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Consideration of Multitrophic Biodiversity and Ecosystem Functions Improves Indices on River Ecological Status

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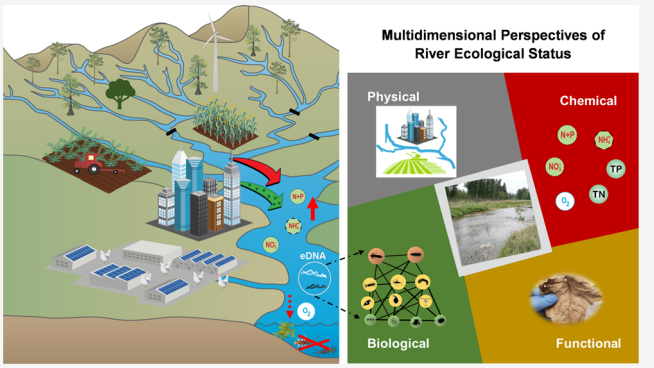
ABSTRACT: Biological quality elements have been developed worldwide to assess whether a water body is in a good status or not. However, current studies mainly focus on a single taxonomic group or a small set of species, often limited by methods of morphological identification, and lack further aspects of biodiversity (e.g., across taxa and multiple attributes) and ecosystem functions. Here, we advance a framework for assessing the river's ecological status based on complete biodiversity data measured by environmental DNA (eDNA) metabarcoding and measurements of ecosystem functions in addition to physicochemical elements across a large riverine system in China. We identified 40 indicators of biodiversity and ecosystem functions, covering five taxonomic groups from bacteria to invertebrates, and associated with multiple attributes of biodiversity and ecosystem functions. Our data show that human impact on ecosystems could be accurately predicted by these eDNA-based indicators and ecosystem functions, using cross-validation with a known stressor gradient. Moreover, indices based on these indicators of biodiversity and ecosystem functions not only distinguish the physicochemical characteristics of the sites but also improve the assessment accuracy of 20−30% for the river's ecological status. Overall, by incorporating eDNA-based biodiversity with physicochemical and ecosystem functional elements, the multidimensional perspectives of ecosystem states provide additional information to protect and maintain a good ecological status of rivers.



KEYWORDS: *eDNA, taxonomic diversity, functional diversity, phylogenetic diversity, litter decomposition*

1. INTRODUCTION

Rivers provide fundamental ecosystem services for humans and their state is closely linked to socioeconomic development.[1−3] Unfortunately, these services are at stake through local to global river degradation, which accelerated within the last decades.[4−6] Establishing an effective assessment method that allows quick and reliable identification of the direction and the degree of ecosystem changes has become an urgent challenge for government managers and stakeholders.[7,8] Biological quality elements (BQEs) are an effective and legally implementable approach to explore human-induced environmental changes. A famous case is the European Union's Water Framework Directive 2000/60/EC (WFD), which uses BQEs as key bioindicators to identify the ecological status of surface water.[9−11] Although the BQE-based system has promoted our understanding of the river's ecological status, the current BQE system indeed does not cover all species present in ecosystems equally.[10,12−14]

Complete biodiversity data, covering many taxonomic groups and multiple attributes, are an important basis for revealing and predicting ecosystem changes.[15,16] A recent study emphasized complex changes in biodiversity among taxa, such that any single or few groups (e.g., fish, diatoms, or macroinvertebrates) cannot completely represent each other.[17] Other measures, such as phylogenetic and functional attributes, capture further aspects of the community. For example, phylogenetic diversity is linked to the evolutionary assembly of species in the natural community,[18] while functional diversity is linked to resource utilization strategies.[19] Thereby, phylogenetic and functional diversity can identify biodiversity changes in different ways,[20] and measurement of biodiversity should cover multiple taxa and attributes associated with ecosystem changes. However, limited by morphological methods, the acquisition of complete biodiversity data has not been effectively solved yet.

Recent advances in environmental DNA (eDNA) metabarcoding provide a new opportunity to capture complete biodiversity.[15,21−23] This is especially the case for meio- and microorganisms (e.g., invertebrates, protozoa, fungi, algae, and

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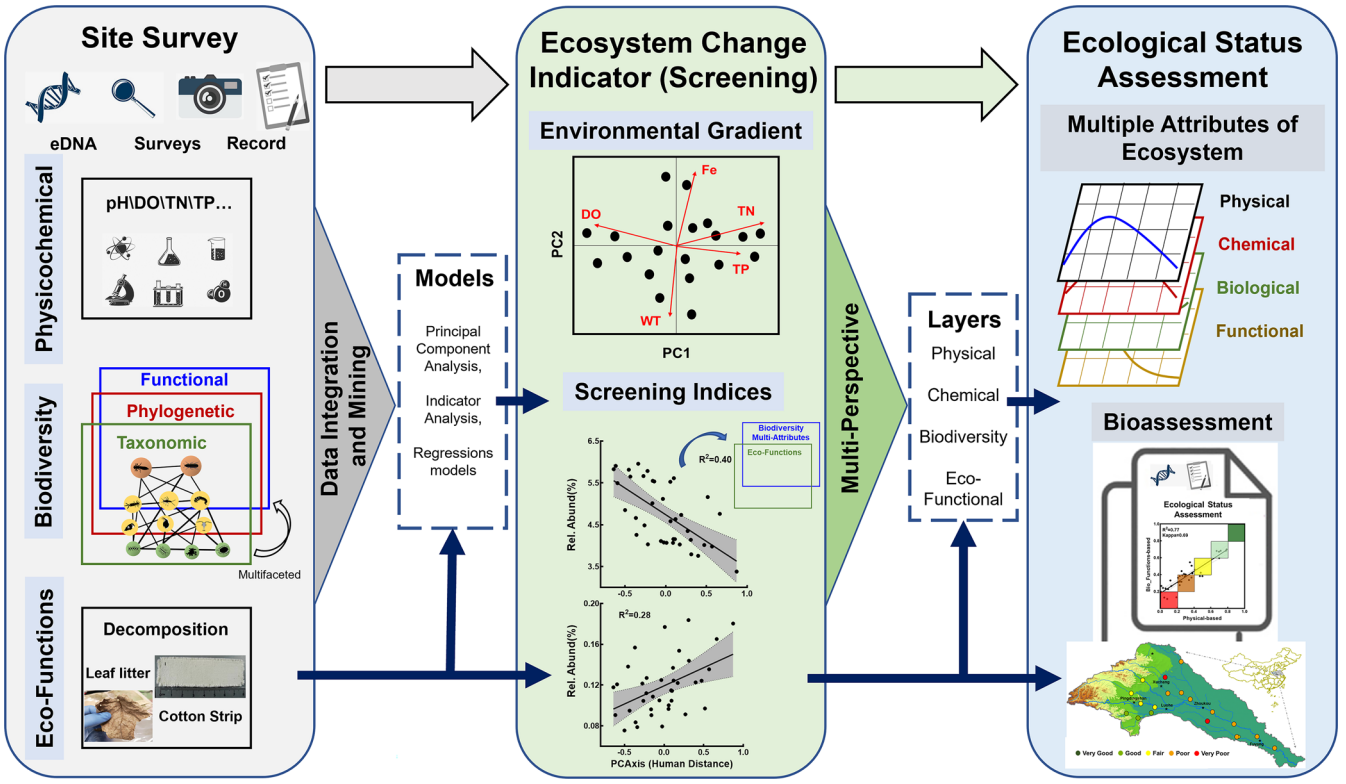

Figure 1. Overview of the analysis from field observations to final human-induced ecosystem change assessment. Integrated data from several observation sources, including physicochemical characteristic data and biodiversity (across taxa and multiple attributes) and ecosystem functions to comprehensively and ecofriendly reveal ecosystem changes. The workflow mainly includes three units: site survey (multivariate data integration), index screening, and multiperspective ecosystem status assessment.

bacteria) that were underrepresented in previous biomonitoring.[24] Meio- and microorganisms not only play an important role in the material and energy transfer of aquatic food webs[25,26] but can also sensitively indicate ecosystem changes.[27,28] A previous study highlighted that 40−90% of the DNA sequences in eDNA data belong to these meio- and microbiota.[29] Recently, researchers have increasingly advocated that molecular fingerprints should be included in bioassessments for detecting ecosystem changes over time.[7,30,31] However, significant gaps remain before the molecular tools are used in routine bioassessments, such as how robust and comparable methods and results are compared to previously validated techniques.

Another critical aspect for identifying the ecological status of rivers is linked to the provisioning of ecosystem functions, such as self-purification and clean water resources.[3,5,32] To some degree, rivers can remove pollutants through a series of physical, chemical, and biological processes such as diffusion, oxidation, and enzyme metabolism to ensure material recycling. As a typical case of riverine ecosystems, carbon decomposition (e.g., leaf litter) has been used to indicate changes in nutrient fluxes in ecosystems.[33,34] Recent studies suggested that carbon decomposition shows clear patterns across spatial scales and has the potential to indicate ecosystem changes.[35,36] Especially, the decomposition of cotton strips has been used as a metric of carbon decomposition, which can greatly promote the standardization of monitoring methods at a large spatial scale.[35,37] However, to our knowledge, there is still a lack of studies that integrate ecosystem functions with physicochemical elements (e.g., habitat and water quality variables) and biodiversity to assess the river's ecological status.

Here, we propose a framework integrating multiple data sources including biodiversity (across taxa and multiple attributes) and ecosystem functions (e.g., carbon decomposition) with physicochemical data to comprehensively reveal human-induced ecosystem changes in riverine ecosystems (Figure 1). Biodiversity fingerprints across taxa (including invertebrates, protozoa, fungi, algae, and bacteria) in the Shaying River in China were obtained by eDNA metabarcoding. Ecosystem functions including leaf litter and cotton strip decomposition were measured. The reliability and comparability of the framework were verified by the following steps: (1) rebuilding a known stressor gradient on the spatial scale using the physicochemical data to screen the candidate indicators of biodiversity and ecosystem functions; (2) analyzing the prediction performance of the framework on the known stressor gradient by the screened indicators; and (3) comparing the consistency between the indices developed by the indicators of biodiversity and ecosystem functions and the physicochemical elements to assess river's ecological status.

2. MATERIALS AND METHODS

2.1. Study Area and Sampling. Shaying River is the largest tributary of Huai River in eastern China (Figure S1), with a length of 620 km and a drainage area of 39 880 km^2. This region inhabits >26.4 million people and has a high rate of urbanization.[38] We set up 20 sites across the Shaying River, which are located in the tributaries and the downstream reaches of their intersection (topological distance >30 km between two sites). Such sampling sites are arranged to capture the hierarchic structure of diversity and ecosystem functions.[15,16] Based on the relative intensity of human disturbance,

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the whole region (and thus the sampling sites) can be divided into three major groups: mild disturbance (five sites) with relatively mild human impacts (e.g., land use), high agriculture and industry (eight sites) characterized by intense agriculture and industry, and high agriculture (seven sites) with intense agriculture and industry, respectively.[16]

Field samples across the three major groups of this region were collected in April (spring) and October 2018 (autumn), respectively. Two sites were abandoned in April due to serious poor traffic conditions. Collection and preservation of field eDNA samples followed the previously published protocol.[27] Briefly, three 1 L surface water per site were collected using sterile bottles (Thermo Fisher Scientific), and 300−500 mL of water was filtered using a 0.45 μm hydrophilic nylon membrane (Merck Millipore). Six field replicates (or subsamples) were obtained at each site. All replicates of membrane discs were placed in 5.0 mL centrifugal tubes and stored at −20 °C until DNA extraction. Blank controls at each site were performed using autoclaved tap water (filtered 300 mL), and were also taken for the DNA extraction, polymerase chain reaction (PCR) amplification, and next-generation sequencing (NGS) step in parallel with replicate samples to monitor possible contaminants.

2.2. eDNA and Biodiversity. All membrane discs (including blank controls) were extracted using a DNeasy PowerWater Kit (QIAGEN, Germany). PCR assays were performed using three primer sets.[39−42] Specifically, a universal primer pair (BF1 and BR2) was used to amplify the 316 bp fragment of the COI genes for invertebrate detection, a universal eukaryotic primer pair (1380F and 1510R) was used to amplify the 130 bp fragment of the V9 region of 18S rRNA genes for protozoa, fungi, and algae detection, and the 180 bp fragment of the V3 region of 16s rRNA genes was amplified using the primer pair (341F and 518R) for bacteria detection. Three PCR replicates were performed on all replicates to minimize potential PCR bias. All purified PCR products were 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 the Illumina MiSeq PE300 platform (Illumina), respectively.

Low-quality sequences were discarded using the "split_libraries.py" script with "−w 50 −s 25 −l 100" parameters in the QIIME toolkit.[43] Sequences were denoised by removing duplicates, the singletons, and PCR chimeras. Cleaned sequences were sorted and distinguished by unique sample tags, and then were clustered into OTUs with 97% nucleotide similarity. For protozoa, fungi, algae, and bacterial communities, taxonomic annotation of each OTU was assigned against the Greengenes database[44] and the Protist Ribosomal Reference database[45] using "align_seqs.py" script, respectively. Each detected invertebrate OTU was assigned against a custom reference database (NCBI Genbank database and indigenous database) using BLASTN pipeline with a ≥98% similarity cutoff to get taxonomic information. Taxonomic (Shannon's diversity and Pielou's evenness) and phylogenetic (Faith's phylogenetic diversity) diversity indices of five taxonomic groups were calculated using the "alpha_diversity.py" script in the QIIME toolkit. Any OTU with relative abundances of <0.001 and a <10% detection frequency in all samples was discarded to clear all OTUs from the extraction and PCR negative controls. Based on the relative abundance of OTUs (that is the proportion of any OTU sequence to the total community sequence), indicative OTUs were identified using a multipatt function in the R package Indispecies, and the Indicator Values (IndVal) were used to reflect the conditional probability of the OTUs as an indicator, followed by 999 permutations for the significance test.[46] Species traits of invertebrates, protozoa, and algae were mainly retrieved from published papers[47−49] and database repositories. Details on species traits of these three groups were summarized in another of our studies in the same region.[16]

2.3. Physicochemical Data. Physical elements, including 10 habitat variables, were investigated referring to the U.S. EPA rapid bioassessment protocols,[50] including substrate cover, habitat complexity, velocity/depth regime, bank stability, channel alteration, channel flow status, vegetative protection, water quality, human activity intensity, and riparian land use. To avoid the potential subjective bias of investigators, no less than three investigators on the site scored and photographed the habitat, and then calculated their mean value to obtain the overall physical habitat status of the corresponding sampling sites. Land use raster maps (30 m resolution) interpreted from Landsat 8 remote-sensing images in 2018 were used to calculate parameters of land use patterns. Details on land use data were summarized in another of our studies in the same region.[16]

Chemical elements, that is 16 water quality variables, were measured at each site. Dissolved oxygen (DO), pH, electrical conductivity (EC), and water temperature (WT) were measured by an AP-2000 Multiparameter Water Quality Instruments (Aquaread, U.K.) on site. For the total nitrogen (TN), total phosphorus (TP), ammonia nitrogen (NH_3−N), and potassium permanganate index (chemical oxygen demand, COD), the collected 1 L surface water was taken back to the laboratory and measured by national standard methods (NEPB, 2002), respectively. For the metals (Zn, Fe, Cu, Ni, Cr, As, Cd, Mn), 1 L surface water was diluted with 2% HNO_3 and filtered through a 2.5 μm membrane filter (Whatman, U.K.), and then the concentration of heavy metals was determined using inductively coupled plasma mass spectrometry (Thermo Fisher).

2.4. Ecosystem Functions. Leaf litter (*Populus alba*) and cotton strip decomposition were measured at all sampling sites. Details on sample collection and treatment in the measurement of ecosystem functions are given in a previous study.[16] Briefly, coarse (10 mm) and fine containing ca. 5 g of air-dried, naturally senescent *P. alba* leaves, cotton strips (ca. 8 cm × 10 cm), and a HOBO InTemp Data Logger (Onset) were tied to a stainless-steel metal frame using plastic wire, which was then put into the river and fixed at the river bottom. Two mesh bags were set to permit or prevent invertebrate colonization, which enabled us to quantify total, microbial, and invertebrate-driven decomposition rates of leaves.[36] All mesh bags were retrieved after 3−4 weeks and the leaves and cotton strips were gently rinsed with running water to remove debris, and then freeze-dried to constant mass (>72 h) and weighed to the nearest 0.01 mg. Decomposition rates of leaves and cotton strips were calculated following the published formula.[16,51−53]

2.5. Candidate Indicators and Prediction. The data sets were randomly divided into (a) a training data set containing the randomly selected two-third of the samples (including reference and impaired sites) and (b) a testing data set including the remaining one-third of the samples. The training data set was used to screen the candidate indicators and develop a prediction model. We used the testing data set to

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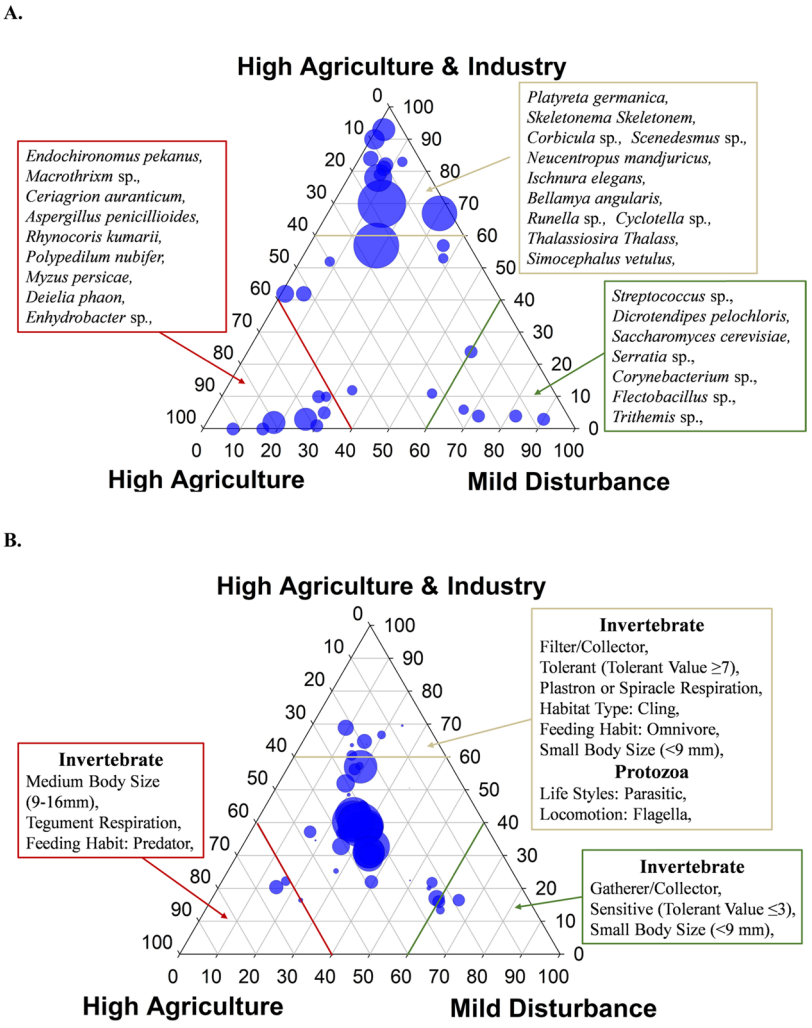

Figure 2. Spatial distribution of OTU-based indicators (A) and species traits (B) in the different taxonomic groups across the three major groups. The dot is the comparison of relative abundance of each indicator (A) and species traits (B) across the three major groups, and the bubble size represents their relative abundance in each taxonomic group.

assess the prediction performance of the models developed by the training data set. First, we defined the "candidate indicators" as a series of metrics on biodiversity and ecosystem functions (e.g., the relative abundance of taxa, diversity, and functions) that must respond sensitively (positively or negatively) to human impacts in the training data set. Then, the screening of candidate indicators followed three steps:

(1) To avoid the redundancy of data information, the correlations among all metrics (including biodiversity and ecosystem functions) were calculated, and the primary screening of redundant metrics was conducted based on the standard of absolute $r > 0.75$.[28] Screening criteria were such that the remaining metrics should maximize the coverage of different attributes.

(2) To further determine significant positive or negative metrics, general linear models were run between the remaining metrics (derived from the first step) and a known human stressor gradient (Figures S2 and S3). The general linear models were selected because they can directly and clearly identify sensitivity and tolerance metrics.[14,54] To rebuild a known human stressor gradient, principal component analysis (PCA) was carried out on the chemical variables; eigenvalues >1 and absolute $r > 0.50$ were taken as a criterion for the extraction of the principal components (PC), the first two PCs (PC1 and PC2) were equally weighted to obtain the new PC (PCAxis) as the gradient descriptor of the human stressor. The PCAxis gradient was validated against the chemical variables and the gradient of human land use (e.g., cropland and impervious cover), and we found significant positive relationships among them (Figure S4 and Table S1). These results suggest that the PCAxis could clearly indicate the intensity of human disturbance.

(3) To verify the reliability of the identified sensitivity metrics (derived from the second step), Student's *t*-tests were used to check the significance of sensitivity metrics between the preset reference ($n = 18$) and impaired sites ($n = 6$). The preset reference sites refer to undisturbed or only minimally disturbed sites by human activities.[55,56] Specifically, the selection criteria of reference sites in this study were as follows: (i) the environmental quality standards for surface water is above grade II in China (GB3838-2002), that is, reference sites should reach and be better than DO ≥6 mg/L, COD ≤4 mg/L, NH_3−N ≤ 0.5 mg/L, TN ≤0.5 mg/L, and TP ≤0.1 mg/L and (ii) the score of the habitat quality is beyond 120,

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Table 1. Screened Indicators for Assessing the River's Ecological Status and Their Response Trends to Human Disturbance (Surrogated by the PCAxis) in the Training Data Set[a]

groups	attributes	response	groups	attributes	response
invertebrates	Invertebrate_Shannon	decrease	algae	Algae_Shannon	decrease
	Insect_OTUs	decrease		Diatom_Shannon	decrease
	Chironomid_OTUs	decrease		Chlorophyta_ OTUs	decrease
	Mollusca_OTUs	decrease		Cryptophyta_ OTUs	decrease
	Oligochaeta_OTUs	decrease		%Dinophyta	increase
	%*Orthocladius* sp.	decrease		%top3 diatom	increase
	%Burrow	increase		%top3 cyanobacteria	increase
protozoa	Protozoa_OTUs	decrease		%*Scenedesmus* sp.	increase
	Opisthokonta_OTUs	increase		%*Cyclotella* sp.	increase
	Stramenopiles_OTUs	increase		%Biovolume Xla	decrease
	%Amoebozoa	decrease		%filamentous	increase
	%Euglenozoa	increase	bacteria	Bacteria_Phylogenetic	increase
	%*Cochliopodium minus*	decrease		Bacteroidetes_OTUs	increase
	%Pseudopods	decrease		%top3 bacteria	increase
	%Cilia	increase		%*Rhodococcus* sp.	increase
fungi	Fungi_OTUs	decrease		%*Corynebacterium* sp.	decrease
	Oomycota_OTUs	increase		%*Flectobacillus* sp.	decrease
	%Ascomycota	decrease		%*Psychrobacter sanguinis*	decrease
	%top3 fungi	increase	ecosystem functions	leaves k_{Total}	decrease
	%*Saccharomyces cerevisiae*	decrease		cotton strips k_{Total}	decrease

[a]"_Shannon", "_OTUs", "_Phylogenetic", "Top3", and "k_{Total}" present Shannon's diversity, OTU number, faith's phylogenetic diversity, percentage of top three dominant taxa, and the total decomposition rates of leaves or cotton strips, respectively.

the human disturbance in the riparian zone is the minimum, and no obvious agricultural and industrial land is in close vicinity (Figure S5). In contrast, impaired sites are defined as those sites where either the water quality standards or habitat quality is lower than the reference site.

Finally, a series of metrics on biodiversity and ecosystem functions meeting the abovementioned three steps were considered as candidate indicators. All indicators of biodiversity and ecosystem functions were standardized by *z*-score transformation (mean of 0 and standard deviation (SD) of 1) to meet the normal distribution. To predict the impacts of human disturbance (surrogated by the PCAxis) on ecosystems using the indicators of biodiversity (e.g., the relative abundance of OTUs-based indicators, richness, diversity) and ecosystem functions (e.g., the decomposition of leaf litter and cotton strips), predictive models were fitted for the training data set using multivariate linear regression (MLR) models. The testing data set was used to examine the accuracy of predicted values derived from MLR models compared with actual measured values.

2.6. Index Development and Comparison. To eliminate the scale differences between candidate indicators and to convert them into measurable and comparable values, all indicators were standardized referring to the formula in Table S2. In the formula, $Q_{95\%}$ and $Q_{5\%}$ are the 95th percentile and 5th percentile of the value at all sites, respectively, and M is the actual measured value of each site. To develop a bioassessment index that represents biodiversity or ecosystem functional elements, including single element and accumulative element index (Bio_Functions, integrating biodiversity, and ecosystem functional elements), all candidate indicators of each type were treated with reference to the formula in Table S3 so that their scores were between 0 and 1, and the higher the values, the better the river's ecological status. Based on the equal division method, the river's ecological status was divided into five levels, namely, "very good [0.8, 1.0]," "good [0.6, 0.8)," "fair [0.4, 0.6)," "poor [0.2, 0.4)", and "very poor [0, 0.2)" (Figure S6 and Table S3). To compare the consistency between the indices developed by the indicators of biodiversity or ecosystem functions and the physicochemical elements to assess river ecological status, the level of agreement between the two was quantified using the kappa2 function in the R package irr;[57] the higher value indicates higher consistency.

3. RESULTS

3.1. Distribution of OTU-Based Indicators and Species Traits at Spatial Scale. We detected a total of 2 002 177 invertebrate reads, 3 026 866 protozoan reads, 1 221 127 fungal reads, 8 695 888 algal reads, and 2 946 353 bacterial reads across all samples after stringent quality filtering (Table S4). These eDNA data were assigned to 806 invertebrate OTUs, 819 protozoan OTUs, 688 fungal OTUs, 1697 algal OTUs, and 1994 bacterial OTUs, annotated to 49 phyla, 119 classes, 227 orders, 297 families, 528 genera, and 411 species, respectively. The most abundant taxa were Insecta and Rotifera in invertebrates; Ciliophora and Amoebozoa in protozoa; Ascomycota and Oomycota in fungi; Ochrophyta, Cryptophyta, and Chlorophyta in algae; and Proteobacteria, Bacteroidetes, and Actinobacteria in bacteria.

A total of 81 OTU-based indicators were identified by the indicator analysis to characterize the intensity of human disturbance. Overall, 37 of the 81 OTU-based indicators have clear taxonomic information at the genus or species level (Figure 2A), such as *Trithemis* sp., *Dicrotendi pespelochloris,* and *Cyclotella* sp. The relative abundance of these indicators fluctuates greatly (range from 1 to 100 times) across the three major groups of this region. For example, the relative abundances of *Trithemis* sp. and *D. pespelochloris* are the highest in mild disturbance; *Ischnura elegans*, *Scenedesmus* sp., and *Cyclotella* sp. belong to the dominant taxa in high agriculture and industry; while *Polypedilum nubifer*, *Enhydro-*

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Table 2. Summary on the Multivariate Linear Regression Models to Predict the Intensity of Human Disturbance (Surrogated by the PCAxis, as the Dependent Variable, y) in the Training Data Set Using Screened Indicators on Biodiversity and Ecosystem Functions (as the Independent Variable, x)[a]

groups	predictor formula	adj-R^2	F
invertebrate	$y = -0.013 - 0.212 \times$ Invertebrate_-Shannon $- 0.118 \times$ Insect_OTUs	0.41	11.697
protozoa	$y = -0.021 - 0.268 \times$ Amoebozoa_OTUs $+ 0.179 \times$ %Cilia	0.37	9.458
fungi	$y = -0.019 + 0.241 \times$ %top3 fungi $- 0.114 \times$ %Ascomycota	0.40	10.049
algae	$y = -0.031 + 0.128 \times$ %*Scenedesmus* sp. $+ 0.162 \times$ %Dinophyta $+ 0.140 \times$ % filamentous	0.57	15.312
bacteria	$y = -0.029 + 0.159 \times$ Verrucomicrobia_OTUs $+ 0.108 \times$ %*Rhodococcus* sp.	0.35	9.253
ecofunctions	$y = -0.023 - 0.175 \times$ leaves k_{Total}	0.32	8.021
Bio_Functions	$y = -0.038 - 0.254 \times$ Invertebrate_-Shannon $+ 0.132 \times$ %filamentous $- 0.105 \times$ leaves $k_{Total} + 0.071 \times$ %top3 fungi	0.62	18.322

[a]"_Shannon", "_OTUs", "Top3", and "k_{Total}" present Shannon's diversity, OTU number, percentage of top three dominant taxa, and the total decomposition rates of leaves, respectively.

bacter sp., and *Macrothrixm* sp. mainly occupy the high agriculture. In addition, some species traits in invertebrates and protozoa have a unique distribution across the three major groups of this region (Figure 2B). Specifically, gatherer/collector, sensitive taxa, and small body size (<9 mm) in invertebrates prefer to live in mild disturbance; filter/collector, tolerant taxa, cling taxa, omnivore, and small body size (<9 mm) in invertebrates, and plastron or spiracle respiration in protozoa could be captured more in high agriculture and industry; and medium body size (9−16 mm), the predator in invertebrates, and tegument respiration in protozoa are more dominant in high agriculture.

3.2. Indicators of Biodiversity and Ecosystem Functions Accurately Predicted the Intensity of Human Disturbance. A total of 40 indicators were screened after the three-step screening (including redundancy analysis, linear regression analysis, and t-test), which cover five taxonomic groups from bacteria to invertebrates, multiple attributes of biodiversity (including taxonomic, phylogenetic, and functional aspects), and OTU-based indicators and ecosystem functions (Table 1). These screened indicators have significant positive or negative relationships with the intensity of human disturbance (surrogated by PCAxis, Table 1). For example, the Shannon diversity index, the relative abundance of OTU-based indicators (e.g., *Orthocladius* sp.), and carbon decomposition have negative responses to human impacts, while some dominant taxa and species traits have positive responses (e.g., burrowing invertebrates and ciliated protozoa).

Using screened indicators of biodiversity and ecosystem functions, we could identify the intensity of human disturbance (surrogated by PCAxis) with 32−62% accuracy on the training data set (R^2 value of MLR models). The accuracy value of the accumulative indicators (Bio_Functions, integrating biodiversity, and ecosystem function metrics) was higher (62%) than any single data (from 32 to 57%, Table 2). Comparing the predicted value (MLR models in the test data set) with the actual value derived from environmental data of human disturbance, there is a good consistency between each value (R^2 = 0.47−0.59, from linear regression models, Figure 3). In addition, we found a positive or negative deviation of the prediction models at the minimum and maximum levels of human disturbance whether single or cumulative indicators, respectively. For example, the predicted value is higher than actual ones in low disturbance, but the lower predicted value occurs in a high disturbance. The predicted values of accumulative indicators of biodiversity and ecosystem functions are closer to the true value than single data (the 1:1 asymptote, Figure 3).

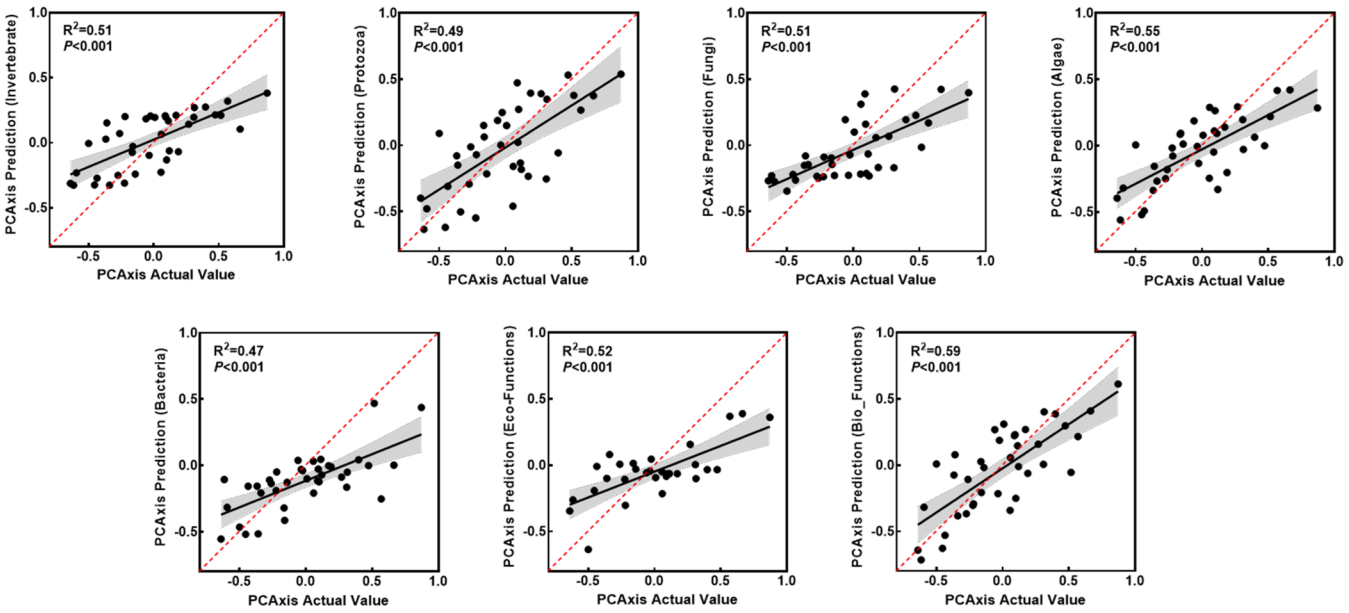

Figure 3. Comparison between the predicted value (R^2 value of multivariate linear regression models) of human disturbance (surrogated by PCAxis) given by the indicators in test samples and the actual value derived from environmental data; the red diagonal dash lines represent the 1:1 ratio between predicted and measured values. Bio_Functions integrate indicators of biodiversity and ecosystem functional elements.

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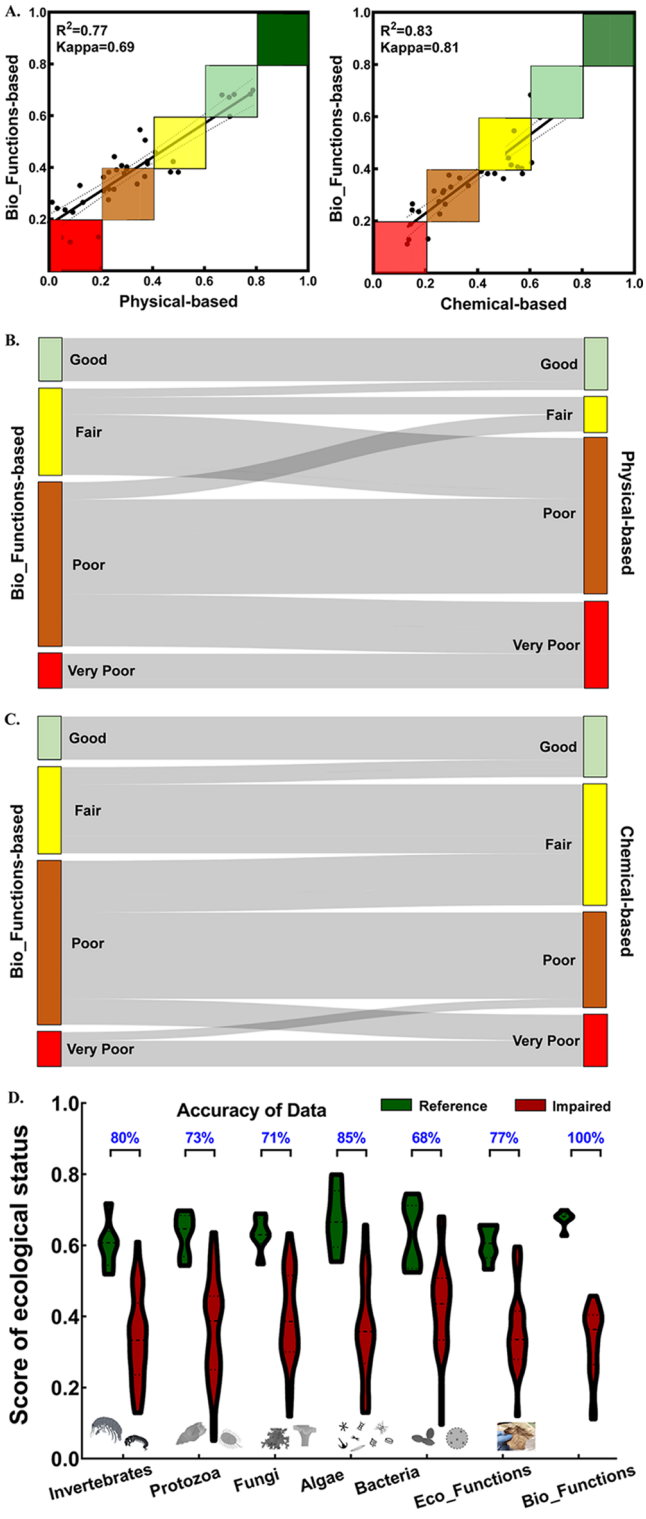

Figure 4. Relationships between the accumulative index (Bio_-Functions, integrating biodiversity, and ecosystem functional elements) and the physicochemical elements (A). Colored boxes represent the score of the ecological status (*y*-axis) given by the framework (B, C): green, very good [0.8, 1.0]; light green, good [0.6, 0.8); yellow, fair [0.4, 0.6); brown, poor [0.2, 0.4); and red, very poor [0, 0.2). The R^2 (derived from multivariate linear regression models) and κ values (derived from κ tests) indicate the linear fit and the level of agreement, respectively. The higher value means the higher consistency. Comparison of identification of reference and impaired sites by different types of indices (D).

3.3. Indices Developed by Indicators Improved the Assessment of the Ecological Status of Shaying River.

We found significant positive correlations between the indices developed by the indicators of biodiversity and ecosystem functions and the physicochemical elements, and these indices can clearly distinguish the physicochemical characteristics of the sites (physical, R^2 = 0.52−0.77, κ = 0.51−0.71; chemical, R^2 = 0.54−0.83, κ = 0.59−0.81) (Figures 4A−C and S6−S11). Compared with any single element index (e.g., invertebrates or algae), the accumulative element index (Bio_Functions) not only has higher consistency with physicochemical elements (physical, R^2 = 0.77, κ = 0.69; chemical, R^2 = 0.83, κ = 0.81) but also improved the assessment accuracy by 20−30% for the identification of reference and impaired sites in Shaying River (Figure 4D). Furthermore, using the multidimensional framework including the indices developed by the indicators of biodiversity and ecosystem functions, we can not only directly distinguish the sites with poor ecological status in the catchment but also completely understand the associated damage degree integrating physicochemical, biodiversity, and ecosystem functional elements (Figure 5).

4. DISCUSSION

Our study showed that OTU-based indicators are an effective taxonomy-free strategy to reveal the status and possible change of ecosystems. For example, we identified that the relative abundance of 81 OTU-based indicators was strongly associated with different human disturbance groups (range from 1 to 100 times), although only 37 of them have clear classification at the genus or species level. This result indicates that OTU-based indicators can make up for the deficiency of the traditional species annotation strategy to a great extent because the traditional strategy is limited to the part of biodiversity with obvious morphological characteristics, which often has high coverage of public reference sequences, such as fish and birds.[58,59] Recent studies have indeed found that for well-studied groups, eDNA-based assessments give comparable results with respect to ecological indices.[60,61] Although eDNA metabarcoding may reduce cost and is a less invasive sampling process,[23,62,63] the deficiency of reference databases and the deviation of OTU species annotation are still one of the biggest shortcomings of this method. An OTU-based indicator strategy obviously reduces or bypasses the dependence of species annotation on reference databases.[7] Relying directly on the occurrence of OTUs in known stressor gradients, it may give indicator values similar to morphology-based species identification, and thus also allow transitioning between the two methods. The advantage of an OTU-based method is that almost 90% of the OTUs can be used to calculate the biological index, while this value drops to less than 30% (or even much lower) when a species annotation method is taken.[7,27,54,61,64,65] Importantly, even without having a complete taxonomic resolution, OTUs can be linked to traits of invertebrates and protozoa and thereby indicate the intensity of human disturbance. For example, the relative abundance of tolerant and small body size species in invertebrates increases with human disturbance. Species traits directly link the functional roles of organisms with environmental factors (e.g., flow, temperature, and nutrients), and some functional traits are not limited by taxonomy and are more stable in space so that they can be applied on a large spatial scale.[19,49]

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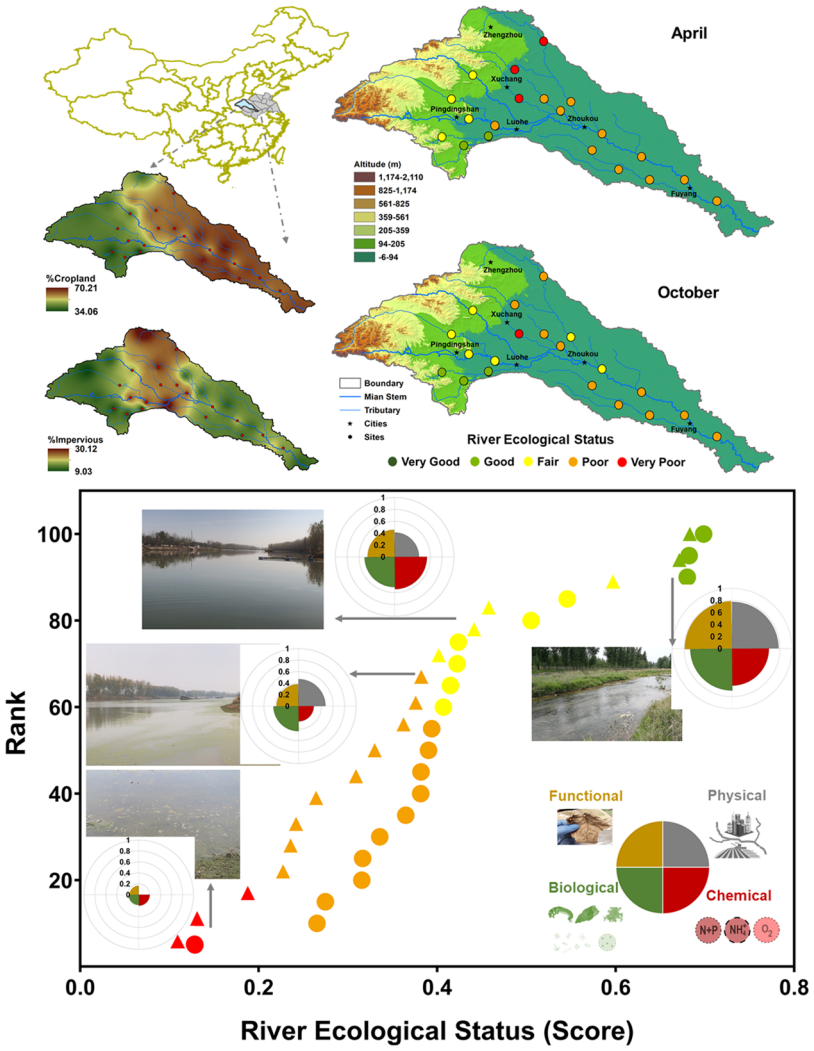

Figure 5. Results of the ecological status assessment in Shaying River based on the multidimensional framework including physicochemical elements, biodiversity (across taxa and multiple attributes), and ecosystem functions. The rose pie chart shows the ecological status assessment of the sampling sites at different elements. Triangles and dots represent April and October data, respectively.

The metrics on ecosystem functions indicate a significant negative impact of human disturbance on ecosystem functions in the Shaying river basin. For example, we found that the decomposition of the leaf litter and cotton strips has a significant negative response to human disturbance (surrogated by PCAxis, Table 1), and there is a significant difference at reference and impaired sites. These two indicators further corroborate that they have great potential to capture functional changes in riverine ecosystems.[36,52] A pan-European continental study supports our data, finding that leaf litter decomposition in impaired rivers is significantly lower than in the reference status.[36] Some studies proved that the decomposition of leaf litter is actually the consequence of the joint regulation of nitrogen and phosphorus concentrations on leaf litter metabolism in ecosystems (including water body and the litter itself), such as the high decomposition rate caused by the low C/N ratio.[66,67] This evidence suggests that the inherent differences of elemental composition (e.g., C/N/P ratios) in the ecosystem may affect the decomposition of leaf litter. To overcome or correct slight defects of leaf litter, we synchronously used the substrate with homogeneous chemical composition (cotton strips, >99% cellulose component). As expected, the response of cotton strips to human impact is consistent with that of leaf litter, and the decomposition of cotton strips decreased significantly with the increase in human disturbance. These data eliminate the relative bias caused by the chemical heterogeneity of leaf litter to a certain extent,[53,68] and also show that the decomposition of cotton strips has great potential to characterize the ecosystem changes and act as functional indicators for assessing the ecosystem status.[35,37]

Our proposed framework provides a comprehensive assessment of the ecological status to identify potential environmental stressors. First, extensive biodiversity data across taxa and multiple attributes provided by the eDNA metabarcoding approach reveal the ecological consequences driven by human impacts in the Shaying river basin, such as shifts of dominant taxa and species traits and the decline of phylogenetic diversity (Table 1). Second, metrics on ecosystem functions directly inform on the degradation or self-purification ability of rivers. This aspect has been largely ignored in the assessment of the river's ecological status in the past decades.[33,36,37] The main contribution of the framework is to integrate complete biodiversity data and ecosystem functions, allowing major complementation of traditional ecosystem monitoring. In general, we developed a multidimensional framework of biomonitoring and ecological status assessment, including

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physicochemical elements, metrics on biodiversity, and ecosystem functions, for a comprehensive and systematical perspective on the changes of riverine ecosystems (Figure 5). To do so, we integrated eDNA metabarcoding into a routine bioassessment approach and linked it to an index linked to ecosystem processes (e.g., exogenous carbon decomposition) to assess the ecological status. Our data showed that together they not only reliably and accurately predict the impact of human disturbance on riverine ecosystems but had also higher accuracy than the traditional physicochemical elements for assessing the ecological status. In general, we highlight the great potential of the framework in future biomonitoring and ecosystem management, which will help to implement ecoenvironmental regulatory reforms aimed at protecting and maintaining the good ecological status of rivers.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.1c05899.

Overview and detailed map of Shaying River, screening of reference sites, main taxa in different taxonomic groups detected by eDNA metabarcoding approach, a framework for ecological status assessment, principal component analysis on chemical variables, screening methods for sensitive and tolerant indicators, relationships between the chemistry-based stressor gradient and human land use, standardization of variables or indicators and their formulae, computational formula and assessment criteria on indices, comparison between the biological or functional indices and the physicochemical elements, and distance-based linear model results (PDF)

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Notes

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■ REFERENCES

(1) Grizzetti, B.; Lanzanova, D.; Liquete, C.; Reynaud, A.; Cardoso, A. C. Assessing water ecosystem services for water resource management. *Environ. Sci. Policy* **2016**, *61*, 194−203.

(2) Gibson, J. F.; Stein, E. D.; Baird, D. J.; Finlayson, C. M.; Zhang, X.; Hajibabaei, M. Wetland Ecogenomics—The Next Generation of Wetland Biodiversity and Functional Assessment. *Wetland Science and Practice*; Society of Wetland Scientists, 2015; Vol. *32*, pp 27−32.

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

(4) Barrett, M.; Belward, A.; Bladen, S.; Breeze, T.; Burgess, N.; Butchart, S.; Clewclow, H.; Cornell, S.; Cottam, A.; Croft, S. *Living Planet Report 2018: Aiming Higher*; WWF, 2018.

(5) 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. Biodiversity loss and its impact on humanity. *Nature* **2012**, *486*, 59−67.

(6) 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. Global threats to human water security and river biodiversity. *Nature* **2010**, *468*, 334.

(7) Cordier, T.; Alonso-Saez, L.; Apotheloz-Perret-Gentil, L.; Aylagas, E.; Bohan, D. A.; Bouchez, A.; Chariton, A.; Creer, S.; Fruhe, L.; Keck, F.; Keeley, N.; Laroche, O.; Leese, F.; Pochon, X.; Stoeck, T.; Pawlowski, J.; Lanzen, A. Ecosystems monitoring powered by environmental genomics: A review of current strategies with an implementation roadmap. *Mol. Ecol.* **2021**, *30*, 2937−2958.

(8) Ratajczak, Z.; Carpenter, S. R.; Ives, A. R.; Kucharik, C. J.; Ramiadantsoa, T.; Stegner, M. A.; Williams, J. W.; Zhang, J.; Turner, M. G. Abrupt Change in Ecological Systems: Inference and Diagnosis. *Trends Ecol. Evol.* **2018**, *33*, 513−526.

(9) Caroni, R.; van de Bund, W.; Clarke, R.; Johnson, R. Combination of multiple biological quality elements into waterbody assessment of surface waters. *Hydrobiologia* **2013**, *704*, 437−451.

(10) Birk, S.; Bonne, W.; Borja, A.; Brucet, S.; Courrat, A.; Poikane, S.; Solimini, A.; van de Bund, W. V.; Zampoukas, N.; Hering, D. Three hundred ways to assess Europe's surface waters: An almost complete overview of biological methods to implement the Water Framework Directive. *Ecol. Indic.* **2012**, *18*, 31−41.

(11) Hering, D.; Borja, A.; Carstensen, J.; Carvalho, L.; Elliott, M.; Feld, C. K.; Heiskanen, A. S.; Johnson, R. K.; Moe, J.; Pont, D.; Solheim, A. L.; van de Bund, W. The European Water Framework Directive at the age of 10: A critical review of the achievements with recommendations for the future. *Sci. Total Environ.* **2010**, *408*, 4007−4019.

(12) Fan, J.; Wang, S.; Li, H.; Yan, Z.; Zhang, Y.; Zheng, X.; Wang, P. Modeling the ecological status response of rivers to multiple stressors using machine learning: A comparison of environmental

DNA metabarcoding and morphological data. *Water Res.* **2020**, *183*, No. 116004.
(13) Pandey, L. K.; Bergey, E. A.; Lyu, J.; Park, J.; Choi, S.; Lee, H.; Depuydt, S.; Oh, Y.-T.; Lee, S.-M.; Han, T. The use of diatoms in ecotoxicology and bioassessment: Insights, advances and challenges. *Water Res.* **2017**, *118*, 39−58.
(14) Yang, J.; Zhang, X. eDNA metabarcoding in zooplankton improves the ecological status assessment of aquatic ecosystems. *Environ. Int.* **2020**, *134*, No. 105230.
(15) Altermatt, F.; Little, C. J.; Mächler, E.; Wang, S.; Zhang, X.; Blackman, R. C. Uncovering the complete biodiversity structure in spatial networks: the example of riverine systems. *Oikos* **2020**, *129*, 607−618.
(16) Li, F.; Altermatt, F.; Yang, J.; An, S.; Li, A.; Zhang, X. Human activities' fingerprint on multitrophic biodiversity and ecosystem functions across a major river catchment in China. *Global Change Biol.* **2020**, *26*, 6867−6879.
(17) Outhwaite, C. L.; Gregory, R. D.; Chandler, R. E.; Collen, B.; Isaac, N. J. B. Complex long-term biodiversity change among invertebrates, bryophytes and lichens. *Nat. Ecol. Evol.* **2020**, *4*, 384−392.
(18) Faith, D. P. The Role of the Phylogenetic Diversity Measure, PD, in Bio-informatics: Getting the Definition Right. *Evol. Bioinf.* **2006**, *2*, 277−283.
(19) Menezes, S.; Baird, D. J.; Soares, A. M. V. M. Beyond taxonomy: a review of macroinvertebrate trait-based community descriptors as tools for freshwater biomonitoring. *J. Appl. Ecol.* **2010**, *47*, 711−719.
(20) Jarzyna, M. A.; Jetz, W. Detecting the Multiple Facets of Biodiversity. *Trends Ecol. Evol.* **2016**, *31*, 527−538.
(21) Mächler, E.; Little, C. J.; Wüthrich, R.; Alther, R.; Fronhofer, E. A.; Gounand, I.; Harvey, E.; Hürlemann, S.; Walser, J.-C.; Altermatt, F. Assessing different components of diversity across a river network using eDNA. *Environ. DNA* **2019**, *1*, 290−301.
(22) Taberlet, P.; Bonin, A.; Zinger, L.; Coissac, E. *Environmental DNA: For Biodiversity Research and Monitoring*; Oxford University Press, 2018.
(23) Cristescu, M. E.; Hebert, P. D. N. Uses and Misuses of Environmental DNA in Biodiversity Science and Conservation. *Annu. Rev. Ecol., Evol., Syst.* **2018**, *49*, 209−230.
(24) Eisenhauer, N.; Bonn, A.; Guerra, C. A. Recognizing the quiet extinction of invertebrates. *Nat. Commun.* **2019**, *10*, No. 50.
(25) Brown, L. E.; Khamis, K.; Wilkes, M.; Blaen, P.; Brittain, J. E.; Carrivick, J. L.; Fell, S.; Friberg, N.; Füreder, L.; Gislason, G. M.; Hainie, S.; Hannah, D. M.; James, W. H. M.; Lencioni, V.; Olafsson, J. S.; Robinson, C. T.; Saltveit, S. J.; Thompson, C.; Milner, A. M. Functional diversity and community assembly of river invertebrates show globally consistent responses to decreasing glacier cover. *Nat. Ecol. Evol.* **2018**, *2*, 325−333.
(26) Grossart, H.-P.; Van den Wyngaert, S.; Kagami, M.; Wurzbacher, C.; Cunliffe, M.; Rojas-Jimenez, K. Fungi in aquatic ecosystems. *Nat. Rev. Microbiol.* **2019**, *17*, 339−354.
(27) Li, F.; Peng, Y.; Fang, W.; Altermatt, F.; Xie, Y.; Yang, J.; Zhang, X. Application of Environmental DNA Metabarcoding for Predicting Anthropogenic Pollution in Rivers. *Environ. Sci. Technol.* **2018**, *52*, 11708−11719.
(28) Xie, Y. W.; Wang, J. Z.; Wu, Y.; Ren, C.; Song, C.; Yang, J. H.; Yu, H. X.; Giesy, J. P.; Zhang, X. W. Using in situ bacterial communities to monitor contaminants in river sediments. *Environ. Pollut.* **2016**, *212*, 348−357.
(29) Cordier, T. Bacterial communities' taxonomic and functional turnovers both accurately predict marine benthic ecological quality status. *Environ. DNA* **2020**, *2*, 175−183.
(30) Sagova-Mareckova, M.; Boenigk, J.; Bouchez, A.; Cermakova, K.; Chonova, T.; Cordier, T.; Eisendle, U.; Elersek, T.; Fazi, S.; Fleituch, T.; Frühe, L.; Gajdosova, M.; Graupner, N.; Haegerbaeumer, A.; Kelly, A. M.; Kopecky, J.; Leese, F.; Nõges, P.; Orlic, S.; Panksep, K.; Pawlowski, J.; Petrusek, A.; Piggott, J. J.; Rusch, J. C.; Salis, R.; Schenk, J.; Simek, K.; Stovicek, A.; Strand, D. A.; Vasquez, M. I.; Vrålstad, T.; Zlatkovic, S.; Zupancic, M.; Stoeck, T. Expanding ecological assessment by integrating microorganisms into routine freshwater biomonitoring. *Water Res.* **2021**, *191*, No. 116767.
(31) Cordier, T.; Lanzén, A.; Apothéloz-Perret-Gentil, L.; Stoeck, T.; Pawlowski, J. Embracing environmental genomics and machine learning for routine biomonitoring. *Trends Microbiol.* **2019**, *27*, 387−397.
(32) UN. *Transforming Our World: The 2030 Agenda for Sustainable Development*; Division for Sustainable Development Goals: New York, NY, 2015.
(33) Marks, J. C. Revisiting the Fates of Dead Leaves That Fall into Streams. *Annu. Rev. Ecol., Evol., Syst.* **2019**, *50*, 547−568.
(34) Brett, M. T.; Bunn, S. E.; Chandra, S.; Galloway, A. W. E.; Guo, F.; Kainz, M. J.; Kankaala, P.; Lau, D. C. P.; Moulton, T. P.; Power, M. E.; Rasmussen, J. B.; Taipale, S. J.; Thorp, J. H.; Wehr, J. D. How important are terrestrial organic carbon inputs for secondary production in freshwater ecosystems? *Freshwater Biol.* **2017**, *62*, 833−853.
(35) Tiegs, S. D.; Costello, D. M.; Isken, M. W.; Woodward, G.; McIntyre, P. B.; Gessner, M. O.; Chauvet, E.; Griffiths, N. A.; Flecker, A. S.; Acuna, V.; Albarino, R.; Allen, D. C.; Alonso, C.; Andino, P.; Arango, C.; Aroviita, J.; Barbosa, M. V. M.; Barmuta, L. A.; Baxter, C. V.; Bell, T. D. C.; Bellinger, B.; Boyero, L.; Brown, L. E.; Bruder, A.; Bruesewitz, D. A.; Burdon, F. J.; Callisto, M.; Canhoto, C.; Capps, K. A.; Castillo, M. M.; Clapcott, J.; Colas, F.; Colon-Gaud, C.; Cornut, J.; Crespo-Perez, V.; Cross, W. F.; Culp, J. M.; Danger, M.; Dangles, O.; de Eyto, E.; Derry, A. M.; Villanueva, V. D.; Douglas, M. M.; Elosegi, A.; Encalada, A. C.; Entrekin, S.; Espinosa, R.; Ethaiya, D.; Ferreira, V.; Ferriol, C.; Flanagan, K. M.; Fleituch, T.; Follstad Shah, J. J.; Frainer Barbosa, A.; Friberg, N.; Frost, P. C.; Garcia, E. A.; Garcia Lago, L.; Garcia Soto, P. E.; Ghate, S.; Giling, D. P.; Gilmer, A.; Goncalves, J. F., Jr.; Gonzales, R. K.; Graca, M. A. S.; Grace, M.; Grossart, H. P.; Guerold, F.; Gulis, V.; Hepp, L. U.; Higgins, S.; Hishi, T.; Huddart, J.; Hudson, J.; Imberger, S.; Iniguez-Armijos, C.; Iwata, T.; Janetski, D. J.; Jennings, E.; Kirkwood, A. E.; Koning, A. A.; Kosten, S.; Kuehn, K. A.; Laudon, H.; Leavitt, P. R.; Lemes da Silva, A. L.; Leroux, S. J.; LeRoy, C. J.; Lisi, P. J.; MacKenzie, R.; Marcarelli, A. M.; Masese, F. O.; McKie, B. G.; Oliveira Medeiros, A.; Meissner, K.; Milisa, M.; Mishra, S.; Miyake, Y.; Moerke, A.; Mombrikotb, S.; Mooney, R.; Moulton, T.; Muotka, T.; Negishi, J. N.; Neres-Lima, V.; Nieminen, M. L.; Nimptsch, J.; Ondruch, J.; Paavola, R.; Pardo, I.; Patrick, C. J.; Peeters, E.; Pozo, J.; Pringle, C.; Prussian, A.; Quenta, E.; Quesada, A.; Reid, B.; Richardson, J. S.; Rigosi, A.; Rincon, J.; Risnoveanu, G.; Robinson, C. T.; Rodriguez-Gallego, L.; Royer, T. V.; Rusak, J. A.; Santamans, A. C.; Selmeczy, G. B.; Simiyu, G.; Skuja, A.; Smykla, J.; Sridhar, K. R.; Sponseller, R.; Stoler, A.; Swan, C. M.; Szlag, D.; Teixeira-de Mello, F.; Tonkin, J. D.; Uusheimo, S.; Veach, A. M.; Vilbaste, S.; Vought, L. B. M.; Wang, C. P.; Webster, J. R.; Wilson, P. B.; Woelfl, S.; Xenopoulos, M. A.; Yates, A. G.; Yoshimura, C.; Yule, C. M.; Zhang, Y. X.; Zwart, J. A. Global patterns and drivers of ecosystem functioning in rivers and riparian zones. *Sci. Adv.* **2019**, *5*, No. eaav0486.
(36) Chauvet, E.; Ferreira, V.; Giller, P. S.; McKie, B. G.; Tiegs, S. D.; Woodward, G.; Elosegi, A.; Dobson, M.; Fleituch, T.; Graça, M. A. et al. Litter Decomposition as an Indicator of Stream Ecosystem Functioning at Local-to-Continental Scales: Insights from the European RivFunction Project. *Advances in Ecological Research*; Academic Press, 2016; Vol. *55*, pp 99−182.
(37) Jabiol, J.; Colas, F.; Guérold, F. Cotton-strip assays: Let's move on to eco-friendly biomonitoring! *Water Res.* **2020**, *170*, No. 115295.
(38) Luo, Z.; Zuo, Q.; Shao, Q.; Ding, X. The impact of socioeconomic system on the river system in a heavily disturbed basin. *Sci. Total Environ.* **2019**, *660*, 851−864.
(39) Elbrecht, V.; Leese, F. Validation and Development of COI Metabarcoding Primers for Freshwater Macroinvertebrate Bioassessment. *Front. Environ. Sci.* **2017**, *5*, No. 11.
(40) Klindworth, A.; Pruesse, E.; Schweer, T.; Peplies, J.; Quast, C.; Horn, M.; Glockner, F. O. Evaluation of general 16S ribosomal RNA

gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Res.* **2013**, *41*, No. e1.
(41) Amaral-Zettler, L. A.; McCliment, E. A.; Ducklow, H. W.; Huse, S. M. A method for studying protistan diversity using massively parallel sequencing of V9 hypervariable regions of small-subunit ribosomal RNA genes. *PLoS One* **2009**, *4*, No. e6372.
(42) Muyzer, G.; De Waal, E. C.; Uitterlinden, A. G. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Appl. Environ. Microbiol.* **1993**, *59*, 695−700.
(43) 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.; et al. QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods* **2010**, *7*, 335−336.
(44) DeSantis, T. Z.; Hugenholtz, P.; Larsen, N.; Rojas, M.; Brodie, E. L.; Keller, K.; Huber, T.; Dalevi, D.; Hu, P.; Andersen, G. L. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Appl. Environ. Microbiol.* **2006**, *72*, 5069−5072.
(45) Guillou, L.; Bachar, D.; Audic, S.; Bass, D.; Berney, C.; Bittner, L.; Boutte, C.; Burgaud, G.; de Vargas, C.; Decelle, J.; et al. The Protist Ribosomal Reference database (PR2): a catalog of unicellular eukaryote small sub-unit rRNA sequences with curated taxonomy. *Nucleic Acids Res.* **2012**, *41*, D597−D604.
(46) Cáceres, M. D.; Legendre, P. Associations between species and groups of sites: indices and statistical inference. *Ecology* **2009**, *90*, 3566−3574.
(47) Bruno, D.; Belmar, O.; Maire, A.; Morel, A.; Dumont, B.; Datry, T. Structural and functional responses of invertebrate communities to climate change and flow regulation in alpine catchments. *Global Change Biol.* **2019**, *25*, 1612−1628.
(48) Abonyi, A.; Horvath, Z.; Ptacnik, R. Functional richness outperforms taxonomic richness in predicting ecosystem functioning in natural phytoplankton communities. *Freshwater Biol.* **2018**, *63*, 178−186.
(49) Usseglio-Polatera, P.; Bournaud, M.; Richoux, P.; Tachet, H. Biomonitoring through biological traits of benthic macroinvertebrates: how to use species trait databases? *Hydrobiologia* **2000**, *422*, 153−162.
(50) Plafkin, J. L. *Rapid Bioassessment Protocols for Use in Streams and Rivers: Benthic Macroinvertebrates and Fish*; Office of Water, United States Environmental Protection Agency, 1989.
(51) Woodward, G.; Gessner, M. O.; Giller, P. S.; Gulis, V.; Hladyz, S.; Lecerf, A.; Malmqvist, B.; McKie, B. G.; Tiegs, S. D.; Cariss, H.; et al. Continental-scale effects of nutrient pollution on stream ecosystem functioning. *Science* **2012**, *336*, 1438−1440.
(52) Rosemond, A. D.; Benstead, J. P.; Bumpers, P. M.; Gulis, V.; Kominoski, J. S.; Manning, D. W. P.; Suberkropp, K.; Wallace, J. B. Experimental nutrient additions accelerate terrestrial carbon loss from stream ecosystems. *Science* **2015**, *347*, 1142−1145.
(53) Vyšná, V.; Dyer, F.; Maher, W.; Norris, R. Cotton-strip decomposition rate as a river condition indicator - Diel temperature range and deployment season and length also matter. *Ecol. Indic.* **2014**, *45*, 508−521.
(54) Tapolczai, K.; Keck, F.; Bouchez, A.; Rimet, F.; Kahlert, M.; Vasselon, V. Diatom DNA Metabarcoding for Biomonitoring: Strategies to Avoid Major Taxonomical and Bioinformatical Biases Limiting Molecular Indices Capacities. *Front. Ecol. Evol.* **2019**, *7*, No. 409.
(55) Harrelson, C. C. *Stream Channel Reference Sites: An Illustrated Guide to Field Technique*; U.S. Department of Agriculture, Forest Service, Rocky Mountain Forest and Range Experiment Station, 1994; Vol. *245*.
(56) Hughes, R. M.; Larsen, D. P.; Omernik, J. M. Regional reference sites: a method for assessing stream potentials. *Environ. Manage.* **1986**, *10*, 629−635.
(57) Garmer, M.; Lemon, J.; Fellows, I.; Singh, S. Various Coefficients of Interrater Reliability and Agreement, 2012, https://CRAN.R-project.org/package=irr.
(58) Weigand, H.; Beermann, A. J.; Čiampor, F.; Costa, F. O.; Csabai, Z.; Duarte, S.; Geiger, M. F.; Grabowski, M.; Rimet, F.; Rulik, B.; Strand, M.; Szucsich, N.; Weigand, A. M.; Willassen, E.; Wyler, S. A.; Bouchez, A.; Borja, A.; Čiamporová-Zaťovičová, Z.; Ferreira, S.; Dijkstra, K.-D. B.; Eisendle, U.; Freyhof, J.; Gadawski, P.; Graf, W.; Haegerbaeumer, A.; van der Hoorn, B. B.; Japoshvili, B.; Keresztes, L.; Keskin, E.; Leese, F.; Macher, J. N.; Mamos, T.; Paz, G.; Pešić, V.; Pfannkuchen, D. M.; Pfannkuchen, M. A.; Price, B. W.; Rinkevich, B.; Teixeira, M. A. L.; Várbíró, G.; Ekrem, T. DNA barcode reference libraries for the monitoring of aquatic biota in Europe: Gap-analysis and recommendations for future work. *Sci. Total Environ.* **2019**, *678*, 499−524.
(59) Kerr, K. C. R.; Stoeckle, M. Y.; Dove, C. J.; Weigt, L. A.; Francis, C. M.; Hebert, P. D. N. Comprehensive DNA barcode coverage of North American birds. *Mol. Ecol. Notes* **2007**, *7*, 535−543.
(60) Brantschen, J.; Blackman, R. C.; Walser, J.-C.; Altermatt, F. Environmental DNA gives comparable results to morphology-based indices of macroinvertebrates in a large-scale ecological assessment. *PLoS One* **2021**, *16*, No. e0257510.
(61) Apothéloz-Perret-Gentil, L.; Cordonier, A.; Straub, F.; Iseli, J.; Esling, P.; Pawlowski, J. Taxonomy-free molecular diatom index for high-throughput eDNA biomonitoring. *Mol. Ecol. Resour.* **2017**, *17*, 1231−1242.
(62) Carraro, L.; Mächler, E.; Wüthrich, R.; Altermatt, F. Environmental DNA allows upscaling spatial patterns of biodiversity in freshwater ecosystems. *Nat. Commun.* **2020**, *11*, No. 3585.
(63) Deiner, K.; Fronhofer, E. A.; Mächler, E.; Walser, J.-C.; Altermatt, F. Environmental DNA reveals that rivers are conveyer belts of biodiversity information. *Nat. Commun.* **2016**, *7*, No. 12544.
(64) Cordier, T.; Esling, P.; Lejzerowicz, F.; Visco, J.; Ouadahi, A.; Martins, C.; Cedhagen, T.; Pawlowski, J. Predicting the ecological quality status of marine environments from eDNA metabarcoding data using supervised machine learning. *Environ. Sci. Technol.* **2017**, *51*, 9118−9126.
(65) Mächler, E.; Walser, J.-C.; Altermatt, F. Decision-making and best practices for taxonomy-free environmental DNA metabarcoding in biomonitoring using Hill numbers. *Mol. Ecol.* **2021**, *30*, 3326−3339.
(66) Demi, L. M.; Benstead, J. P.; Rosemond, A. D.; Maerz, J. C. Litter P content drives consumer production in detritus-based streams spanning an experimental N: P gradient. *Ecology* **2018**, *99*, 347−359.
(67) Manning, D. W.; Rosemond, A. D.; Kominoski, J. S.; Gulis, V.; Benstead, J. P.; Maerz, J. C. Detrital stoichiometry as a critical nexus for the effects of streamwater nutrients on leaf litter breakdown rates. *Ecology* **2015**, *96*, 2214−2224.
(68) Tiegs, S. D.; Clapcott, J. E.; Griffiths, N. A.; Boulton, A. J. A standardized cotton-strip assay for measuring organic-matter decomposition in streams. *Ecol. Indic.* **2013**, *32*, 131−139.

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