ChinaRxiv 中X

AI translation · View original & related papers at
chinarxiv.org/items/chinaxiv-201912.00032

Effectiveness assessment of using riverine water eDNA to simultaneously monitor the riverine and riparian biodiversity information postprint

Authors: Yang, Haile, Du, Hao, Qi, Hongfang, Yu, Luxian, Hou, Xindong, Zhang, Hui, Li, Junyi, Wei, Qiwei

Date: 2023-07-06T00:00:00+00:00

Abstract

In recent years, the application of environmental DNA (eDNA) technology in ecological research has become increasingly widespread. Based on watershed ecosystem processes driven by natural runoff, utilizing aquatic eDNA enables monitoring upstream river segment biological composition from downstream sampling sites, as well as monitoring biological composition within catchment areas from water sampling sites. However, the effectiveness of such monitoring lacks systematic validation, affecting the credibility of its application. To investigate the effectiveness of aquatic eDNA analysis in watershed ecosystem biodiversity monitoring, we employ watershed biological information flow as an analytical framework to explore the two types of aquatic eDNA monitoring applications mentioned above and their respective effectiveness. We selected the Shaliu River, a major tributary of Qinghai Lake, as our study area and conducted comparative analysis of microbial composition in river water and riparian soil using eDNA technology. The results show that: (1) The effectiveness of monitoring upstream microbial biodiversity using downstream eDNA is primarily influenced by temperature and discharge. Monitoring effectiveness is relatively low in spring (only approximately 76% of organisms at 1 km upstream can be detected), while it is significantly higher in summer and autumn (over 96% of organisms at 1 km upstream can be detected); (2) The effectiveness of monitoring terrestrial microbial biodiversity within catchment areas using aquatic eDNA is primarily influenced by season and rainfall. Monitoring effectiveness is relatively low in spring (only approximately 17% of organisms in adjacent riparian soils can be detected), while it is significantly higher in summer and autumn (the proportion of detected organisms in adjacent riparian soils can exceed 62%). Based on these findings, we propose that: (1) For monitoring upstream biodiversity from downstream sites, the summer-autumn breeding season represents the most cost-effective monitoring period, while sampling site

density should be appropriately increased during spring; (2) For monitoring terrestrial biodiversity from aquatic sampling sites, rainfall days and post-rain periods with turbid water during the active biological period in summer and autumn represent the most cost-effective monitoring windows, though the issue of detection effectiveness warrants continuous attention.

Full Text

Preamble

Effectiveness Assessment of Using Riverine Water eDNA to Simultaneously Monitor Riverine and Riparian Biodiversity

Haile Yang[1], Hao Du[1], *Hongfang Qi[2], Luxian Yu[2], Xindong Hou[3], Hui Zhang[1], Junyi Li[1], Jinming Wu[1], Chengyou Wang[1], Qiong Zhou[1] & Qiwei Wei[1]*

Both aquatic and terrestrial biodiversity information can be detected in riverine water environmental DNA (eDNA). However, the effectiveness of using riverine water eDNA to simultaneously monitor riverine and terrestrial biodiversity information remains unidentified. Here, we propose that monitoring effectiveness could be approximated by the transportation effectiveness of land-to-river and upstream-to-downstream biodiversity information flows, and described by three new indicators.

We conducted a case study in a watershed on the Qinghai–Tibet Plateau. The results demonstrated higher monitoring effectiveness on summer or autumn rainy days than in other seasons and weather conditions. Monitoring of bacterial biodiversity information was more efficient than monitoring of eukaryotic biodiversity information. On summer rainy days, 43–76% of species information from riparian sites could be detected in adjacent riverine water eDNA samples, 92–99% of species information from riverine sites could be detected in a 1-km downstream eDNA sample, and half of dead bioinformation (the bioinformation labeling biological material that lacked life activity and fertility) could be monitored 4–6 km downstream for eukaryotes and 13–19 km downstream for bacteria. This study provides reference methods and data for future monitoring project design and results evaluation.

Biodiversity monitoring is the foundation of ecological research, biodiversity conservation, and ecosystem management[1],[2]. Traditional biodiversity monitoring methods are costly and time-consuming and require high levels of expertise. These methods often study biodiversity from a local perspective with low spatio-temporal resolution and are generally not available at wide taxonomic breadth, high spatio-temporal resolution, and large spatio-temporal scale[3]– . This limits the development of ecological research, biodiversity conservation, and ecosystem management. Currently, metabarcoding and high-throughput sequencing of environmental DNA (eDNA, DNA extracted from environmental samples such as water, soil, and air) provide novel opportunities to monitor biodiversity – . As an efficient and easy-to-standardize non-invasive monitoring approach ,[1] –[12],

and with continuous advancements in DNA sequencing technology, using eDNA metabarcoding to monitor biodiversity is an appropriate method to revolutionize biodiversity monitoring by enabling census of wide taxonomic species at high spatio-temporal resolution and large spatio-temporal scale , ,[13],[1] .

Streams and rivers connect upstream and downstream regions, link land with waterbodies, and transport materials and information through extensive and heterogeneous network systems ,[1] ,[1] . Riverine water eDNA incorporates biodiversity information across terrestrial and aquatic biomes ,[1] . Therefore, samples of riverine water eDNA have the potential to simultaneously monitor both aquatic and terrestrial biodiversity information of a watershed for biodiversity research, conservation, and management. However, its viability and monitoring effectiveness (represented by the proportion of aquatic and terrestrial biodiversity information that can be detected using limited riverine water eDNA samples) has not been systematically identified.

The effectiveness of using riverine water eDNA to simultaneously monitor both aquatic and terrestrial biodiversity depends on the land-to-river and upstream-to-downstream transportation effectiveness of terrestrial and upstream biodiversity information ,[1] -[2] . Biodiversity information monitoring effectiveness could be approximated by assessing the land-to-river and upstream-to-downstream transportation effectiveness of the corresponding bioinformation (eDNA). Here we define the land-to-river and upstream-to-downstream bioinformation transportation (including organisms, nucleic acids, peptides, and other biomarkers), which is driven by hydrologic processes of watershed systems, as the watershed biological information flow (WBIF). WBIF integrates the ecological processes of eDNA, including the origin, state, transport, and fate of eDNA[1] ,[1] ,[21–23]. The transportation effectiveness of WBIF mainly relies on the transport capacity, degradation rate, and environmental filtration of WBIF[1] ,[21–23]. The transport capacity of WBIF mainly depends on erosion and runoff[12],[1] ,[2] . Additionally, the degradation rate of WBIF mainly depends on environmental features[21],[2] ,[2] , and the environmental filtration of WBIF mainly depends on environmental changes that restrict organisms. Collectively, all these factors are related to seasons and weather conditions[2] . Therefore, we hypothesized that the monitoring effectiveness of riverine water eDNA would vary with seasons and weather conditions. Moreover, due to taxonomy-specific eDNA degradation rates[2] , species-specific eDNA degradation rates[1] , and form-specific eDNA degradation rates[2] , we hypothesized that the monitoring effectiveness of riverine water eDNA would vary with taxonomic communities.

To identify the effectiveness of using riverine water eDNA to simultaneously monitor riverine and terrestrial biodiversity information, we needed to assess the transportation effectiveness of land-to-river and upstream-to-downstream WBIF for different taxonomic communities in different seasons and weather conditions. In the present study, we conducted a case study in a watershed on the Qinghai–Tibet Plateau to test the eDNA monitoring effectiveness assessment framework. We estimated the monitoring effectiveness, as indicated by

the biodiversity information of three taxonomic communities in three seasons and weather conditions. Our objectives were threefold: (1) to identify variation in biodiversity information monitoring effectiveness across different seasons and weather conditions; (2) to identify variation in effectiveness for monitoring biodiversity information of different taxonomic communities; and (3) to test the monitoring effectiveness assessment framework.

Results

WBIF of the Three Seasonal Groups

A total of 10,602, 13,766, and 16,500 bacterial OTUs were detected from the samples (including 9 riverine water samples and 9 riparian soil samples, [Figure 1: see original paper]) of the spring group (sampled on frozen days), summer group (sampled on rainy days), and autumn group (sampled on cloudy days), respectively ([Figure 2: see original paper], Supplementary Fig. S1 and Supplementary Tables S1, S2). The total OTUs detected from riparian soil eDNA samples were similar among seasons (Figs. 2, 3, Supplementary Fig. S1). The total OTUs detected from riverine water eDNA samples were richest in autumn ([Figure 2: see original paper], 3, Supplementary Fig. S1). The common OTUs shared between riparian soil eDNA and riverine water eDNA samples accounted for 36.30%, 71.98%, and 67.58% of the total OTUs detected in riparian soil eDNA samples in the spring, summer, and autumn groups, respectively ([Figure 3: see original paper]).

The transportation effectiveness values of WBIF, as indicated by bacterial OTUs from the riparian sampling site to the adjacent riverine sampling site, were 16.62%, 62.76%, and 48.09% on spring frozen, summer rainy, and autumn cloudy days, respectively, among which there was the highest transport capacity and lowest environmental filtration on summer rainy days (, Supplementary Table S3). The transportation effectiveness of WBIF indicated by bacterial OTUs from upstream to downstream was 75.86%, 97.41%, and 96.07% per km on spring frozen, summer rainy, and autumn cloudy days, respectively (, Supplementary Table S4), among which the transport capacity exceeded 99% in all three seasons and the least noneffective WBIF (dead bioinformation) occurred; the longest half-life distance of the noneffective WBIF occurred on summer rainy days ().

WBIF of the Three Taxonomic Groups

A total of 13,766, 7,098, and 17,316 OTUs and 3,532, 1,032, and 6,836 species were detected among the 18 summer samples, as indicated by the 16S rRNA gene, ITS gene, and CO1 gene, respectively ([Figure 4: see original paper], Supplementary Fig. S2 and Supplementary Table S5). The OTUs and species detected in riverine water eDNA samples were generally higher than in riparian soil eDNA samples for all three taxonomic communities ([Figure 4: see original paper]). The common OTUs and species shared between riparian soil and riverine water eDNA samples accounted for 71.98% and 87.95%, 60.40% and 76.18%,

and 37.93% and 53.52% of the total OTUs and species in the bacterial, fungal, and eukaryotic groups, respectively.

The transportation effectiveness of bacterial, fungal, and eukaryotic WBIF from the riparian sampling site to the adjacent riverine sampling site was 62.76%, 44.79%, and 22.64% at the OTU level, respectively, and 80.75%, 65.62%, and 43.38% at the species level, respectively, among which both transport capacity and environmental filtration significantly declined across bacterial, fungal, and eukaryotic communities (, Supplementary Tables S6, S7). The transportation effectiveness of bacterial, fungal, and eukaryotic WBIF from upstream to downstream was 97.41%, 92.64%, and 89.83% per km at the OTU level, and 98.69%, 95.71%, and 92.41% per km at the species level, respectively, among which the noneffective WBIF decreased across bacterial, fungal, and eukaryotic communities (, Supplementary Tables S8, S9), and the half-life distance of the noneffective WBIF was 14.52, 4.93, and 4.07 km at the OTU level and 17.82, 5.96, and 5.02 km at the species level for the bacterial, fungal, and eukaryotic groups, respectively ().

Discussion

Driven by land-to-river and upstream-to-downstream WBIF, biodiversity information across terrestrial and aquatic biomes could be detected in riverine water eDNA ,[1] , and the monitoring effectiveness of riverine water eDNA relies on the transportation effectiveness of corresponding WBIF ,[1] -[2] . The transportation effectiveness of WBIF mainly relies on transport capacity, degradation rate, and environmental filtration of WBIF[1] ,[21]-[23], which can vary with different seasons and weather conditions[2] . We hypothesized that monitoring effectiveness would vary with seasons and weather conditions. In the present case, bacterial community richness in riparian soil did not vary with season, whereas bacterial community composition in riverine water was richest in autumn, followed by summer (Figs. 2, 3). The transportation effectiveness of riparian-to-river and upstream-to-downstream WBIF on spring frozen days was significantly lower than on summer rainy days and autumn cloudy days (Tables 1, 2, Supplementary Tables S3, S4). Considering the insufficient read depth in riverine water samples of summer and autumn groups (Supplementary Fig. S1), the riverine water bacterial community richness and riparian-to-river transportation effectiveness in summer and autumn were already underestimated. This indicates that monitoring effectiveness varied with different seasons and weather conditions, and summer and autumn were the optimal seasons, with rainy days being the optimal weather condition, for using riverine water eDNA to simultaneously monitor holistic biodiversity information in riverine and riparian sites.

The biodiversity information detected by water eDNA could originate from living and dead organisms[23],[2] . Detection of biodiversity information from living organisms mainly depends on dispersal of these living organisms[11],[2] . Detection of biodiversity information from dead organisms mainly depends on transport capacity and degradation rate[12],[22],[2] . In summer and autumn, driven by active

organisms, more eDNA was input into the river system. In particular, surface runoff caused by rain can input more eDNA from terrestrial soil into the river system and preserve it in soil aggregates[3] . In the present study, the highest proportion of bacteria from riparian soil was detected in riverine water in summer and autumn, and rain promoted this phenomenon ([Figure 3: see original paper] and , Supplementary Table S3). The proportion of effective upstream-to-downstream WBIF was significantly higher in summer and autumn than in spring, as well as being higher on rainy days than on cloudy days (). eDNA (originated from dead organisms) degrades over time in a logistic manner (a half-life time)[12,22,2] , which we described as degrading by half-life distance in a lotic system, integrating transport capacity and degradation rate. In the present work, driven by runoff discharge and flow velocity (Supplementary Table S1), the half-life distance of noneffective WBIF was significantly farther in summer than in autumn and spring ().

The biodiversity information monitoring effectiveness of riverine water eDNA, as approximated by WBIF transportation effectiveness, was impacted by the eDNA degradation rate in WBIF, and there were taxonomy-specific eDNA degradation rates[2] , species-specific eDNA degradation rates[1] , and form-specific eDNA degradation rates[2] . We hypothesized that monitoring effectiveness of riverine water eDNA would vary with taxonomic communities. In the present case, results revealed significantly higher monitoring effectiveness of riverine water eDNA (both riparian-to-river and downstream-to-upstream) for bacterial communities than for eukaryotic communities (Tables 3, 4). Considering the insufficient read depth for the bacterial community (16S rRNA gene, Supplementary Fig. S2), detection capacity for the bacterial group was already underestimated. A significantly higher monitoring effectiveness of riverine water eDNA was found for micro-eukaryotic communities (fungi) than for overall eukaryotic communities (including micro- and macro-organisms) (Tables 3, 4). This indicates that monitoring effectiveness varied with different taxonomic communities, and the effectiveness of monitoring eukaryotic communities was significantly lower than for bacterial communities; additionally, the effectiveness of monitoring macrobe communities was significantly lower than for microbe communities. eDNA surveys based on metabarcoding can actually acquire information across the tree of life , ,[11,32,33]. However, eDNA originating from different taxonomic groups has different probabilities of being left in the environment and input into water , , ,[3] . van Bochove et al. inferred that eDNA contained inside cells and mitochondria is especially resilient against degradation (i.e., intracellular vs. extracellular effects)[2] . In the present case, more bacteria than eukaryotes and more microorganisms than macroorganisms (at both OTU and species levels) from riparian soil could be detected in riverine water (). The half-life distance of noneffective WBIF for bacteria (detected by the 16S rRNA gene) was much farther than that for unicellular eukaryotes (detected by the ITS gene, which is mainly unicellular), which in turn was farther than that for multicellular eukaryotes (detected by the CO1 gene, which is mainly multicellular) (). We inferred that eDNA contained inside bacterial cells was more resilient against degrada-

tion than that contained inside unicellular eukaryotic cells (i.e., prokaryotic cells vs. eukaryotic cells), as well as compared to eDNA contained inside multicellular eukaryotic cells or extracellular mitochondria (i.e., unicellular eukaryotic cells vs. multicellular eukaryotic cells or extracellular mitochondria).

In previous studies, the effectiveness of using water eDNA to monitor terrestrial organisms was indicated by detection probability , ,[3] , and the effectiveness of using downstream water eDNA to monitor upstream organisms was indicated by detectable distance ,[12],[1] ,[1] ,[2] ,[3] . In this study, we approximated biodiversity information monitoring effectiveness by WBIF transportation effectiveness and proposed its assessment framework, in which we described riparian-to-river monitoring effectiveness by the proportion of biodiversity information in riparian soil detected using riverine water eDNA samples. Additionally, we described downstream-to-upstream monitoring effectiveness by the proportion of biodiversity information in upstream site water eDNA samples detected by 1-km downstream site water eDNA samples, and the runoff distance at which 50% of dead bioinformation (i.e., bioinformation labeling biological material lacking life activity and fertility) could be monitored. These indicators provide new usable assessment tools for designing monitoring projects and evaluating monitoring results.

In the optimal monitoring season and weather condition (a summer rainy day) in the Shaliu River basin on the Qinghai–Tibet Plateau, using riverine water eDNA we were able to monitor as much as 87.95% of bacterial species, 76.18% of fungal species, and 53.52% of eukaryotic species from riparian soil, along with as much as 98.69% of bacterial species, 95.71% of fungal species, and 92.41% of eukaryotic species from 1 km upstream (). The half-life distance of noneffective WBIF was respectively 17.82 km, 5.96 km, and 5.02 km for bacteria, fungi, and metazoans at the species level (). Considering that monitoring effectiveness of eDNA can vary not only with season, weather, and taxonomic communities, but also with rivers and watersheds with different environmental conditions[12],[1] ,[1] ,[23], more studies on monitoring effectiveness for each taxonomic community in other watersheds with different environmental conditions are needed. eDNA metabarcoding surveys are relatively cheaper, more efficient, and more accurate than traditional surveys in aquatic systems[1] ,[13], although this is certainly not true in all circumstances[3] . Sales et al. showed that the detection probability of using riverine water eDNA to monitor semi-aquatic and terrestrial mammals in natural lotic ecosystems in the UK was 40–67%, providing comparable results to conventional survey methods per unit of survey effort for three species (water vole, field vole, and red deer); in other words, results from 3 to 6 water replicates would be equivalent to results from 3 to 5 latrine surveys and 5–30 weeks of single camera deployment . In the current case, riverine water eDNA samples detected 53.52% of eukaryotic species from riparian soil samples. As bioinformation in WBIF includes biodiversity information of all taxonomic communities, information of all taxonomic communities could be monitored using riverine water eDNA, although variability in monitoring effectiveness exists among different taxonomic communities. We anticipate that in future biodi-

versity research, conservation, and management, we will be able to efficiently monitor and assess aquatic and terrestrial biodiversity by simply using riverine water eDNA samples.

In summary, to test the idea of using riverine water eDNA to simultaneously monitor aquatic and terrestrial biodiversity, we proposed a monitoring effectiveness assessment framework, in which land-to-river monitoring effectiveness was indicated by detection probability, and upstream-to-downstream monitoring effectiveness was described by detection probability per kilometer runoff distance and by the half-life distance of dead bioinformation. In our case study in the Shaliu River watershed on the Qinghai-Tibet Plateau, on summer rainy days, 43–76% of species information from riparian sites could be detected in adjacent riverine water eDNA samples, 92–99% of species information from upstream sites could be detected in a 1-km downstream eDNA sample, and the half-life distances of dead bioinformation were approximately 13–19 km for bacteria and approximately 4–6 km for eukaryotes. The indicators in the assessment framework that describe monitoring effectiveness provide usable assessment tools for designing monitoring projects and evaluating monitoring results. In future ecological research, biodiversity conservation, and ecosystem management, riverine water eDNA may serve as a general diagnostic procedure for routine watershed biodiversity monitoring and assessment.

Materials and Methods

Study Area

The Shaliu River basin (37°10 -37°52 N, 100°17 -99°32 E), as a sub-basin of the Qinghai Lake basin, is located 3,196 m above sea level on the Qinghai–Tibet Plateau ([Figure 1: see original paper]). The Shaliu River is 106 km long, with a catchment area of 1,320 km^2. Grassland is the main land cover type, accounting for more than 90% of the watershed area. Less than 5% of the watershed area has been seriously altered by human activity, such as conversion to cultivated land and building land (http://www.gangcha.gov.cn/html/2125/item.html). Due to its simple ecosystem assemblages (only grassland, aquatic ecosystem, and building land) and minimal disturbance by human activity, the Shaliu River basin serves as a natural simplified model for investigating the effectiveness of monitoring aquatic and terrestrial biodiversity information using riverine water eDNA.

Sampling and Sequencing

To identify seasonal variation in monitoring effectiveness, we collected eDNA samples on April 8–9, June 25–26, and September 19–20, 2019 (spring group, summer group, and autumn group, respectively), including 27 riparian soil eDNA samples and 27 riverine water eDNA samples. The samples were collected from 9 transects (including riverine sampling sites and riparian sampling sites) of the Shaliu River ([Figure 1: see original paper]). Weather and hydrological conditions of each group are summarized in Supplementary Table S1.

A 5-mL surface soil sample was collected using a 5-mL sterilized centrifuge tube from the riparian site (5 m from the river) of each transect. A 1.5-L surface water sample was collected using a 1.5-L sterilized bottle (rinsed three times with sampling water) from the riverine site of each transect. Because keeping samples cool can reduce the rate of eDNA decay and is a convenient and efficient method for conserving eDNA samples[3] , field samples were transported in an ice bath (0 °C) to the laboratory of the Rescue and Rehabilitation Center of Naked Carps of Qinghai Lake. To obtain eDNA from most taxonomic communities[2] ,[3] , riverine water samples (with purified water used as a negative control) were filtered using 0.2- m membrane filters (JinTeng, Tianjin, PRC) to obtain eDNA samples in the laboratory (with every step following molecular biology operation specifications to control contamination and using bleach to wash experimental apparatus). Subsequently, filter membranes from each riverine water sample were placed in a 50-mL sterilized centrifuge tube. Samples were transported at −20 °C (in a dry ice bath) and stored at −80 °C (in an ultra-low temperature freezer) until DNA extraction. Further details are provided in and Supplementary Material 1.

To identify taxonomic variation in monitoring effectiveness, we analyzed three taxonomic communities using metabarcoding of the 16S rRNA, ITS, and mitochondrial CO1 genes[3] - [1]. As long DNA fragments show higher decay rates than short fragments[22], short fragments better reflect community richness than long fragments[22],[31]. We restricted amplified fragment length to 300–500 bp and selected primers 338F/806R, ITS1F/ITS2R, and mlCOIintF/jgHCO2198R to detect bacteria, fungi, and metazoans, respectively[3] - [1]. As eDNA extraction [2], [3], amplification approach, and sequencing can impact eDNA monitoring results, a consistent DNA extraction method and amplification approach should be used for comparisons among samples [1], , . Commercial eDNA labs can help[11], as all approaches (including eDNA extraction, primer synthesis, amplification, sequencing, and contamination control) could be standardized.

In our work, samples were processed by Shanghai Majorbio Bio-pharm Technology Co., Ltd (Shanghai, China). Details are provided in and Supplementary Material 1.

On the free online Majorbio Cloud Platform (www.majorbio.com), we analyzed raw sequence data and obtained operational taxonomic unit (OTU) types, sequence numbers for each OTU, and taxonomic features of each sample; additionally, we examined community richness (Chao richness index at the OTU level).

WBIF Analysis

The WBIF (including land-to-river and upstream-to-downstream WBIF) of each group was assessed to reveal the effectiveness of using riverine water eDNA to monitor biodiversity information in riverine and riparian sites. In the current WBIF analysis, all statistical analyses used the OTUs and species in each sample. The processing approach is described below (indicated by OTU type).

Transportation effectiveness of WBIF was indicated by the proportion of input OTUs (i.e., common types between the source site sample and pool site sample) to output OTUs (total types of source site sample) (Eq. 1).

$$e = \frac{\text{Num}(SOTU \cap POTU)}{\text{Num}(SOTU)}$$

where e denotes transportation effectiveness of WBIF; $SOTU$ denotes the OTU assemblage of the source site sample (i.e., the adjacent riparian soil eDNA sample in land-to-river WBIF or the adjacent upstream water eDNA sample in upstream-to-downstream WBIF); and $POTU$ denotes the OTU assemblage of the pool site sample (i.e., the adjacent riverine water eDNA sample in land-to-river WBIF or the adjacent downstream water eDNA sample in upstream-to-downstream WBIF).

As the distance of land-to-river WBIF was less than 5 m in the present case study, transportation effectiveness of land-to-river WBIF was assumed to be constructed by transport capacity and environmental filtration (no degradation rate). Transportation effectiveness of land-to-river WBIF could be indicated by the proportion of common types shared between adjacent riparian soil eDNA samples and riverine water eDNA samples to total types of riparian soil eDNA samples (Eq. 1). Transport capacity of land-to-river WBIF could be indicated by the proportion of common types shared between adjacent riparian soil eDNA samples and riverine water eDNA samples to common types shared between the riparian soil eDNA sample and all riverine water eDNA samples in the corresponding group (Eq. 2). Environmental filtration of land-to-river WBIF could be indicated by the proportion of types included in the riparian soil eDNA sample but not in any riverine water eDNA sample to total types in the riparian soil eDNA sample (Eq. 3).

$$t = \frac{\text{Num}(SOTU \cap POTU)}{\text{Num}(SOTU \cap WOTU)}$$

$$f = \frac{\text{Num}(SOTU \cap WOTU)}{\text{Num}(SOTU)}$$

where t denotes transport capacity; f denotes environmental filtration; $SOTU$ denotes the OTU assemblage of the source site sample (i.e., the riparian soil eDNA sample); and $WOTU$ denotes the OTU assemblage of all riverine water eDNA samples.

WBIF included effective WBIF (i.e., flow or migration of living organisms) and noneffective WBIF (i.e., flow of bioinformation labeling biological material lacking life activity and fertility [dead bioinformation]). Transportation effectiveness of upstream-to-downstream WBIF was determined by different features of effective WBIF and noneffective WBIF. Effective WBIF was impacted by transport

capacity and environmental filtration. Noneffective WBIF was impacted by transport capacity and degradation rate. We established the following presuppositions: (1) transport capacity was consistent in a defined runoff condition of a definite season and weather condition; (2) the proportion of noneffective WBIF at each site was consistent; (3) noneffective WBIF degraded over time (i.e., distance) in a logistic manner; and (4) environmental filtration was consistent in a definite environmental change. These four presuppositions do not exactly describe the factual complex WBIF processes driven by various environmental factors, but they provide a means to construct a model that approximately addresses these complex processes. Transportation effectiveness of upstream-to-downstream WBIF could be described by an equation (Eq. 4), in which transportation effectiveness is a function of runoff distance, and transport capacity, environmental filtration, and degradation rate are parameters that can be estimated according to sets of transportation effectiveness and runoff distance. In practice, as WBIF are impacted by varying factors at any site and time, analytical solutions for the parameters in Eq. (4) are impossible. Therefore, we suggest that Eq. (4) could be solved through programming according to the evolutionary algorithm in Microsoft Excel. As there are only approximate solutions for the parameters in Eq. (4), we recommend obtaining several sets (such as 30 sets) of approximate solutions, after which statistical analysis can be performed for each parameter.

$$e = t^d \times \left[(1-k) \times \frac{1}{1+f} + k \times \frac{1}{1+f \times 2^{d/D}}\right]$$

where e denotes transportation effectiveness of WBIF; t denotes transport capacity; d denotes distance of WBIF; k denotes proportion of noneffective WBIF; f denotes environmental filtration; and D denotes half-life distance of noneffective WBIF.

Data Availability

The datasets generated for this study can be found in the China National GeneBank Sequence Archive (CNSA, https://db.cngb.org/cnsa/) of the China National GeneBank database (CNGBdb) under accession number CNP0001046.

Received: 6 September 2021; Accepted: 9 December 2021

References

1. Hooper, D. U. et al. A global synthesis reveals biodiversity loss as a major driver of ecosystem change. Nature 486, 105 (2012).
2. Dixon, K. M., Cary, G. J., Worboys, G. L., Banks, S. C. & Gibbons, P. Features associated with effective biodiversity monitoring and evaluation. Biol. Conserv. 238, 108221 (2019).
3. Anderson, C. B. Biodiversity monitoring, earth observations and the ecology of scale. Ecol. Lett. 21, 1572 (2018).

4. Altermatt, F. et al. Uncovering the complete biodiversity structure in spatial networks: The example of riverine systems. Oikos 129, 607 (2020).
5. Pawlowski, J., Apothéloz-Perret-Gentil, L. & Altermatt, F. Environmental DNA: What' s behind the term? Clarifying the terminology and recommendations for its future use in biomonitoring. Mol. Ecol. 29, 4258 (2020).
6. Deiner, K., Fronhofer, E. A., Mächler, E., Walser, J. & Altermatt, F. Environmental DNA reveals that rivers are conveyer belts of biodiversity information. Nat. Commun. 7, 12544 (2016).
7. Carraro, L., Hartikainen, H., Jokela, J., Bertuzzo, E. & Rinaldo, A. Estimating species distribution and abundance in river networks using environmental DNA. Proc. Natl. Acad. Sci. U. S. A. 115, 11724 (2018).
8. Seeber, P. A. et al. Terrestrial mammal surveillance using hybridization capture of environmental DNA from African waterholes. Mol. Ecol. Resour. 19, 1486 (2019).
9. Sales, N. G. et al. Fishing for mammals: Landscape-level monitoring of terrestrial and semi-aquatic communities using eDNA from riverine systems. J. Appl. Ecol. 57, 707 (2020).
10. Lugg, W. H., Griffiths, J., van Rooyen, A. R., Weeks, A. R. & Tingley, R. Optimal survey designs for environmental DNA sampling. Methods Ecol. Evol. 9, 1049 (2018).
11. Ravindran, S. Turning discarded DNA into ecology gold. Nature 570, 543 (2019).
12. Seymour, M. Rapid progression and future of environmental DNA research. Commun. Biol. 2, 80 (2019).
13. Valentini, A. et al. Next-generation monitoring of aquatic biodiversity using environmental DNA metabarcoding. Mol. Ecol. 25, 929 (2016).
14. Cristescu, M. E. & Hebert, P. D. N. Uses and misuses of environmental DNA in biodiversity science and conservation. Annu. Rev. Ecol. Evol. Syst. 49, 209 (2018).
15. Shogren, A. J. et al. Controls on eDNA movement in streams: Transport, retention, and resuspension. Sci. Rep.-UK 7, 5065 (2017).
16. Matsuoka, S. et al. Spatial structure of fungal DNA assemblages revealed with eDNA metabarcoding in a forest river network in western Japan. Metabarcoding Metagenom. 3, e36335 (2019).
17. Deiner, K. & Altermatt, F. Transport distance of invertebrate environmental DNA in a natural river. PLoS One 9, e88786 (2014).
18. Jerde, C. L. et al. Influence of stream bottom substrate on retention and transport of vertebrate environmental DNA. Environ. Sci. Technol. 50, 8770 (2016).
19. Sansom, B. J. & Sassoubre, L. M. Environmental DNA (eDNA) shedding and decay rates to model freshwater mussel eDNA transport in a river. Environ. Sci. Technol. 51, 14244 (2017).
20. Pont, D. et al. Environmental DNA reveals quantitative patterns of fish biodiversity in large rivers despite its downstream transportation. Sci. Rep.-UK 8, 10361 (2018).

21. Barnes, M. A. & Turner, C. R. The ecology of environmental DNA and implications for conservation genetics. Conserv. Genet. 17, 1 (2016).
22. Jo, T. et al. Rapid degradation of longer DNA fragments enables the improved estimation of distribution and biomass using environmental DNA. Mol. Ecol. Resour. 17, e25 (2017).
23. Tillotson, M. D. et al. Concentrations of environmental DNA (eDNA) reflect spawning salmon abundance at fine spatial and temporal scales. Biol. Conserv. 220, 1 (2018).
24. Fremier, A. K., Strickler, K. M., Parzych, J., Powers, S. & Goldberg, C. S. Stream transport and retention of environmental DNA pulse releases in relation to hydrogeomorphic scaling factors. Environ. Sci. Technol. 53, 6640 (2019).
25. Eichmiller, J. J., Best, S. E. & Sorensen, P. W. Effects of temperature and trophic state on degradation of environmental DNA in lake water. Environ. Sci. Technol. 50, 1859 (2016).
26. Nukazawa, K., Hamasuna, Y. & Suzuki, Y. Simulating the advection and degradation of the environmental DNA of common carp along a river. Environ. Sci. Technol. 52, 10562 (2018).
27. Barnes, M. A. et al. Environmental conditions influence eDNA persistence in aquatic systems. Environ. Sci. Technol. 48, 1819 (2014).
28. Bochove, K. et al. Organic matter reduces the amount of detectable environmental DNA in freshwater. Ecol. Evol. 10, 3647 (2020).
29. Seymour, M. et al. Acidity promotes degradation of multi-species environmental DNA in lotic mesocosms. Commun. Biol. 1, 4 (2018).
30. Wilpiszeski, R. L. et al. Soil aggregate microbial communities: Towards understanding microbiome interactions at biologically relevant scales. Appl. Environ. Microbiol. 85, e319 (2019).
31. Wei, N., Nakajima, F. & Tobino, T. A microcosm study of surface sediment environmental DNA: Decay observation, abundance estimation, and fragment length comparison. Environ. Sci. Technol. 52, 12428 (2018).
32. Stat, M. et al. Ecosystem biomonitoring with eDNA: Metabarcoding across the tree of life in a tropical marine environment. Sci. Rep.-UK 7, 12211 (2017).
33. Djurhuus, A. et al. Environmental DNA reveals seasonal shifts and potential interactions in a marine community. Nat. Commun. 11, 254 (2020).
34. Harper, L. R. et al. Environmental DNA (eDNA) metabarcoding of pond water as a tool to survey conservation and management priority mammals. Biol. Conserv. 238, 108225 (2019).
35. Carraro, L., Mächler, E., Wüthrich, R. & Altermatt, F. Environmental DNA allows upscaling spatial patterns of biodiversity in freshwater ecosystems. Nat. Commun. 11, 3585 (2020).
36. Beng, K. C. & Corlett, R. T. Applications of environmental DNA (eDNA) in ecology and conservation: Opportunities, challenges and prospects. Biodivers. Conserv. 29, 2089 (2020).
37. Sales, N. G., Wangensteen, O. S., Carvalho, D. C. & Mariani, S. Influence of preservation methods, sample medium and sampling time on eDNA

recovery in a neotropical river. Environ. DNA 1, 119 (2019).
38. Li, J., Lawson Handley, L., Read, D. S. & Hänfling, B. The effect of filtration method on the efficiency of environmental DNA capture and quantification via metabarcoding. Mol. Ecol. Resour. 18, 1102 (2018).
39. Wangensteen, O. S., Palacín, C., Guardiola, M. & Turon, X. DNA metabarcoding of littoral hard-bottom communities: High diversity and database gaps revealed by two molecular markers. PeerJ 6, e4705 (2018).
40. Heeger, F., Wurzbacher, C., Bourne, E. C., Mazzoni, C. J. & Monaghan, M. T. Combining the 5.8S and ITS2 to improve classification of fungi. Methods Ecol. Evol. 10, 1702 (2019).
41. Giebner, H. et al. Comparing diversity levels in environmental samples: DNA sequence capture and metabarcoding approaches using 18S and COI genes. Mol. Ecol. Resour. 20, 1333 (2020).
42. Hermans, S. M., Buckley, H. L. & Lear, G. Optimal extraction methods for the simultaneous analysis of DNA from diverse organisms and sample types. Mol. Ecol. Resour. 18, 557 (2018).
43. Armbrecht, L. et al. An optimized method for the extraction of ancient eukaryote DNA from marine sediments. Mol. Ecol. Resour. 20, 906 (2020).
44. Nichols, R. V. et al. Minimizing polymerase biases in metabarcoding. Mol. Ecol. Resour. 18, 927 (2018).
45. Nicholson, A. et al. An analysis of metadata reporting in freshwater environmental DNA research calls for the development of best practice guidelines. Environ. DNA 2, 343 (2020).
46. Dopheide, A., Xie, D., Buckley, T. R., Drummond, A. J. & Newcomb, R. D. Impacts of DNA extraction and PCR on DNA metabarcoding estimates of soil biodiversity. Methods Ecol. Evol. 10, 120 (2019).

Acknowledgements

This work was supported by the Central Public-Interest Scientific Institution Basal Research Fund, Chinese Academy of Fishery Sciences (Grant numbers 2019HY-XKQ02, 2020TD08) and the Department of Science and Technology of Qinghai Province (Grant number 2018-ZJ-703).

Author Contributions

H.Y., H.D., and H.Q. contributed to the conception of the study. H.Y. and L.Y. performed field sampling. H.Y. and X.H. conducted laboratory work. H.Y., H.Z., J.L., J.W., and C.W. contributed to data acquisition, analysis, and interpretation. Q.Z. administered the project. H.Y. led manuscript writing, and H.Z., J.L., J.W., C.W., and Q.W. contributed critically to drafts and the final manuscript version. Q.W. supervised and validated this study.

Competing Interests

The authors declare no competing interests.

Additional Information

ChinaRxiv 中X

Supplementary Information is available for this paper at https://doi.org/10.1038/s41598-021-03733-7.

Correspondence

Correspondence and requests for materials should be addressed to H.D. or Q.W.

Reprints and Permissions

Reprints and permissions information is available at www.nature.com/reprints.

Publisher' s Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access

This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article' s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article' s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/. © The Author(s) 2021

Note: Figure translations are in progress. See original paper for figures.

Source: ChinaXiv —Machine translation. Verify with original.