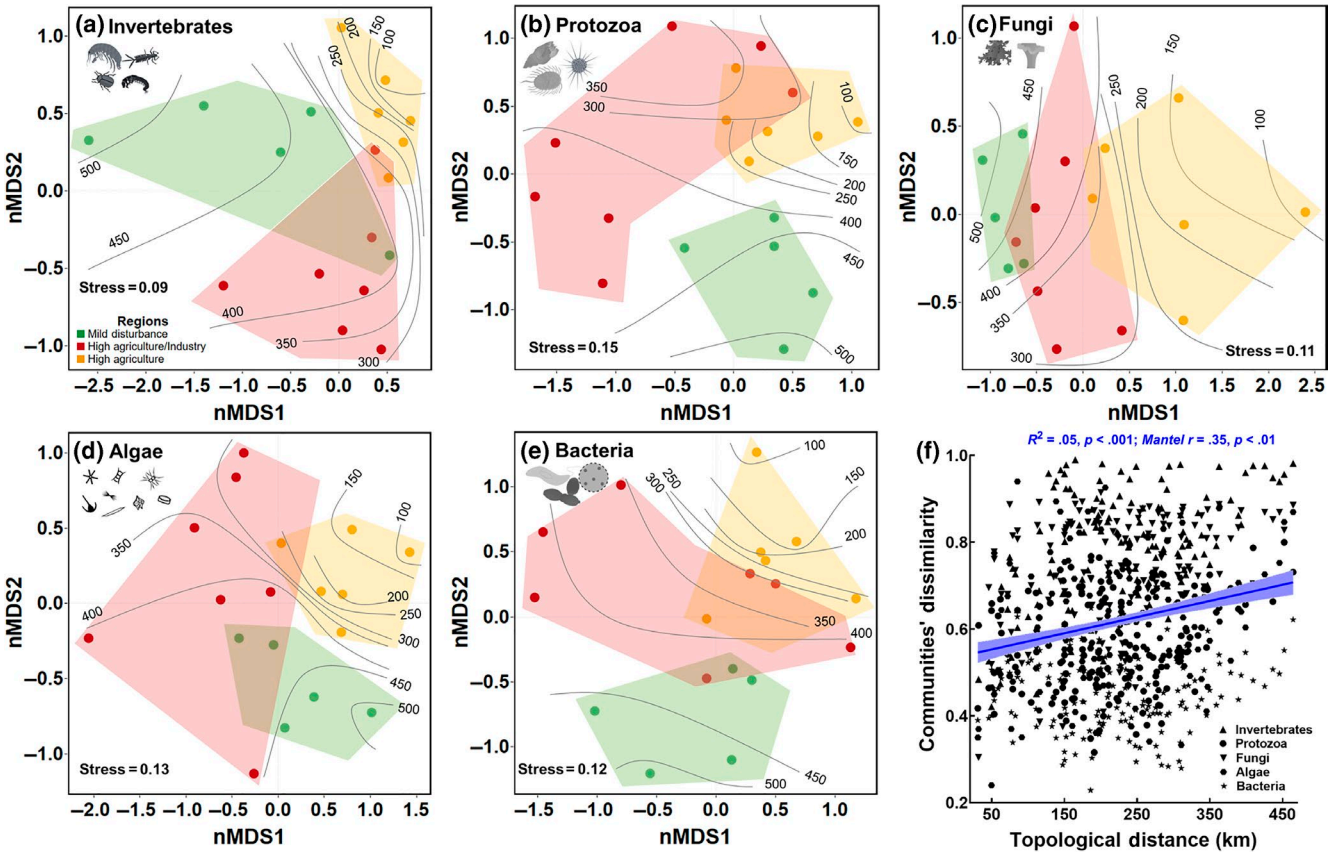

FIGURE 2 Ordination plots of invertebrates (a), protozoa (b), fungi (c), algae (d), and bacteria (e) community structure and the relationships between communities' dissimilarity (β-diversity) and spatial distance (f) across the river networks. The dots in 2D ordination space indicate communities' structure at one site in relationship to other sites. Sites located closer to each other share a larger percentage of taxa. Polygons contain all sites in the same region, and lines in the background show the contour lines of spatial distance (distance to the outlet of each site). Solid lines indicate the relationships by linear regression fit, shade represents the 95% confidence interval (95% CI), the r^2 and r values in the panel are derived from linear regressions and Mantel's tests, respectively [Colour figure can be viewed at wileyonlinelibrary.com]

(Table S12). Invertebrate-driven decomposition rates of leaf litter were higher in *Mild Disturbance* and *High Agriculture* regions, compared to High Agriculture/Industry regions. Furthermore, leaf litter decomposition in *High Agriculture/Industry* regions and the cotton strips decomposition were more associated to microbiota (Figure S8). Compared with leaf litter and cotton strip decomposition, the difference in activities of four enzymes among three regions was not obvious (Table S12). Overall, the reduced ecosystem functions were correlated with an increased land-use intensity, however, the intensity and direction of the response of individual function were not consistent (negative, positive, or flat; Figure S9). For example, decomposition and C-cycling enzymes' activities decreased with the increase of human land use, while alkaline phosphatase and chitinase activity increased or showed no dependency, respectively.

Metrics on biodiversity were directly and positively related to most ecosystem functions, but diverse dependencies were observed (Figure 4a). For example, most metrics in taxonomic, phylogenetic, and functional diversity were positively correlated with functional metrics (e.g., organic matter decomposition, C/N cycling), but were negatively correlated with metrics on *P* cycling and functional redundancy. For single groups, invertebrates, fungi, and bacteria were the stronger predictors of the organic matter decomposition and *C* cycling than protozoa and algae. Phylogenetic and functional diversity were more strongly associated to ecosystem functions than taxonomic diversity. For example, functional diversity of protozoa and phylogenetic diversity of fungi could better predict the *N* cycling in ecosystems than others. In addition, the direction of these dependencies in single groups was largely consistent with the direction of multitrophic drivers (e.g., MultiTaxa, MultiPhyl and MultiFunc) in multifunctionality analyses. The B-EF relationships at an integrative level were concave-up (non-saturating) over the parameter space looked at (Figure 4b–d).

3.3 | Effects of human land use on ecosystem functions via biodiversity-mediated pathways

Human land use indirectly affects ecosystem functions through multiple pathways of biodiversity changes (Figure 5). Firstly, human land use had negative impacts on diversity of all taxonomic groups, which reduced ecosystem functions. For example, cropland had a stronger negative drive on invertebrates (standardized path

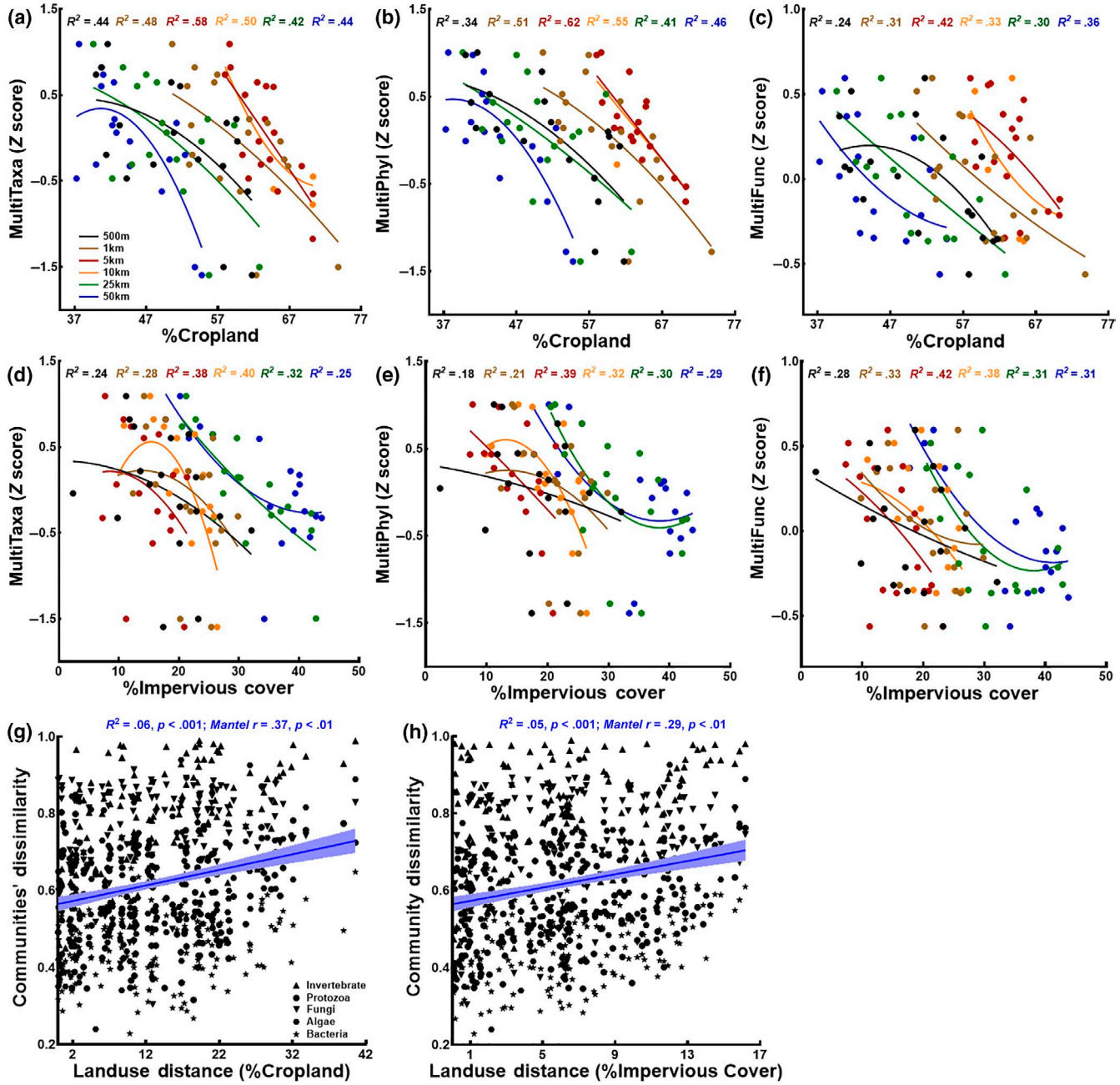

FIGURE 3 Effects of human land use on taxonomic (a, d), phylogenetic (b, e), and functional (c, f) diversity at multitrophic groups at six buffer regions, and relationships between β diversity (based on Jaccard or Bray–Curtis dissimilarity matrix) and land-use distance in 5 km buffer region (g, h). Land-use distance refers to the difference between any two sites of land-use data. Solid lines indicate the relationships by polynomial regression fit, shaded areas represent the 95% CI, the r^2 and r values in the panel are derived from linear regressions and Mantel's tests, respectively. "MultiTaxa," "MultiPhyl," and "MultiFunc" are the integrated metrics reflecting taxonomic, phylogenetic, and functional diversity across multitrophic groups, respectively [Colour figure can be viewed at wileyonlinelibrary.com]

coefficient = 0.41; $p < .001$) and fungi (standardized path coefficient = 0.46; $p = .021$), and impervious cover had more restrictions on invertebrates (standardized path coefficient = 0.38; $p < .001$) and algae (standardized path coefficient = 0.30; $p = .007$). Secondly, human land use affected the community interdependence in multitrophic groups, thereby reducing ecosystem functions. For instance, invertebrates and protozoa were directly involved in shaping algae and fungi in these regulatory pathways and thus indirectly mediated ecosystem functions. We still found that invertebrates, fungi, and algae were at the central (have strong and dense paths) of all the regulatory pathways by which human land use affects ecosystem functions. The *C* cycling was driven directly and positively by invertebrates, fungi, and algae, the *N* cycling was positively influenced by more groups (except invertebrates), and only fungi and bacteria are significantly linked to *P* cycling, but the links were negative. Overall, the data fitted our model well (RMSEA = 0.028, CFI = 0.977,

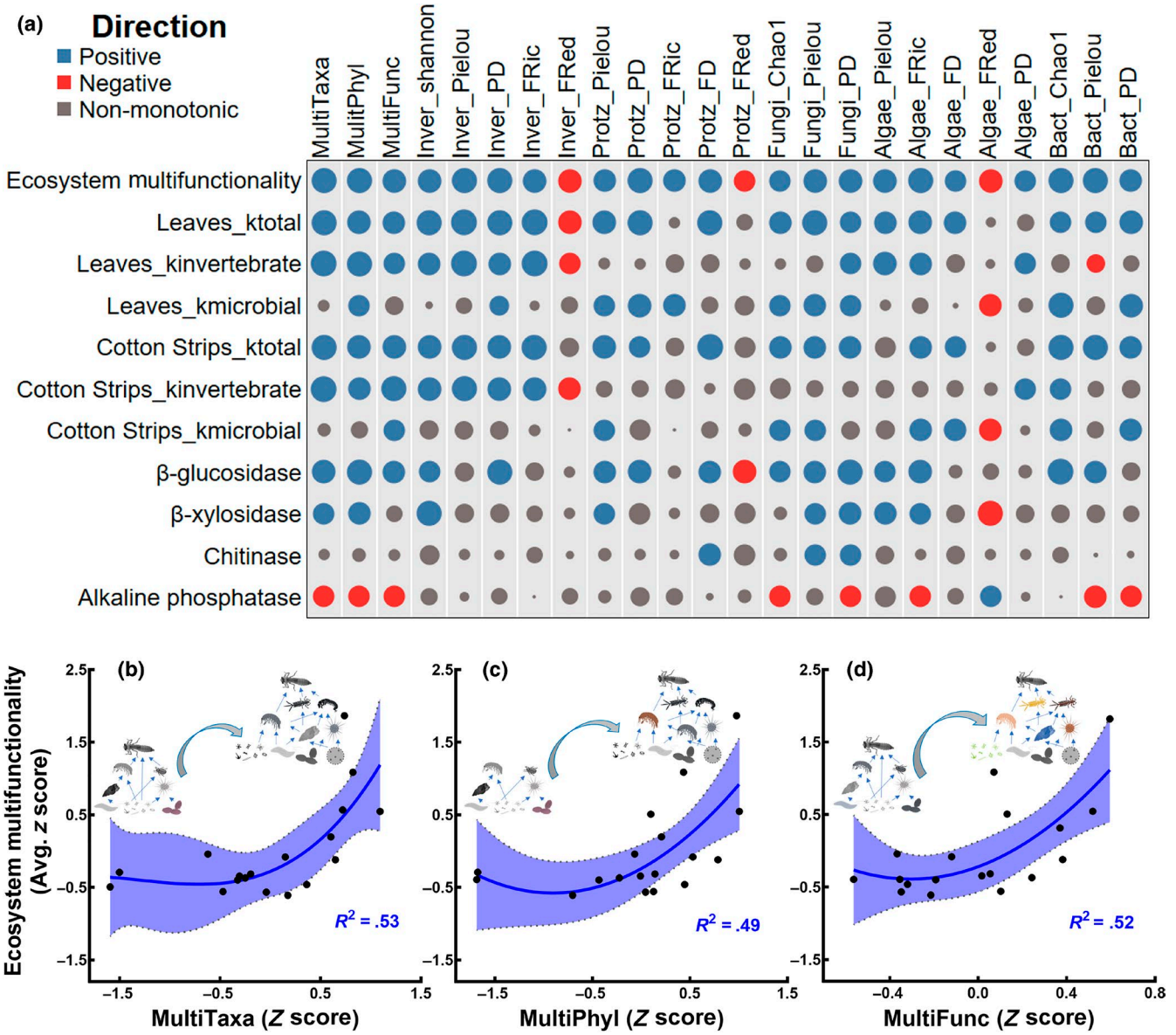

FIGURE 4 Biodiversity and ecosystem function relationships. The top panel shows the predictors' standardized estimate for ecosystem multifunctionality and carbon, nitrogen, and phosphorus cycling (a). Red bubbles represent monotone negative effects, blue bubbles indicate monotone positive effects, gray bubbles belong to non-monotone effects, and the size of bubbles gives the strength of the relationships. "MultiTaxa," "MultiPhyl," and "MultiFunc" are the integrated metrics on taxonomic, phylogenetic, and functional diversity across multitrophic groups, respectively. "Inver," "Protz," and "Bact" present the invertebrates, protozoa, and bacteria, respectively. "FRic," "FD," and "Fred" are for the functional richness, functional diversity, and functional redundancy of community, respectively. "-ktotal," "-kinvertebrate," and "-kmicrobial" are for the total, invertebrate-driven, and microbial-driven decomposition rates of leaves and cotton strips, respectively. Solid lines in the below panels show the relationships between taxonomic (b), phylogenetic, (c) and funcional (d) diversity of multitrophic groups and ecosystem multifunctionality with the 95% CI in shade [Colour figure can be viewed at wileyonlinelibrary.com]

SRMR = 0.053), all the pathways accounted for 65% of C cycling variation, 46% of N cycling variation, and 45% of P cycling variation in riverine ecosystems, respectively.

4 | DISCUSSION

Using eDNA-based data on multitrophic biodiversity in the Shaying river basin, we found strong and consistent fingerprints of human activities on biodiversity. Cross-taxon congruences of spatial patterns of communities' structure occurred among five organismal groups at large spatial scales from headwater to the outlet. Most measures of taxonomic, phylogenetic, and functional diversity of invertebrates, protozoa, fungi, algae, and bacteria consistently decreased, while functional redundancy and β diversity increased along an increasing level of human land-use intensity. In a traditional understanding, different taxonomic groups are thought to have unique spatial patterns in river networks due to the distinct dispersal properties of certain

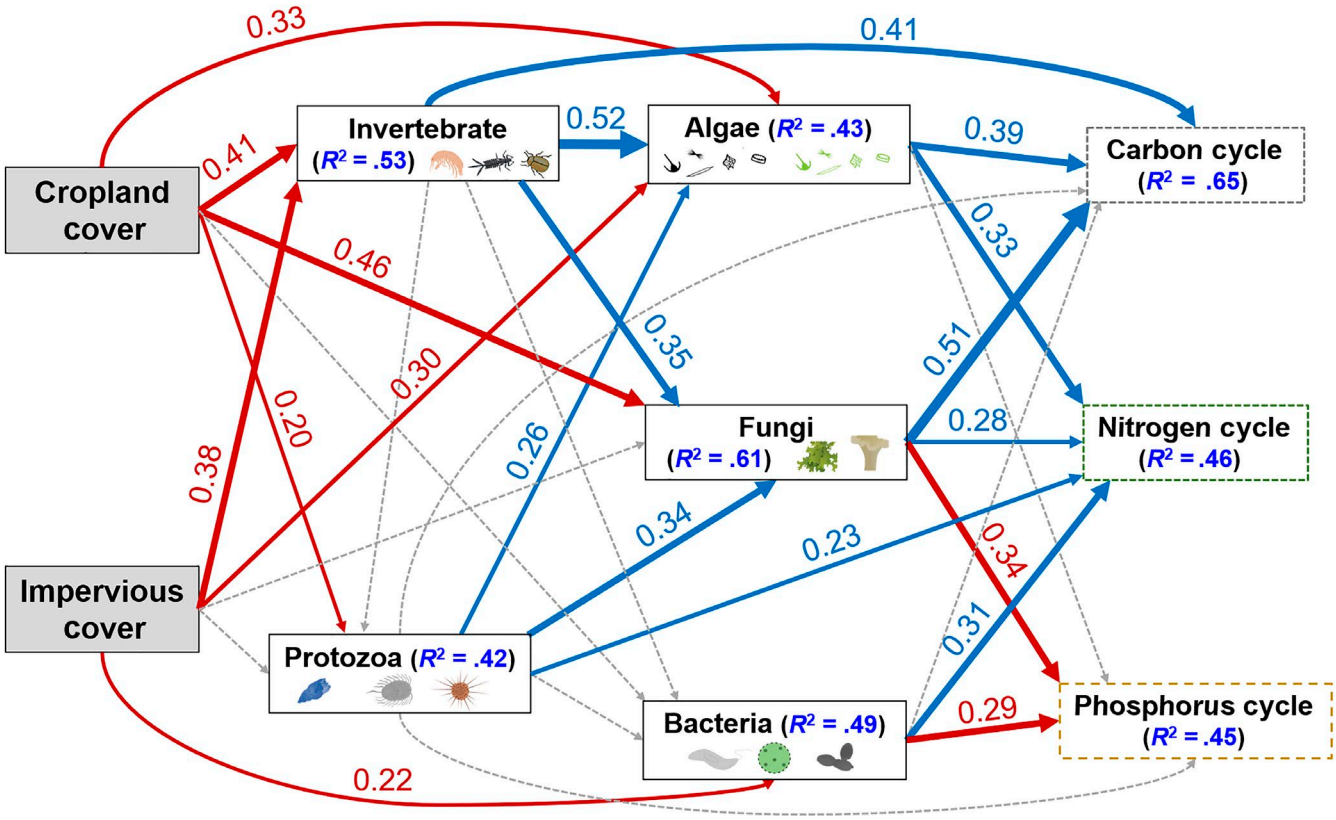

FIGURE 5 Structural equation modeling (SEM) shows the direct and indirect effects of human land use (cropland and impervious cover in 5 km buffer region) on river biodiversity and ecosystem functions. We hypothesized that human land use indirectly affects ecosystem functions by changes in biodiversity. The model fit the data well (root mean square error of approximation, RMSEA = 0.028; comparative fit index, CFI = 0.977; standardized root-mean-squared residual, SRMR = 0.053). The red and blue arrows indicate the negative and positive effects, respectively, and the dashed gray arrows represent non-significant effects. The numbers on the arrows are the standardized coefficients, which represent the strength of the effect of one factor on another. The width of arrows is weighted according to standardized path coefficients, and the percentage values in the bracket indicate the total explanation of the total variation based on the endogenous variables modeled [Colour figure can be viewed at wileyonlinelibrary.com]

taxonomic groups (He et al., 2020; Liu et al., 2013; Tonkin et al., 2018). For example, microbiota are mainly controlled by local environmental factors and thus exhibit a spatial pattern of greater species diversity in headwater than downstream (Besemer et al., 2013; Savio et al., 2015), while fish and insects have a higher biodiversity in the downstream than in the upstream due to their strong dispersal capacity in the river networks (Altermatt, 2013; Shao et al., 2019). However, it has recently been suggested that environmental variables outperform the spatial factors in structuring algal community in river networks (Jamoneau et al., 2018; Wu et al., 2018). A microcosm model study has also demonstrated that disturbances reverse the commonly expected spatial pattern of diversity in river-like networks (Harvey et al., 2018). Together, these human-induced new spatial patterns do not only affect single taxonomic groups, but may also restructure the entire food web (Bartley et al., 2019).

Ecosystem functions were significantly reduced by intensive human land use. For example, leaf litter or cotton strip decomposition significantly decreased with an increase in human land use. The results could be explained by multiple mechanisms. Firstly, the direct effect is that an increasing human land use is changing availability of resources/nutrients for a certain ecosystem function. For example, leaf litter diversity promotes the litter decomposition (Santschi et al., 2017). However, an increase of human land use reduces the riparian vegetation coverage and diversity, followed by a decrease of leaf litter diversity, ventually leading to a decline of leaf litter decomposition. Secondly, human land use could negatively affect biodiversity, which subsequently has negative effects on ecosystem functions. Actually, biodiversity and ecosystem functions are directly and positively correlated across the whole Shaying riverine network (Figure 4), and the highest values of biodiversity and ecosystem functions were spatially highly correlated (Tables S11 and S12). Human land-use change (e.g., deforestation and increased impervious cover) reduces the supply of terrestrial C (e.g., leaf litter) to riverine ecosystems and declines the retention of organic matter in watercourses, which leads to a simultaneous decline in biodiversity, such as invertebrate and fungi, and then ecosystem function such as metabolism (Kominoski & Rosemond, 2012; Marks, 2019). Furthermore, the negative relationships between functional metrics and human land use provide an important indicative information for the assessment of the ecological status using functional indicators. The functional metrics are more spatially stable, and often reflect the endpoint effect of

ecosystem processes. For example, enzyme activities (including β-glucosidase, β-xylosidase, and chitinase) had strong positive correlations with organic matter decomposition (see Figure S1e). The alkaline phosphatase was almost negatively correlated with metrics on diversity and functions. This unique property is associated with changes in water quality. For example, alkaline phosphatase positively correlated with turbidity, chemical oxygen demand, and chlorophyll *a* (Yuan et al., 2017), meaning that regions with high disturbance may have higher phosphatase activity. Our data confirm this hypothesis, and show that phosphatase activity was highest in *High Agriculture/Industry* region (see Table S12).

Biodiversity and ecosystem functions were positively correlated at integrative levels, and even have a non-saturating (concave-up) shape over the parameter range of B-EF relationships observed in the Shaying riverine ecosystem. This is closely linked to the increase of comprehensive metrics on multitrophic and multifaceted biodiversity. For example, taxonomic, phylogenetic, and functional diversity had positive associations with functional metrics. Phylogenetic and functional diversity had better prediction for C/N enzymes activities than taxonomic diversity, and the multitrophic metrics have stronger dependence on ecosystem functions (Figure 4). While ecosystem functions must logically saturate at some level, our results indicate that the parameter space studied is not yet reaching this eventual saturation (similarly to initial dynamics in a functional type III response setting). It means that under the given environmental conditions, even a slightly higher biodiversity could lead to substantially higher ecosystem functions in the Shaying riverine ecosystems.

Human land use affected ecosystem functions through two potential pathways mediated by biodiversity change. Firstly, cropland and impervious cover reduced the diversity of different taxa groups, which inhibited common ecosystem processes of aquatic communities (Figure 5). Leaf litter decomposition in rivers is mainly through the direct feeding of invertebrates and the breakdown of extracellular enzymes derived from fungi and bacteria (Marks, 2019). Human-induced biodiversity loss directly affects these two processes, thereby reducing ecosystem functions. Secondly, a decline in ecosystem function could then even be aggravated by changes in community interdependence or food web structure, in addition to loss of biodiversity. For instance, invertebrate and protozoa communities are directly affecting the structure of algal and fungal communities. These interactions or interdependencies are particularly important for the decomposition we measured. Invertebrates mechanically break litter into fine particles, thus promoting the microbial mineralization, in turn, the microbes are invertebrate prey and use their secretions to stimulate or inhibit the growth of invertebrates (Marks, 2019; Weitere et al., 2018). In addition, multitrophic communities in rivers jointly play a regulatory role in the process of external stressor changing ecosystem function, this may be related to the niche partitioning or functional role of species in ecosystems (Cardinale, 2011). For example, invertebrates, fungi, and algae belong to the strong predictors in ecosystem functions and are at the central of all the regulatory pathways (Figure 5), which indicate the potential top-down and bottom-up regulations of ecosystem functions by biodiversity in the river's diverse food web.

5 | CONCLUSIONS

The Shaying riverine ecosystem has been affected by human land use in several ways: (a) a spatial cross-taxon congruence pattern was formed by the homogenization of multitrophic communities; (b) multitrophic and multifaceted biodiversity were reduced, but functional redundancy was increased; (c) ecosystem functions declined indirectly due to the biodiversity loss and altered community interdependence in multitrophic groups. Importantly, these effects by human land use were not mutually exclusive or completely synergistic in riverine ecosystems. Overall, our study illustrates that the complex interactions between human land use and biotic factors jointly shape multitrophic communities and ecosystem functions across an entire catchment, and how the outcome of those interactions generates the specific context that allow each taxonomic group to survive or not, leading to large spatial-scale variation in communities' structure and ecosystem functions.

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AUTHOR CONTRIBUTION

F.L., X.Z., and F.A. designed the study. A.L., S.A., and X.Z. provided the funding. F.L. and J.Y. collected the data and conducted the statistical analyses. F.L. and X.Z. wrote the first draft of the manuscript. F.A., A.L., and S.A. contributed to the discussion and revisions.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in the Supporting Information and are available from the corresponding author upon reasonable request.

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REFERENCES

Abonyi, A., Horvath, Z., & Ptacnik, R. (2018). Functional richness outperforms taxonomic richness in predicting ecosystem functioning in natural phytoplankton communities. *Freshwater Biology*, *63*, 178–186.

Altermatt, F. (2013). Diversity in riverine metacommunities: A network perspective. *Aquatic Ecology*, *47*, 365–377.

Altermatt, F., Little, C. J., Mächler, E., Wang, S. P., Zhang, X. W., & Blackman, R. C. (2020). Uncovering the complete biodiversity structure in spatial networks: The example of riverine systems. *Oikos*, *129*, 607–618. https://doi.org/10.1111/oik.06806

Amaral-Zettler, L. A., McCliment, E. A., Ducklow, H. W., & Huse, S. M. (2009). A method for studying protistan diversity using massively parallel sequencing of V9 hypervariable regions of small-subunit ribosomal RNA genes. *PLoS ONE*, *4*, e6372. https://doi.org/10.1371/journal.pone.0006372

Anderson, M. J. (2001). A new method for non-parametric multivariate analysis of variance. *Austral Ecology*, *26*, 32–46.

Bagousse-Pinguet, L. E., Yoann, S. S., Gross, N., Torices, R., Berdugo, M., & Maestre, F. T. (2019). Phylogenetic, functional, and taxonomic richness have both positive and negative effects on ecosystem multifunctionality. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(17), 8419–8424. https://doi.org/10.1073/pnas.1815727116

Bartley, T. J., McCann, K. S., Bieg, C., Cazelles, K., Granados, M., Guzzo, M. M., MacDougall, A. S., Tunney, T. D., & McMeans, B. C. (2019). Food web rewiring in a changing world. *Nature Ecology & Evolution*, *3*, 345–354.

Besemer, K., Singer, G., Quince, C., Bertuzzo, E., Sloan, W., & Battin, T. J. (2013). Headwaters are critical reservoirs of microbial diversity for fluvial networks. *Proceedings of the Royal Society B: Biological Sciences*, *280*, 20131760.

Best, J. (2019). Anthropogenic stresses on the world's big rivers. *Nature Geoscience*, *12*, 7–21.

Bodmer, P., Heinz, M., Pusch, M., Singer, G., & Premke, K. (2016). Carbon dynamics and their link to dissolved organic matter quality across contrasting stream ecosystems. *Science of the Total Environment*, *553*, 574–586.

Bond, E. M., & Chase, J. M. (2002). Biodiversity and ecosystem functioning at local and regional spatial scales. *Ecology Letters*, *5*, 467–470.

Bruno, D., Belmar, O., Maire, A., Morel, A., Dumont, B., & Datry, T. (2019). Structural and functional responses of invertebrate communities to climate change and flow regulation in alpine catchments. *Global Change Biology*, *25*, 1612–1628.

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., Fierer, N., Pena, A. G., Goodrich, J. K., Gordon, J. I., Huttley, G. A., Kelley, S. T., Knights, D., Koenig, J. E., Ley, R. E., Lozupone, C. A., McDonald, D., Muegge, B. D., Pirrung, M., … Knight, R. (2010). QIIME allows analysis of high-throughput community sequencing data. *Nature Methods*, *7*, 335–336.

Cardinale, B. J. (2011). Biodiversity improves water quality through niche partitioning. *Nature*, *472*, 86–89.

Cardinale, B. J., Duffy, J. E., Gonzalez, A., Hooper, D. U., Perrings, C., Venail, P., Narwani, A., Mace, G. M., Tilman, D., Wardle, D. A., Kinzig, A. P., Daily, G. C., Loreau, M., Grace, J. B., Larigauderie, A., Srivastava, D. S., & Naeem, S. (2012). Biodiversity loss and its impact on humanity. *Nature*, *486*, 59–67.

Cardinale, B. J., Palmer, M. A., & Collins, S. L. (2002). Species diversity enhances ecosystem functioning through interspecific facilitation. *Nature*, *415*, 426–429.

Carraro, L., Mächler, E., Wüthrich, R., & Altermatt, F. (2020). Environmental DNA allows upscaling spatial patterns of biodiversity in freshwater ecosystems. *Nature Communications*, *11*, 3585.

Casanoves, F., Pla, L., Di Rienzo, J. A., & Diaz, S. (2011). FDiversity: A software package for the integrated analysis of functional diversity. *Methods in Ecology and Evolution*, *2*, 233–237.

Chase, J. M., & Leibold, M. A. (2002). Spatial scale dictates the productivity–biodiversity relationship. *Nature*, *416*, 427–430.

Chauvet, E., Ferreira, V., Giller, P. S., McKie, B. G., Tiegs, S. D., Woodward, G., Elosegi, A., Dobson, M., Fleituch, T., & Graça, M. A. S. (2016). Litter decomposition as an indicator of stream ecosystem functioning at local-to-continental scales: Insights from the European RivFunction Project. *Advances in Ecological Research*, *55*, 99–182.

Clarke, K. R. (1993). Non-parametric multivariate analyses of changes in community structure. *Australian Journal of Ecology*, *18*, 117–143.

Craven, D., Eisenhauer, N., Pearse, W. D., Hautier, Y., Isbell, F., Roscher, C., Bahn, M., Beierkuhnlein, C., Bönisch, G., Buchmann, N., Byun, C., Catford, J. A., Bruno, E. L., Cerabolini, J. H., Cornelissen, C., Craine, J. M., De Luca, E., Ebeling, A., Griffin, J. N., … Manning, P. (2018). Multiple facets of biodiversity drive the diversity–stability relationship. *Nature Ecology & Evolution*, *2*, 1579–1587.

Daam, M. A., Teixeira, H., Lillebø, A. I., & Nogueira, A. J. A. (2019). Establishing causal links between aquatic biodiversity and ecosystem functioning: Status and research needs. *Science of the Total Environment*, *656*, 1145–1156.

Deiner, K., Bik, H. M., Machler, E., Seymour, M., Lacoursiere-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*, 5872–5895.

Deiner, K., Fronhofer, E. A., Machler, E., Walser, J. C., & Altermatt, F. (2016). Environmental DNA reveals that rivers are conveyer belts of biodiversity information. *Nature Communications*, *7*, 1–9.

DeSantis, T. Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E. L., Keller, K., Huber, T., Dalevi, D., Hu, P., & Andersen, G. L. (2006). Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Applied and Environment Microbiology*, *72*, 5069–5072.

Dudgeon, D., Arthington, A. H., Gessner, M. O., Kawabata, Z., Knowler, D. J., Leveque, C., Naiman, R. J., Prieur-Richard, A. H., Soto, D., Stiassny, M. L., & Sullivan, C. A. (2006). Freshwater biodiversity: Importance, threats, status and conservation challenges. *Biological Reviews of the Cambridge Philosophical Society*, *81*, 163–182.

Duffy, J. E., Cardinale, B. J., France, K. E., McIntyre, P. B., Thebault, E., & Loreau, M. (2007). The functional role of biodiversity in ecosystems: Incorporating trophic complexity. *Ecology Letters*, *10*, 522–538. https://doi.org/10.1111/j.1461-0248.2007.01037.x

Eisenhauer, N., Schielzeth, H., Barnes, A. D., Barry, K., Bonn, A., Brose, U., Bruelheide, H., Buchmann, N., Buscot, F., Ebeling, A., Ferlian, O., Freschet, G. T., Giling, D. P., Hättenschwiler, S., Hillebrand, H., Hines, J., Isbell, F., Koller-France, E., König-Ries, B., … Jochum, M. (2019). A multitrophic perspective on biodiversity–ecosystem functioning research. *Advances in Ecological Research*, *61*, 1–54. https://doi.org/10.1016/bs.aecr.2019.06.001

Elbrecht, V., & Leese, F. (2017). Validation and development of COI metabarcoding primers for freshwater macroinvertebrate bioassessment. *Frontiers in Environmental Science*, *5*, 11. https://doi.org/10.3389/fenvs.2017.00011

Faith, D. P. (2006). The role of the phylogenetic diversity measure, PD, in bio-informatics: Getting the definition right. *Evolutionary Bioinformatics*, *2*, 277–283.

Fuß, T., Behounek, B., Ulseth, A. J., & Singer, G. A. (2017). Land use controls stream ecosystem metabolism by shifting dissolved organic matter and nutrient regimes. *Freshwater Biology*, *62*, 582–599.

Gibbs, H. K., Ruesch, A. S., Achard, F., Clayton, M. K., Holmgren, P., Ramankutty, N., & Foley, J. A. (2010). Tropical forests were the primary sources of new agricultural land in the 1980s and 1990s. *Proceedings of the National Academy of Sciences of the United States of America*, *107*, 16732–16737.

Gounand, I., Little, C. J., Harvey, E., & Altermatt, F. (2018). Cross-ecosystem carbon flows connecting ecosystems worldwide. *Nature Communications*, *9*, 1–8.

Gounand, I., Little, C. J., Harvey, E., & Altermatt, F. (2020). Global quantitative synthesis of ecosystem functioning across climatic zones and ecosystem types. *Global Ecology and Biogeography*, *29*, 1139–1176.

Grill, G., Lehner, B., Thieme, M., Geenen, B., Tickner, D., Antonelli, F., Babu, S., Borrelli, P., Cheng, L., Crochetiere, H., Ehalt Macedo, H., Filgueiras, R., Goichot, M., Higgins, J., Hogan, Z., Lip, B., McClain, M. E., Meng, J., Mulligan, M., … Zarfl, C. (2019). Mapping the world's free-flowing rivers. *Nature*, *569*, 215–221.

Guillou, L., Bachar, D., Audic, S., Bass, D., Berney, C., Bittner, L., Boutte, C., Burgaud, G., de Vargas, C., Decelle, J., Del Campo, J., Dolan, J. R., Dunthorn, M., Edvardsen, B., Holzmann, M., Kooistra, W. H., Lara, E., Le Bescot, N., Logares, R., … Christen, R. (2013). The Protist Ribosomal Reference database (PR2): A catalog of unicellular eukaryote small sub-unit rRNA sequences with curated taxonomy. *Nucleic Acids Research*, *41*, D597–D604.

Hansen, M. C., Potapov, P. V., Moore, R., Hancher, M., Turubanova, S. A., Tyukavina, A., Thau, D., Stehman, S. V., Goetz, S. J., & Loveland, T. R. (2013). High-resolution global maps of 21st-century forest cover change. *Science*, *342*, 850–853.

Harvey, E., Isabelle Gounand, A., Emanuel, F., & Altermatt, F. (2018). Disturbance reverses classic biodiversity predictions in river-like landscapes. *Proceedings of the Royal Society B: Biological Sciences*, *285*, 20182441.

He, S., Soininen, J., Deng, G., & Wang, B. (2020). Metacommunity structure of stream insects across three hierarchical spatial scales. *Ecology and Evolution*, *10*, 2874–2884.

Jabiol, J., Colas, F., & Guérold, F. (2020). Cotton-strip assays: Let's move on to eco-friendly biomonitoring! *Water Research*, *170*, 115295.

Jabiol, J., McKie, B. G., Bruder, A., Bernadet, C., Gessner, M. O., & Chauvet, E. (2013). Trophic complexity enhances ecosystem functioning in an aquatic detritus-based model system. *Journal of Animal Ecology*, *82*, 1042–1051.

Jamoneau, A., Passy, S. I., Soininen, J., Leboucher, T., & Tison-Rosebery, J. (2018). Beta diversity of diatom species and ecological guilds: Response to environmental and spatial mechanisms along the stream watercourse. *Freshwater Biology*, *63*, 62–73.

Jarzyna, M. A., & Jetz, W. (2016). Detecting the multiple facets of biodiversity. *Trends in Ecology & Evolution*, *31*, 527–538.

Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., & Glockner, F. O. (2013). Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Research*, *41*, e1.

Kominoski, J. S., & Rosemond, A. D. (2012). Conservation from the bottom up: Forecasting effects of global change on dynamics of organic matter and management needs for river networks. *Freshwater Science*, *31*, 51–68. https://doi.org/10.1899/10-160.1

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*, *52*, 11708–11719.

Little, C. J., Fronhofer, E. A., & Altermatt, F. (2020). Nonlinear effects of intraspecific competition alter landscape-wide scaling up of ecosystem function. *The American Naturalist*, *195*, 432–444.

Liu, J., Soininen, J., Han, B.-P., & Declerck, S. A. J. (2013). Effects of connectivity, dispersal directionality and functional traits on the metacommunity structure of river benthic diatoms. *Journal of Biogeography*, *40*, 2238–2248.

Mächler, E., Little, C. J., Wüthrich, R., Alther, R., Fronhofer, E. A., Gounand, I., Harvey, E., Hürlemann, S., Walser, J.-C., & Altermatt, F. (2019). Assessing different components of diversity across a river network using eDNA. *Environmental DNA*, *1*, 290–301.

Maestre, F. T., Quero, J. L., Gotelli, N. J., Escudero, A., Ochoa, V., Delgado-Baquerizo, M., Garcia-Gomez, M., Bowker, M. A., Soliveres, S., Escolar, C., Garcia-Palacios, P., Berdugo, M., Valencia, E., Gozalo, B., Gallardo, A., Aguilera, L., Arredondo, T., Blones, J., Boeken, B., … Zaady, E. (2012). Plant species richness and ecosystem multifunctionality in global drylands. *Science*, *335*, 214–218.

Mantel, N. (1967). The detection of disease clustering and a generalized regression approach. *Cancer Research*, *27*, 209–220.

Marks, J. C. (2019). Revisiting the fates of dead leaves that fall into streams. *Annual Review of Ecology, Evolution, and Systematics*, *50*, 547–568.

Menezes, S., Baird, D. J., & Soares, A. M. V. M. (2010). Beyond taxonomy: A review of macroinvertebrate trait-based community descriptors as tools for freshwater biomonitoring. *Journal of Applied Ecology*, *47*, 711–719.

Muyzer, G., de Waal, E. C., & Uitterlinden, A. G. (1993). Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Applied and Environment Microbiology*, *59*, 695–700. https://doi.org/10.1128/AEM.59.3.695-700.1993

Pennekamp, F., Pontarp, M., Tabi, A., Altermatt, F., Alther, R., Choffat, Y., Fronhofer, E. A., Ganesanandamoorthy, P., Garnier, A., Griffiths, J. I., Greene, S., Horgan, K., Massie, T. M., Machler, E., Palamara, G. M., Seymour, M., & Petchey, O. L. (2018). Biodiversity increases and decreases ecosystem stability. *Nature*, *563*, 109–112.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., R Core Team. (2020). nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-149. Retrieved from https://CRAN.R-project.org/package=nlme

Rodrigues, A. C. M., Machado, A. L., Bordalo, M. D., Saro, L., Simao, F. C. P., Rocha, R. J. M., Golovko, O., Zlabek, V., Barata, C., Soares, A., & Pestana, J. L. T. (2018). Invasive species mediate insecticide effects on community and ecosystem functioning. *Environmental Science & Technology*, *52*, 4889–4900.

Rosseel, Y. (2012). lavaan: An R Package for Structural Equation Modeling. *Journal of Statistical Software*, *48*(2), 1–36.

Santschi, F., Gounand, I., Harvey, E., & Altermatt, F. (2017). Leaf litter diversity and structure of microbial decomposer communities modulate litter decomposition in aquatic systems. *Functional Ecology*, *32*(2), 522–532. https://doi.org/10.1111/1365-2435.12980

Savio, D., Sinclair, L., Ijaz, U. Z., Parajka, J., Reischer, G. H., Stadler, P., Blaschke, A. P., Bloschl, G., Mach, R. L., Kirschner, A. K. T., Farnleitner, A. H., & Eiler, A. (2015). Bacterial diversity along a 2600 km river continuum. *Environmental Microbiology*, *17*, 4994–5007.

Schuldt, A., Assmann, T., Brezzi, M., Buscot, F., Eichenberg, D., Gutknecht, J., Hardtle, W., He, J. S., Klein, A. M., Kuhn, P., Liu, X., Ma, K., Niklaus, P. A., Pietsch, K. A., Purahong, W., Scherer-Lorenzen, M., Schmid, B., Scholten, T., Staab, M., … Bruelheide, H. (2018). Biodiversity across trophic levels drives multifunctionality in highly diverse forests. *Nature Communications*, *9*, 2989.

Seymour, M., Deiner, K., & Altermatt, F. (2016). Scale and scope matter when explaining varying patterns of community diversity in riverine metacommunities. *Basic and Applied Ecology*, *17*, 134–144.

Shao, X., Fang, Y. U., Jawitz, J. W., Yan, J., & Cui, B. (2019). River network connectivity and fish diversity. *Science of the Total Environment*, *689*, 21–30.

Soliveres, S., van der Plas, F., Manning, P., Prati, D., Gossner, M. M., Renner, S. C., Alt, F., Arndt, H., Baumgartner, V., Binkenstein, J., Birkhofer, K., Blaser, S., Bluthgen, N., Boch, S., Bohm, S., Borschig, C., Buscot, F., Diekotter, T., Heinze, J., … Allan, E. (2016). Biodiversity at multiple trophic levels is needed for ecosystem multifunctionality. *Nature*, *536*, 456–459.

Song, X.-P., Hansen, M. C., Stehman, S. V., Potapov, P. V., Tyukavina, A., Vermote, E. F., & Townshend, J. R. (2018). Global land change from 1982 to 2016. *Nature*, *560*, 639–643.

Tlili, A., Jabiol, J., Behra, R., Gil-Allue, C., & Gessner, M. O. (2017). Chronic exposure effects of silver nanoparticles on stream microbial decomposer communities and ecosystem functions. *Environmental Science & Technology*, *51*, 2447–2455.

Tonkin, J. D., Altermatt, F., Finn, D. S., Heino, J., Olden, J. D., Pauls, S. U., & Lytle, D. A. (2018). The role of dispersal in river network metacommunities: Patterns, processes, and pathways. *Freshwater Biology*, *63*, 141–163.

Usseglio-Polatera, P., Bournaud, M., Richoux, P., & Tachet, H. (2000). Biomonitoring through biological traits of benthic macroinvertebrates: How to use species trait databases? *Hydrobiologia*, *422*(423), 153–162.

Vaughn, C. C. (2010). Biodiversity losses and ecosystem function in freshwaters: Emerging conclusions and research directions. *BioScience*, *60*, 25–35. https://doi.org/10.1525/bio.2010.60.1.7

Vörösmarty, C. J., McIntyre, P. B., Gessner, M. O., Dudgeon, D., Prusevich, A., Green, P., Glidden, S., Bunn, S. E., Sullivan, C. A., Reidy Liermann, C., & Davies, P. M. (2010). Global threats to human water security and river biodiversity. *Nature*, *467*, 555.

Wang, S., & Brose, U. (2018). Biodiversity and ecosystem functioning in food webs: The vertical diversity hypothesis. *Ecology Letters*, *21*, 9–20. https://doi.org/10.1111/ele.12865

Wang, Y., Cadotte, M. W., Chen, Y., Fraser, L. H., Zhang, Y., Huang, F., Luo, S., Shi, N., & Loreau, M. (2019). Global evidence of positive biodiversity effects on spatial ecosystem stability in natural grasslands. *Nature Communications*, *10*, 3207.

Weitere, M., Erken, M., Majdi, N., Arndt, H., Norf, H., Reinshagen, M., Traunspurger, W., Walterscheid, A., & Wey, J. K. (2018). The food web perspective on aquatic biofilms. *Ecological Monographs*, *88*, 543–559.

Wu, N., Yueming, Q. U., Guse, B., Makarevičiūtė, K., To, S., Riis, T., & Fohrer, N. (2018). Hydrological and environmental variables outperform spatial factors in structuring species, trait composition, and beta diversity of pelagic algae. *Ecology and Evolution*, *8*, 2947–2961.

WWF. (2018). In M. Grooten & R. E. A. Almond (Eds.), *Living planet report–2018: Aiming higher*. Gland, Switzerland: WWF.

Yao, J. M., Colas, F., Solimini, A. G., Battin, T. J., Gafny, S., Morais, M., Puig, M. A., Marti, E., Pusch, M. T., Voreadou, C., Sabater, F., Julien, F., Sanchez-Perez, J. M., Sauvage, S., Vervier, P., & Gerino, M. (2017). Macroinvertebrate community traits and nitrate removal in stream sediments. *Freshwater Biology*, *62*, 929–944.

Yuan, Y., Bi, Y., & Zhengyu, H. U. (2017). Phytoplankton communities determine the spatio-temporal heterogeneity of alkaline phosphatase activity: Evidence from a tributary of the Three Gorges Reservoir. *Scientific Reports*, *7*, 1–9. https://doi.org/10.1038/s41598-017-16740-4

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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