Simultaneously monitoring aquatic and terrestrial biodiversity using riverine water eDNA: seasonal variation of monitoring effectiveness

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Abstract

Both aquatic and terrestrial biodiversity information can be detected in riverine water environmental DNA (eDNA). However, the monitoring effectiveness (i.e., the proportion of aquatic and terrestrial biodiversity information detected in riverine water eDNA samples) is unknown. To investigate the monitoring effectiveness, we introduced the concept of watershed biological information flow (WBIF) and proposed that the monitoring effectiveness depended on the transportation effectiveness of the WBIF. Then, the monitoring effectiveness could be assessed in the WBIF framework. Here, we conducted a monitoring effectiveness assessment case study in a watershed on the Qinghai-Tibet Plateau according to analysis of the bacterial operational taxonomic unit (OTU) assemblages detected in riverine water eDNA samples and riparian soil eDNA samples during three seasons. The results showed that (1) the downstream-to-upstream monitoring effectiveness: only 76% of the bacterial OTUs could be detected 1 km downstream in spring and more than 97% and 96% could be detected in summer and autumn, respectively. (2) The river-to-land monitoring effectiveness: more than 62% of the bacterial OTUs in riparian soil eDNA samples could be detected in adjacent riverine water eDNA samples on rainy summer days and 16% and 48% could be detected on cloudy spring and autumn days, respectively. These results suggested that riverine water eDNA was viable for simultaneously monitoring aquatic and terrestrial bacterial biodiversity and that rainy days in summer or autumn were suitable sampling times on the Qinghai-Tibet Plateau. More studies on monitoring effectiveness in other taxonomies and in other watersheds with different climatic conditions are needed.

Introduction

Biodiversity monitoring is the basal content for ecological research, ecosystem management and conservation action (Cardinale, et al.,2012; Hooper, et al.,2012; Carrizo, et al.,2017; Mallick & Chakraborty,2018). While abiotic environmental conditions and ecosystem property information are now available at highly resolved spatial and temporal scales (Vina, et al.,2013; Jetz, et al.,2016), biodiversity is still often studied from a local and accumulative perspective and is generally not available at a wide taxonomic breadth, high-resolution temporal scale and spatial coverage (Anderson,2018; Chase, et al.,2018; Mcglinn, et al.,2019; Altermatt, et al.,2020). This limitation slows the development of ecological research, ecosystem management and conservation action. Now, meta-barcoding and high-throughput sequencing of environmental DNA (eDNA, i.e., DNA extracted from environmental samples such as water, soil, and air) provide novel opportunities

to monitor biodiversity (Deiner, et al.,2016; Cristescu & Hebert,2018; Altermatt, et al.,2020), and this approach is nonlethal for most classically sampled taxonomic groups, minimizes habitat disruption and can assess diversity across the tree of life with a single-field sampling protocol, making it extremely cost effective (Deiner, et al.,2016; Valentini, et al.,2016; Stat, et al.,2017). As an efficient and easy-to-standardize monitoring approach (Thomsen & Willerslev,2015; Valentini, et al.,2016; Lugg, et al.,2018; Seymour,2019), and with the continuous advancements in DNA sequencing technology, using eDNA metabarcoding to monitor biodiversity would be an appropriate method to revolutionize biodiversity monitoring by enabling the census of wide taxonomic species on a highly resolved spatial and temporal scale in near real time (Thomsen & Willerslev,2015; Cristescu & Hebert,2018; Altermatt, et al.,2020).

Streams and rivers connect upstream regions with downstream regions, connect land with waterbodies, and transport natural and anthropogenic materials and information through extensive and heterogeneous network systems (Luo, et al.,2011; Deiner, et al.,2016; Wang, et al.,2016; Matsuoka, et al.,2019). Riverine water eDNA incorporates biodiversity information across terrestrial and aquatic biomes (Deiner, et al.,2016; Matsuoka, et al.,2019; Yang, et al.,2019). By analyzing riverine water eDNA, information on terrestrial and aquatic biological compositions and even their spatial structures can be obtained (Deiner, et al.,2016; Matsuoka, et al.,2019). Therefore, the eDNA that is transported in river networks offers a novel and spatially integrated component that can be used to simultaneously monitor aquatic and terrestrial biodiversity and will transform biodiversity data acquisition in research, management and conservation (Deiner, et al.,2016; Yang, et al.,2019). However, to achieve this objective, the monitoring effectiveness (i.e., the proportion of aquatic and terrestrial biodiversity information that could be detected using limited riverine water eDNA samples) needs to be assessed first. Until now, there has been no systemic research on monitoring effectiveness.

The concept of watershed biological information flow (WBIF) (Yang, et al.,2019) is a good conceptual framework to assist with the assessment of monitoring effectiveness. In the WBIF framework, the monitoring effectiveness depends on the transportation effectiveness of the WBIF from the upstream to downstream regions and from the land to river (Deiner & Altermatt,2014; Sansom & Sassoubre,2017; Pont, et al.,2018; Seymour,2019; Yang, et al.,2019). The WBIF framework integrates the processes of origin, state, transport, and fate of eDNA (Barnes & Turner,2016; Shogren, et al.,2017). Many studies have shown that eDNA degrades over time in a logistic manner (Barnes, et al.,2014; Nukazawa, et al.,2018; Seymour,2019; M, et al.,2020), and the detection distance varies from less than 1 km in a small stream to more than 100 km in a large river (Deiner & Altermatt,2014; Stoeckle, et al.,2016; Pont, et al.,2018; Seymour,2019). Deiner and colleagues (2016) indicated that much terrestrial biological information has been input to rivers and that a large number of eukaryotic phyla from terrestrial taxa can be detected from riverine water eDNA (Deiner, et al.,2016). Our previous WBIF study conducted on the Qinghai-Tibet Plateau in summer showed that the transportation effectiveness from adjacent riparian sites to river sites was 62.76% on rainy days and 44.16% on sunny days; additionally, the transportation effectiveness from adjacent upstream sites to downstream sites (distance varied from 7 km to 23.5 km) was approximately 80.30% (Yang, et al.,2019). Moreover, it was shown that the transportation effectiveness of WBIF relied on transport capacity, degradation rate and environmental filtration (Yang, et al.,2019). The transport capacity mainly depended on erosion and runoff, and degradation rate mainly depended on environmental features, and environmental filtration mainly depended on environmental change, all of which were related to season and weather conditions (Yang, et al.,2019). Because the monitoring effectiveness depends on the transportation effectiveness of WBIF and WBIF is related to season and weather conditions, we propose that the monitoring effectiveness varies with season and weather conditions.

The aim of this study was to identify the effectiveness of monitoring the biodiversity information of upstream and riparian zones using riverine water eDNA in different seasons and weather conditions. In this study, based on the WBIF framework, we conducted a case study in the Shaliu River basin, which is a typical watershed on the Qinghai-Tibet Plateau; specifically, we analyzed the transportation effectiveness of two types of WBIFs, i.e., the WBIF from upstream to downstream regions and the WBIF from riparian zones to rivers, in different seasons and weather conditions, as indicated by environmental microbes. Then, we used the transportation effectiveness of the WBIF to identify the corresponding monitoring effectiveness. Our

objectives were twofold: first, we sought to establish whether riverine water eDNA was viable for monitoring the biodiversity information of upstream and riparian zones. Second, we sought to identify which season and weather conditions were optimal for monitoring the biodiversity information of the upstream and riparian zones.

Materials and Methods

Study Area

The Shaliu River basin (37°10'-37deg52' N, 100deg17'-99deg32' E), as a sub-basin of the Qinghai Lake basin, is located 3196 m above sea level on the Qinghai-Tibet Plateau (Fig. 1). The mean annual precipitation is 423.4 mm, and the average annual evaporation is 1674.7 mm. The annual mean temperature is -0.6degC, the monthly mean temperature of January is -17.5degC and that of July is 11.0degC. The Shaliu River freezes in October and unfreezes in the following April. The Shaliu River is 106 km long, with a catchment area of 1320 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 was seriously changed by human activity, such as transformation into cultivated land and building land11http://www.gangcha.gov.cn/html/2125/item.html. Due to its simple ecosystem assemblages and weak disturbance by human activity, the Shaliu River basin is a natural simplified model for investigating the effectiveness of monitoring aquatic and terrestrial biodiversity information using riverine water eDNA.

Field Sampling

We collected eDNA samples three times, including 27 soil eDNA samples and 27 water eDNA samples, from 9 transects (Fig. 1) in the Shaliu River on April 8 and 9, June 25 and 26 and September 19 and 20, 2019. A 1.5 L surface water sample was collected from the river site of each transect and transported at 0degC to the laboratory of the Rescue and Rehabilitation Center of Naked Carps of Qinghai Lake. Then, water samples were filtered using 0.2-μm membrane filters to obtain the eDNA sample in the laboratory, and the water eDNA samples were frozen, transported at -20°C and stored at -80°C until DNA extraction. A 5 mL soil eDNA sample was collected from the riparian zone site (5 m distance from the river) of each transect and transported to the laboratory at 0°C. Then, the soil eDNA samples were frozen, transported at -20°C and stored at -80°C until DNA extraction.

In the first sampling period (spring group), during April 8 and 9, the air temperature was -6˜8°C, the water temperature was -0.5˜0.7°C, the frozen river was starting to thaw, the runoff volume was 1.8˜3.9 m^3/s, the soil was still frozen, and both days were cloudy with freezing, heavy winds. On the frozen days of April 8 and 9, the river was clear; on these days, we sampled 18 samples (9 water samples and 9 soil samples) at 9 transects from the downstream to upstream regions.

In the second sampling period (summer group), during June 25 and 26, the air temperature was 7˜17°C, the water temperature was 4.3˜16.4°C, and the runoff volume was 29.9˜45.5 m^3/s. On the sunny day of June 25, the river was clear; on this day, we collected 4 samples (2 water samples and 2 soil samples) from the transects of SL1 and SL2. It began to rain on the night of June 25, and on the rainy day of June 26, the river was turbid; we collected samples (7 water samples and 7 soil samples) from the last 7 transects from the downstream to upstream regions.

In the third sampling period (autumn group) on September 19 and 20, the air temperature was 0˜10°C, the water temperature was 0.2˜8.8°C, part of the transects started to freeze, and the runoff volume was 5.7˜12.8 m^3/s. On the rainy day of September 19, the river was turbid; we collected 4 samples (2 water samples and 2 soil samples) from the SL1 and SL2 transects. On the cloudy day of September 20, the river was clear, and we collected 14 samples (7 water samples and 7 soil samples) from the last 7 transects from the downstream to upstream regions.

DNA Extraction and Sequence Analysis

Microbial DNA was extracted from eDNA samples using an E.Z.N.A.® Stool DNA Kit (Omega BioTek, Norcross, GA, USA) according to the manufacturer's protocols. Then, the final DNA concentration and purity were determined by a NanoDrop 2000 UV-vis spectrophotometer (Thermo Scientific, Wilmington, USA), and DNA quality was checked by 1% agarose gel electrophoresis. The V3-V4 hypervariable regions of the bacterial 16S rRNA gene were amplified with the primers 338F (5'- ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') by using a PCR thermocycler system (GeneAmp 9700, ABI, USA). The PCRs were conducted using the following program: 3 min of denaturation at 95°C; 29 cycles of 30 s at 95°C, 30 s for annealing at 55°C, and 45 s for elongation at 72°C; and a final extension at 72°C for 10 min. The PCRs were performed in triplicate 20-μL mixtures containing 4 μL of 5× FastPfu Buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each primer (5 μM), 0.4 μL of FastPfu Polymerase, 0.2 μL of BSA and 10 ng of template DNA. The resulting PCR products were extracted from a 2% agarose gel, further purified using an AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) and quantified using QuantiFluor-ST (Promega, USA) according to the manufacturer's protocol.

Purified amplicons were pooled in equimolar amounts and subjected to paired-end sequencing on an Illumina MiSeq platform (Illumina, San Diego, USA) according to standard protocols by Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China). Raw fastq files were demultiplexed, quality-filtered by Trimmomatic and merged by FLASH. Operational taxonomic units (OTUs) were clustered with a 97% similarity cutoff using UPARSE, and chimeric sequences were identified and removed using UCHIME. The taxonomy of each 16S rRNA gene sequence was analyzed by the RDP classifier Bayesian algorithm against the Silva132/16S_Bacteria database using a confidence threshold of 70%. The data were analyzed on the Majorbio Cloud Platform (*www.majorbio.com*). The raw data have been deposited in the CNSA (https://db.cngb.org/cnsa/) of CNGBdb with accession number CNP0001046.

Statistical Analysis

The OTU numbers, types and taxonomic features of the samples of the three groups, i.e., those sampled on April 8 and 9, June 25 and 26 and September 19 and 20, 2019, were analyzed. Community richness (Chao richness index) was examined to reveal the variation among the three groups. The WBIF (including the WBIF from the upstream to downstream regions and the WBIF from land to river) of each group was assessed to reveal the effectiveness of monitoring the biodiversity information of the upstream and riparian zones using riverine water eDNA. The analysis of the WBIF follows the processing approach proposed by Yang et al. (2019). In the analysis of the WBIF, all statistics used the types of OTUs of each sample rather than the numbers of OTUs of each sample. The processing approach can be described simply as follows.

As the WBIF driven by watershed ecosystem processes was transported from land to river and from upstream to downstream regions, the transportation effectiveness of the WBIF could be estimated by comparing the OTU assemblages between adjacent soil eDNA samples and water eDNA samples and by comparing the OTU assemblages between two adjacent water eDNA samples. The transportation effectiveness of the WBIF was indicated by the proportion of input OTU types (i.e., the common types between the source site sample and the pool site sample) to output OTU types (the total types of source site sample) (Eq. 1).

$$e = \mathrm{Num}(S_{OTU}\ [?]P_{OTU}\ )/\mathrm{Num}(S_{OTU}\ ) \quad \text{(Eq. 1)}$$

where e denotes the transportation effectiveness of the WBIF;S_{OTU} denotes the OTU assemblage of the source site sample (i.e., the adjacent soil eDNA sample in the land-river WBIF or the adjacent upstream water eDNA sample in the upstream-downstream WBIF); and P_{OTU} denotes the OTU assemblage of the pool site sample (i.e., the adjacent water eDNA sample in the land-river WBIF or the adjacent downstream water eDNA sample in the upstream-downstream WBIF).

As the transportation effectiveness of the WBIF relied on transport capacity, degradation rate and environmental filtration and the distance of the land-to-river WBIF was less than 5 m, the transportation

effectiveness of the land-to-river WBIF was assumed to be constructed by transport capacity and environmental filtration. The transportation effectiveness of the land-to-river WBIF could be indicated by the proportion of the common types shared between adjacent soil eDNA samples and water eDNA samples to the total types of soil eDNA samples (Eq. 1). The transport capacity of the land-to-river WBIF could be indicated by the proportion of the common types shared between adjacent soil eDNA samples and water eDNA samples to the common types shared between the soil eDNA sample and all water eDNA samples in the corresponding group (Eq. 2). The environmental filtration of the land-to-river WBIF could be indicated by the proportion of the types included in the soil eDNA sample but not in any water eDNA sample to the total types in the soil eDNA sample (Eq. 3).

t = Num(S_{OTU} [?] P_{OTU})/Num(S_{OTU} [?] W_{OTU}) (Eq. 2)

f = 1 - Num(S_{OTU} [?] W_{OTU})/Num(S_{OTU}) (Eq. 3)

where t denotes the transport capacity; f denotes the environmental filtration; S_{OTU} denotes the OTU assemblage of the source site sample (i.e., the soil eDNA sample); and W_{OTU} denotes the OTU assemblage of all water eDNA samples.

The upstream-to-downstream WBIF that is indicated by environmental microbes includes the effective WBIF (i.e., the flow of living organisms) and the noneffective WBIF (i.e., the flow of dead organisms). The effective WBIF was impacted by transport capacity and environmental filtration. The noneffective WBIF was impacted by transport capacity and degradation rate. We established the following assumptions: the transport capacity was consistent in a defined runoff condition; the proportion of noneffective WBIF at each site was consistent; the noneffective WBIF degraded over time (i.e., distance) in a logistic manner; and the environmental filtration was consistent in a definite environmental change. The transportation effectiveness of the upstream-to-downstream WBIF could be constructed by the transport capacity of the WBIF, the environmental filtration of the effective WBIF and the degradation rate of the noneffective WBIF (Eq. 4). In practice, as watershed ecosystem processes are impacted by varied influencing factors at any site and time, the analytical solution of Eq. 4 is impossible. Therefore, Eq. 4 will be programming-solved according to the evolutionary algorithm.

$e = (t \hat{} d)[(1\text{-}k)(1\text{-}f) + k\ (1/2)\hat{}(d\ /D)]$ (Eq. 4)

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

Results

Biological Information Features of the Samples of the Three Groups

A total of 1,030,826, 968,122 and 842,317 clean sequences were obtained, respectively, from the 18 samples of the spring group, summer group and autumn group (more details in Fig. 2a), and the average lengths of these sequences were 447.54 bp, 416.06 bp, and 416.32 bp, respectively. A total of 10,602, 13,766 and 16,500 types of bacterial OTUs were detected (UPARSE, 97% cutoff), respectively, from the 18 samples of the three groups (more details in Fig. 2b and Fig. 3), which belonged to 58 phyla, 141 classes, 424 orders, 782 families, 1,895 genera and 4,537 species. The OTU counts and compositions of each group were highly variable (Fig. 2b and Fig. 2c). The rarefaction curves of each sample showed that the number of clean sequences of each sample in five groups (except for the spring water eDNA samples) was lower than it should be, especially for the water eDNA samples in summer and autumn (Fig. 3).

The total types of OTUs detected in the soil eDNA samples estimated using the species accumulation curve were similar among the spring, summer and autumn groups (Fig. 2d), although there were some spatially and temporally heterogeneous OTU compositions (Fig. 2c and Fig. 4). The total types of OTUs detected in the water eDNA samples estimated using the species accumulation curve showed that the richest OTU

types occurred in autumn (Fig. 2d). Moreover, the temporal heterogeneity of OTU composition among the three groups was obvious (Fig. 4). The common OTUs shared between the soil and water eDNA samples totaled 2,834, 5,651 and 5,279 in the spring group, summer group and autumn group, respectively, which accounted for 36.30%, 71.98% and 67.58% of the total types of OTUs detected in the soil eDNA samples of each group, respectively.

Land-river WBIF Features of the Three Groups

The transportation effectiveness of the WBIF from the riparian zone to the river was 16.62% (0.1662±0.1254, 95%) in the frozen spring, 62.76% (0.6276±0.0873, 95%) in the rainy summer, and 48.09% (0.4809±0.0522, 95%) in the cloudy autumn. The transport capacity of the WBIF from the riparian zone to the river was 26.88% (0.2688±0.2024, 95%) in the frozen spring, 68.49% (0.6849±0.0913, 95%) in the rainy summer, and 57.36% (0.573579±0.052897, 95%) in the cloudy autumn. The environmental filtration of the WBIF from the riparian zone to the river was 38.54% (0.3854±0.0293, 95%) in the frozen spring, 8.38% (0.0838±0.0206, 95%) in the rainy summer, and 16.18% (0.1618±0.0451, 95%) in the cloudy autumn. More details are shown in Table 1.

During the spring sampling period, the ice water in the estuary (transect SL1) covered the riparian grassland. Because the water sample was likely to be the eluate from the soil at SL1, the transportation effectiveness and transport capacity of the WBIF from the riparian zone to the river were high. As Qinghai Lake is saline, the environmental filtration of the WBIF from the riparian zone to the river in the estuary (SL1) was high. During the summer sampling period, because the samples from SL1 and SL2 were sampled on sunny days and the other samples were sampled on rainy days, the transport capacity of the WBIF from the riparian zone was low, the environmental filtration of the WBIF from the riparian zone was high, and the transportation effectiveness of the WBIF from the riparian zone was low at SL1 and SL2. During the autumn sampling period, because the samples from SL1 and SL2 were sampled on rainy days and the other samples were sampled on cloudy days, the transport capacity of the WBIF from the riparian zone was high at SL2. Because of the environmental stress caused by estuary saline water, the environmental filtration of the WBIF from the riparian zone was high at SL1.

Upstream-downstream WBIF Features of the Three Groups

Along with the flow from upstream to downstream, there were 4 chains that connected the sampling transects. Their cumulative transportation effectiveness of the upstream-to-downstream WBIF varied with cumulative distance (Table 2). The transportation effectiveness of the upstream-to-downstream WBIF relied on transport capacity, degradation rate and environmental filtration. In spring, the transport capacity of the upstream-to-downstream WBIF was 99.97% (0.9997±0.0003, 95%) per km, of which there was 66.85% (0.6685±0.0034, 95%) noneffective WBIF, the half-life distance of the noneffective WBIF was 1.55 (1.5490±0.1269, 95%) km, and the environmental filtration from SL2 to SL1 (from the freshwater ecosystem to saline water ecosystem) was 16.04% (0.1604±0.0082, 95%). In summer, the transport capacity of the upstream-to-downstream WBIF was 99.42% (0.9942±0.0009, 95%) per km, of which there was 43.46% (0.4346±0.0417, 95%) noneffective WBIF, the half-life distance of the noneffective WBIF was 14.52 (14.5234±1.4405, 95%) km, the environmental filtration from SL3 to SL2 (from rainy sampling conditions to sunny sampling conditions) was 0.57% (0.0057±0.0055, 95%) and the environmental filtration from SL2 to SL1 (from the freshwater ecosystem to saline water ecosystem) was 54.42% (0.5442±0.0100, 95%). In autumn, the transport capacity of the upstream-to-downstream WBIF was 99.23% (0.9923±0.0016, 95%) per km, of which there was 49.35% (0.4935±0.0410, 95%) noneffective WBIF, the half-life distance of the noneffective WBIF was 10.40 (10.3981±0.7111, 95%) km, and the environmental filtration from SL2 to SL1 (from the freshwater ecosystem to saline water ecosystem) was 12.87% (0.1287±0.0171, 95%). The transportation effectiveness of the upstream-to-downstream WBIF was 75.86%, 97.41% and 96.07% in spring, summer and autumn, respectively, without regard to environmental filtration in defined areas and times, with environmental change.

Discussion

Effectiveness of Monitoring Riparian Biodiversity Information Using Riverine Water eDNA

Because riverine water eDNA incorporates terrestrial biodiversity information (Deiner, et al.,2016; Yang, et al.,2019), by analyzing riverine water eDNA, information can be obtained on terrestrial biological compositions (Deiner, et al.,2016; Matsuoka, et al.,2019). The effectiveness of monitoring riparian biodiversity information depends on the transportation effectiveness of the WBIF from the riparian zone to the river. Following the WBIF framework (Yang, et al.,2019), the transportation effectiveness of the WBIF from the riparian zone to the river is mainly constructed by the capacity of transporting eDNA in the riparian zone into the river (driven by runoff, animals, wind, etc.) and environmental filtration (driven by soil/water environmental shifts, soil aggregates, etc.). Therefore, the effectiveness of monitoring riparian biodiversity information is also impacted by these factors.

In the frozen spring period, the maximum effectiveness of monitoring riparian biodiversity information was 16.62%, as the transportation effectiveness of the WBIF from the riparian zone to the river was 16.62% (estimated based on the OTU compositions detected from the soil eDNA sample and the water eDNA sample at the same sampling transect). In other words, a water eDNA sample could include information on as much as 16.62% of the biodiversity information from the adjacent riparian zone. Moreover, nearly 36.30% of the types of OTUs detected from the soil eDNA samples could be detected in water eDNA samples. This result suggested that as much as 36.30% of the riparian zone biodiversity information could be monitored using water eDNA samples. This effectiveness was mainly controlled by the low transport capacity of the WBIF (26.88%) and by the high environmental filtration (38.54%).

In the rainy summer period, the maximum effectiveness of monitoring the riparian biodiversity information was 62.76%, as the transportation effectiveness of the WBIF from the riparian zone to the river was 62.76% (estimated based on the OTU compositions detected from the soil eDNA sample and the water eDNA sample at the same sampling transect). In other words, a water eDNA sample could monitor as much as 62.76% of biodiversity information from the adjacent riparian zone. Moreover, nearly 71.98% of the types of OTUs detected from the soil eDNA samples could be detected in the water eDNA samples. This result suggested that as much as 71.98% of the biodiversity information in the riparian zone could be monitored using water eDNA samples. This effectiveness was mainly controlled by the transport capacity of the WBIF (68.49%).

In the cloudy autumn period, the maximum effectiveness of monitoring riparian biodiversity information was 48.09%, as the transportation effectiveness of the WBIF from the riparian zone to the river was 48.09% (estimated based on the OTU compositions detected from the soil eDNA sample and the water eDNA sample at the same sampling transect). In other words, a water eDNA sample could monitor as much as 48.09% of the biodiversity information from the adjacent riparian zone. Moreover, nearly 67.58% of the types of OTUs detected from the soil eDNA samples could be detected in the water eDNA samples. This result suggested that as much as 67.58% of the biodiversity information in the riparian zone could be monitored using water eDNA samples. This effectiveness was mainly controlled by the transport capacity of the WBIF (57.36%) and by environmental filtration (16.18%).

The transport capacity of the WBIF from the riparian zone to the river was driven by animal movement, wind erosion and transport, rainfall and surface runoff erosion, and surface runoff transport (Yang, et al.,2019). In the frozen spring period, there was little animal movement, as most animals were hibernating during Qinghai-Tibet Plateau's long winter. Although there was strong wind, the frozen soil limited the amount of wind erosion and transport. Moreover, the precipitation form was snow, and there was little surface runoff, which provided suboptimal conditions for significant erosion and transport. In summer and autumn, animal movement was frequent, wind erosion and transport were permitted, and rainfall and surface runoff erosion and surface runoff transport were significant. Therefore, the transport capacity of the WBIF from the riparian zone to the river in spring was obviously lower than that in summer and autumn and that on sunny or cloudy days was obviously lower than that on rainy days.

The environmental filtration of the WBIF was impacted by soil-to-water environmental shifts and soil aggre-

gates (Wilpiszeski, et al.,2019; Yang, et al.,2019). In the frozen spring period, the microbial activities were low in both soil and water, and most microbes (input to rivers from soil) could not be effectively preserved in water. Because there was no significant surface runoff, there was no soil aggregate to conserve the microbial input to the river. In summer and autumn, the microbial activities were high in both soil and water, and most microbes (input to rivers from soil) could be effectively preserved in water. Rainfall and surface runoff promoted the input of soil aggregates into rivers and promoted the conservation of the microbes living in them. Therefore, the environmental filtration of the WBIF in spring was obviously higher than that in summer and autumn and that on sunny or cloudy days was obviously higher than that on rainy days.

Sales et al. (2020) indicated that although the detection probability of riverine water eDNA was 40%˜67, it provided comparable results to conventional survey methods per unit of survey effort for three species (water vole, field vole and red deer); in other words, the results from 3˜6 water replicates would be equivalent to the results from 3˜5 latrine surveys and 5˜30 weeks of single camera deployment (Sales, et al.,2020). Considering that the number of clean sequences of each summer water eDNA sample and autumn water eDNA sample is obviously lower than it should be (Fig. 3), it is probable that the water eDNA samples contain more types of OTUs than the current results indicate. In other words, the transportation effectiveness of the WBIF from the riparian zone to the river in summer and autumn was obviously underestimated; therefore, the effectiveness of monitoring riparian biodiversity information in summer and autumn was obviously underestimated. However, the monitoring effectiveness was still 62%˜72%. This result suggests that riverine water eDNA is viable for monitoring riparian biodiversity information on rainy days in summer or autumn.

Effectiveness of Monitoring Upstream Biodiversity Information Using Downstream Water eDNA

The fact that species information at some distance upstream could be detected is the key for eDNA application in lotic systems (Stoeckle, et al.,2016; Carraro, et al.,2018; Pont, et al.,2018). The effectiveness of monitoring upstream biodiversity information depends on the transportation effectiveness of the upstream-to-downstream WBIF (Deiner & Altermatt,2014; Sansom & Sassoubre,2017; Pont, et al.,2018; Seymour,2019; Yang, et al.,2019). eDNA could originate from living and dead organisms and could be detected at distances downstream, which determined the eDNA transport and degradation processes (Stoeckle, et al.,2016; Nukazawa, et al.,2018; Tillotson, et al.,2018; Yang, et al.,2019). Following the framework of the WBIF (Yang, et al.,2019), the transportation effectiveness of the upstream-to-downstream WBIF was mainly constructed by the transport capacity of the upstream-to-downstream WBIF (impacted by eDNA evenness dispersed in water) and the degradation rate (constructed by the proportion and the half-life distance of noneffective WBIF). Therefore, the effectiveness of monitoring upstream biodiversity information was also impacted by these factors.

In spring, the maximum effectiveness of biodiversity information monitoring 1 km upstream was 75.86%, as the transportation effectiveness of the upstream-to-downstream WBIF was 75.86% per km. This result suggested that a water eDNA sample could monitor as much as 75.86% of the biodiversity information 1 km upstream. This effectiveness was mainly controlled by the high proportion of the noneffective WBIF (66.85%) and the small half-life distance of the noneffective WBIF (1.55 km).

In summer, the maximum effectiveness of biodiversity information monitoring 1 km upstream is 97.41%, as the transportation effectiveness of the upstream-to-downstream WBIF is 97.41% per km. This result suggested that a water eDNA sample could monitor as much as 97.41% of the biodiversity information 1 km upstream. This effectiveness was mainly controlled by the proportion of the noneffective WBIF (43.46%) and its half-life distance (14.52 km).

In autumn, the maximum effectiveness of biodiversity information monitoring 1 km upstream was 96.07%, as the transportation effectiveness of the upstream-to-downstream WBIF was 96.07% per km. This result suggested that a water eDNA sample could monitor as much as 96.07% of the biodiversity information 1 km upstream. This effectiveness was mainly controlled by the proportion of the noneffective WBIF (49.35%) and its half-life distance (10.40 km).

The transport capacity of the upstream-to-downstream WBIF was impacted by the even dispersal of eDNA in water. When the community richness is too high to spread each type of OTU in every liter of water, the transport capacity of the upstream-to-downstream WBIF declines. Therefore, along with the increase in the types of bacterial OTUs from spring to summer to autumn (Fig. 2d), the transport capacity declined from spring to summer to autumn. Fortunately, in the Shaliu River basin, the transport capacity was high (more than 99%) in the three seasons.

The degradation rate in the present study was constructed by the proportion and the half-life distance of the noneffective WBIF. The proportion of noneffective WBIF relies on microbial activity, which is impacted by temperature (Lin, et al.,2016). Therefore, the highest proportion of noneffective WBIF was detected in the frozen spring period, and the lowest proportion as detected in summer. The half-life distance of noneffective WBIF was mainly impacted by the flow rate of river runoff and the half-life period of eDNA degradation (Yang, et al.,2019). The half-life period of eDNA degradation is impacted by many environmental conditions, such as biochemical oxygen demand, temperature, pH, and organic matter (Barnes, et al.,2014; Eichmiller, et al.,2016; Nukazawa, et al.,2018; Seymour, et al.,2018; van Bochove, et al.,2020). However, the highest flow rate and the largest half-life distance of noneffective WBIF are found in summer. In other words, the half-life distance of the noneffective WBIF was mainly controlled by the flow rate in the Shaliu River.

The number of clean sequences of each water eDNA sample in summer and autumn was obviously lower than it should be (Fig. 3), and this result indicated that the water eDNA samples contained more types of OTUs than the current results presented; in other words, the transportation effectiveness of the upstream-to-downstream WBIFs in summer and autumn were obviously underestimated. Therefore, the effectiveness of monitoring upstream biodiversity information in summer and autumn was also obviously underestimated. However, the monitoring effectiveness was greater than 96% 1 km downstream in summer and autumn. This result suggested that riverine water eDNA was viable for monitoring upstream biodiversity information in summer and autumn.

Cost-effective Proposal for Monitoring the Biodiversity Information of Upstream and Riparian Zones Using Water eDNA

Because eDNA can be used to assess diversity across the tree of life with a single-field sampling protocol (Deiner, et al.,2016), eDNA sampling is more cost-effective (including cost and time) than conventional biodiversity monitoring methods (Mächler, et al.,2014; Valentini, et al.,2016; Seymour,2019). Moreover, simultaneously monitoring aquatic and terrestrial biodiversity using riverine water eDNA represents one step toward obtaining a more cost-effective biodiversity monitoring method.

As the highest effectiveness of monitoring riparian biodiversity information (62.76% for adjacent sites, or 71.98% for overall sites) appeared on rainy days in summer, the cost-effective sampling time for monitoring riparian biodiversity information using water eDNA is a rainy day in summer. Considering that the effectiveness was mainly impacted by rainfall and surface runoff, a rainy day in autumn would also be a cost-effective sampling time. Considering the temporal heterogeneity of biodiversity information, although there was variation in the OTU composition (Fig. 4), we do not suggest monitoring riparian biodiversity information using water eDNA during the frozen spring period because the monitoring effectiveness would be too low to detect the different biodiversity information in spring from that in summer and autumn. If sampling is required in the frozen spring period, it would be better to monitor riparian biodiversity information using soil eDNA directly.

As the effectiveness of biodiversity information monitoring 1 km upstream was 97.41% in summer, 96.07% in autumn, and higher than 75.86% in spring, the cost-effective sampling time for monitoring riparian biodiversity information using water eDNA is summer or autumn. In term of the temporal heterogeneity of biodiversity information, the water samples in autumn had the highest community richness, followed by those collected in summer and spring (Figs. 2, 3). Considering the degradation of noneffective WBIF, the flow rate mainly controlled the half-life distance of noneffective WBIF. We suggest that, to monitor microbial biodiversity information, autumn is the first choice for sampling, followed by summer because of the relatively

high flow rate. Because there were low monitoring effectiveness and high OTU composition differences with other seasons in spring, the microbial biodiversity in spring should be monitored with a high sampling site density, if spring sampling is required.

After examining the rarefaction curves of each sample, it was obvious that the number of clean sequences of each sample in the five groups (except for the spring water eDNA samples) was lower than it should be (Fig. 3). Perhaps more than 60,000 clean sequences of each sample or more than one duplicate sample is needed, especially for the water eDNA samples in summer and autumn. Therefore, to monitor the biodiversity information of upstream and riparian zones using riverine water eDNA, a rainy day in autumn or summer would be the most cost-effective sampling time, and more than one duplicate sample would be a better sampling choice for water eDNA samples in summer and autumn. If sampling is needed in spring, riparian biodiversity information should be monitored by soil eDNA rather than by water eDNA, and aquatic biodiversity information should be monitored using a high-density sampling method. All biodiversity assessments based on the monitoring results using riverine water eDNA should be revalued based on the monitoring effectiveness.

Moreover, the monitoring effectiveness in this study is indicated by environmental microbes, and whether the effectiveness of monitoring other taxonomies using riverine water eDNA is higher or lower is still unknown, and more research is required. Although the seasonal variation in monitoring effectiveness was delineated based on the results indicated by environmental microbes, it would be effective for other taxonomic groups. In other words, a rainy day in autumn or summer is the most cost-effective sampling time for monitoring the biodiversity information of upstream and riparian zones using riverine water eDNA for other taxonomic groups. Therefore, a study on monitoring the effectiveness assessment of other taxonomic groups would be useful for implementation on rainy days in autumn or summer to assess a cost-effective monitoring proposal. If the monitoring effectiveness in all taxonomic groups was verified to be higher than that obtained using conventional methods, the claim of revolutionizing biodiversity science (Thomsen & Willerslev,2015; Cristescu & Hebert,2018; Altermatt, et al.,2020) would come true.

The results showed that the effectiveness of monitoring the biodiversity information of the upstream and riparian zones was 96%˜97% (1 km upstream) and 62%˜72% (5 m distance from the river) on rainy days in summer or autumn, respectively, which was investigated in the Shaliu River basin (a typical watershed on the Qinghai-Tibet Plateau), and this information is likely a useful reference for other watersheds on the Qinghai-Tibet Plateau. Moreover, the following points may be applicable to watersheds in other regions: (1) rainfall, surface runoff and animal movements increased the effectiveness of monitoring riparian biodiversity information using riverine water eDNA; (2) frozen soil limited the effectiveness of monitoring riparian biodiversity information using riverine water eDNA; and (3) a high flow rate increased the effectiveness of monitoring upstream biodiversity information using downstream water eDNA. We believe that our framework for assessing monitoring effectiveness is reasonable and general. We encourage more research on monitoring effectiveness in other watersheds with different climatic conditions to support simultaneous aquatic and terrestrial biodiversity assessments.

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Data Accessibility

The datasets generated for this study can be found in the CNSA (https://db.cngb.org/cnsa/) of CNGBdb with accession number CNP0001046.

Author Contributions

HY acquired fund, designed and performed research, analyzed data, wrote and edited the paper.

HD acquired fund, designed and supervised research, reviewed and edited the paper.

HQ acquired fund, designed research, reviewed and edited the paper.

LY performed field sampling.

XH performed laboratory experiment.

HZ, JL, JW and CW reviewed and edited the paper.

QZ administrated project.

QW supervised and validated research.

Tables and Figures (with captions)

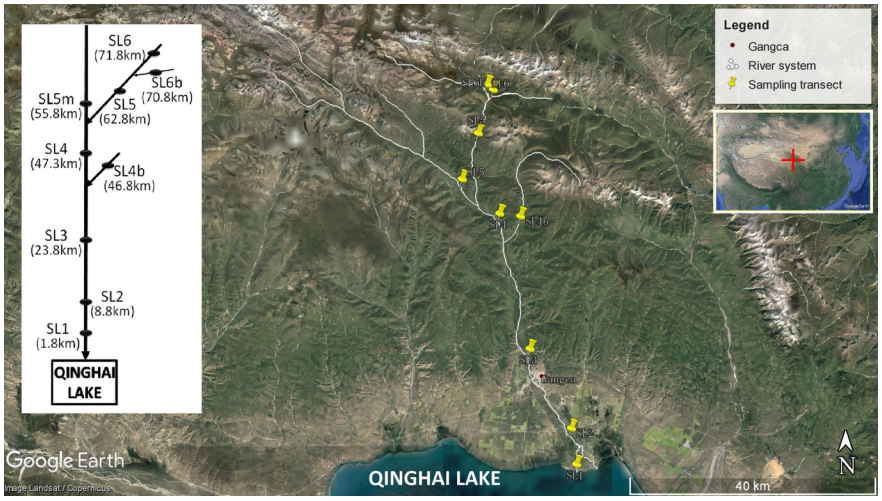

Fig. 1 Sampling transects.

SL1 denotes the first sampling transect on the Shaliu River. The distances labeled in parentheses under the tags of sampling transects denote the distances from the estuary to the sampling transects, such as SL1 (1.8 km), which means the distance from the estuary to SL1 is 1.8 km.

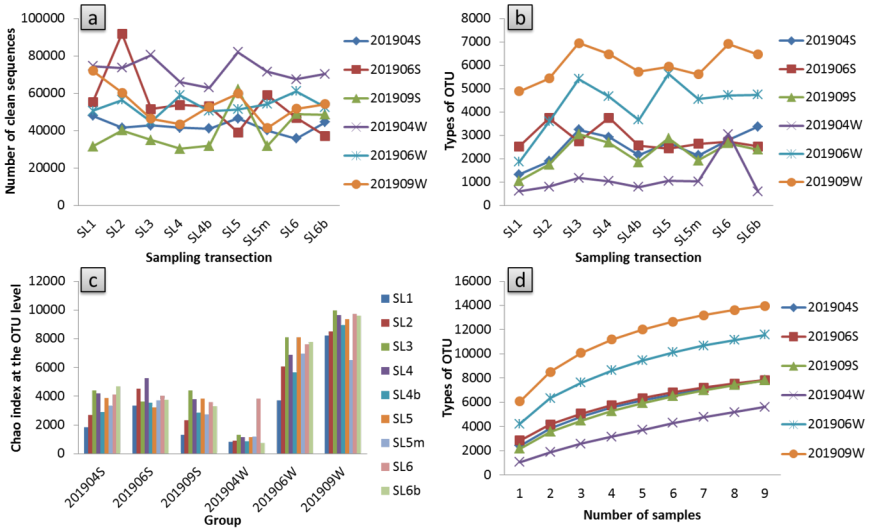

Fig. 2 Biological information features of the samples: numbers of clean sequences in each sample (a), types of OTUs in each sample (b), community richness of each sample at the OTU level (c) and species accumulation curves at the OTU level (d).

201904S denotes the soil eDNA samples that were sampled during April 2019; 201909W denotes the water eDNA samples that were sampled during September 2019.

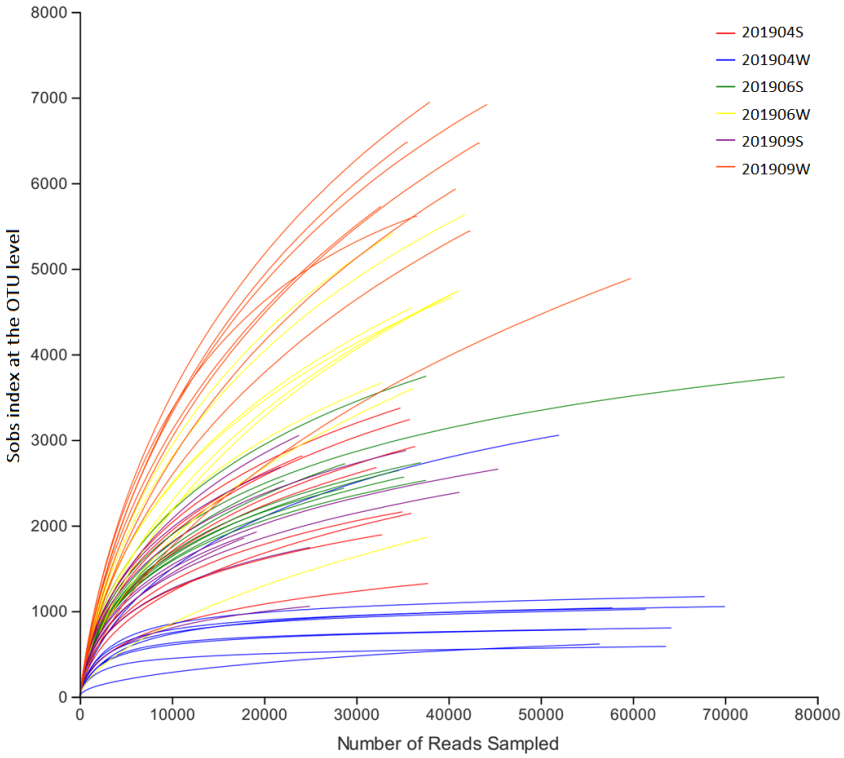

Fig. 3 Rarefaction curves of each sample.

201904S denotes the soil eDNA samples that were sampled during April 2019; 201909W denotes the water eDNA samples that were sampled during September 2019.

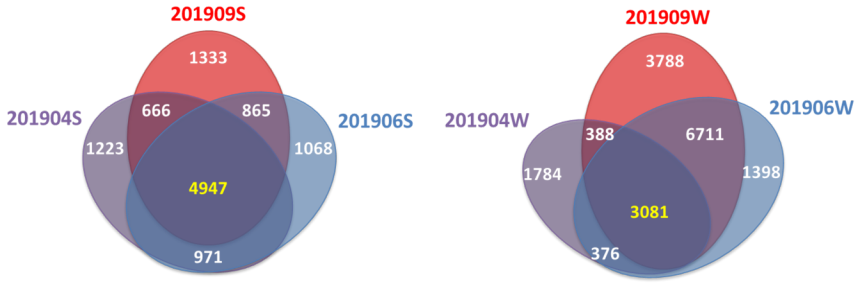

Fig. 4 The OTU types in riparian soil samples and riverine water samples shared by three groups.

201904S denotes the soil eDNA samples that were sampled during April 2019; 201909W denotes the water eDNA samples that were sampled during September 2019.

Table 1 Transport capacity, environmental filtration and transportation effectiveness of watershed biological information flow (WBIF) from the riparian zone to the river in each sampling transect

	201904	201904	201904	201906
	Transport capacity	**Environmental filtration**	**Transportation effectiveness**	**Transport capacit**
S-W_SL6b	0.099499	0.414501	0.058256	0.504529
S-W_SL6	0.859828	0.383092	0.530435	0.756467
S-W_SL5m	0.221932	0.311564	0.152786	0.776533
S-W_SL5	0.226740	0.360500	0.145000	0.655574
S-W_SL4b	0.175403	0.401328	0.105009	0.667238
S-W_SL4	0.184110	0.388603	0.112564	0.784735
S-W_SL3	0.205840	0.409836	0.121480	0.649059
S-W_SL2	0.176972	0.414116	0.103685	0.568211
S-W_SL1	0.428305	0.503238	0.212766	0.257329

S-W denotes the WBIF from the soil eDNA sample (riparian zone) to the water eDNA sample (river). SL1, SL2, SL3, SL4, SL4b, SL5m, SL5, SL6 and SL6b denote the sampling transections. S-W_SL1 denotes the WBIF from the riparian zone to the river at SL1. 201904, 201906, and 201909 indicate the groups that were sampled in April, June and September 2019, respectively. Transport capacity indicates the proportion of soil microbes that were transported into the river. Environmental filtration indicates that the proportion of soil microbes that could not be kept in rivers. Transportation effectiveness indicates the proportion of soil microbes that could be detected in rivers.

Table 2 The accumulative runoff distance and accumulative transportation effectiveness from the first sampling transection in each chain of the watershed biological information flow (WBIF)

Transport Chain	Cumulative Distance/km	Cumulative Effectiveness	Cumulative Effectiveness	Cumulative Effectiveness
		201904	**201906**	**201909**

Transport Chain	**Cumulative Distance/km**	**Cumulative Effectiveness**	**Cumulative Effectiveness**	**Cumulative Effectiveness**
Chain A				
SL6b-SL5	9	0.521352	0.798283	0.598885
##-SL4	24.5	0.412811	0.534830	0.471711
##-SL3	48	0.368327	0.486959	0.415639
##-SL2	63	0.330961	0.383295	0.308563
##-SL1	70	0.119217	0.197425	0.257389
Chain B				
SL6-SL5	8	0.217131	0.762412	0.605484
##-SL4	23.5	0.111554	0.590463	0.469171
##-SL3	47	0.093625	0.517251	0.408623
##-SL2	62	0.067231	0.414025	0.299731
##-SL1	69	0.032371	0.192146	0.248692
Chain C				
SL5m-SL4	8.5	0.430074	0.765880	0.667929
##-SL3	32	0.334385	0.579820	0.540136
##-SL2	47	0.228181	0.448004	0.361037
##-SL1	54	0.072555	0.196740	0.298750
Chain D				
SL4b-SL3	23	0.521333	0.738029	0.719376
##-SL2	38	0.352000	0.555864	0.437211
##-SL1	45	0.108000	0.237335	0.335324

Chains A, B, C and D denote the 4 chains of the upstream-to-downstream WBIF. In Chain A, SL6b-SL5 denotes the WBIF from sampling transects SL6b to SL5; ##-SL4 denotes the WBIF from sampling transects SL6b to SL5 and then to SL4; ##-SL3 denotes the WBIF from sampling transects SL6b to SL5, SL4 and then to SL3; ##-SL2 denotes the WBIF from sampling transects SL6b to SL5, SL4, SL3 and then to SL2; ##-SL1 denotes the WBIF from sampling transects SL6b to SL5, SL4, SL3, SL2 and then to SL1 (estuary). 201904, 201906, and 201909 indicate the groups that were sampled in April, June and September 2019, respectively.