**Title**

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

**Running title**

Monitoring aquatic & terrestrial biodiversity

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## Abstract

Both aquatic and terrestrial biodiversity information can be detected in riverine water environmental DNA (eDNA). However, whether riverine water eDNA can be used to simultaneously monitor aquatic and terrestrial biodiversity remains unverified. To assess the effectiveness of using riverine water eDNA to simultaneously monitor the riverine and terrestrial biodiversity information, we proposed that the monitoring effectiveness could be approximated by the transportation effectiveness of land-to-river and upstream-to-downstream biodiversity information flows. Subsequently, we conducted a case study in a watershed on the Qinghai-Tibet Plateau. The case demonstrated that there was higher monitoring effectiveness on summer or autumn rainy days than in other seasons and weather conditions. The monitoring of the bacterial biodiversity information was more efficient than the monitoring of the eukaryotic biodiversity information. On summer rainy days, 43-76% of species information in riparian sites could be detected in water eDNA samples, 92-99% of species information in riverine sites could be detected in a 1-km downstream eDNA sample, and half of dead bioinformation (i.e., the bioinformation labeling the biological material that lacked life activity and fertility) could be monitored 4-6 km downstream for eukaryotes and 13-19 km downstream for bacteria. In this case, we tested the eDNA monitoring effectiveness assessment framework, in which the land-to-river monitoring effectiveness was indicated by detection probability, and the upstream-to-downstream monitoring effectiveness was described by the detection probability per kilometer runoff distance and by the half-life distance of dead bioinformation. It provided a new and usable tool for designing monitoring projects and for evaluating monitoring results.

**Keywords**

biodiversity information, biodiversity monitoring, detection probability, environmental DNA, monitoring effectiveness, watershed biological information flow

## Introduction

Biodiversity monitoring is the basis of ecological research, biodiversity conservation and ecosystem management (Hooper et al., 2012; Dixon et al. 2019). Traditional biodiversity monitoring methods are cost- and time-consuming and require high levels of expertise, in which biodiversity is often studied from a local and low spatio-temporal resolution perspective and is generally not available at a wide taxonomic breadth, high spatio-temporal resolution and large spatio-temporal scale (Anderson, 2018; Altermatt et al., 2020; Pawlowski et al. 2020). 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 (Deiner et al., 2016; Carraro et al., 2018; Seeber et al. 2019; Pawlowski et al. 2020; Sales et al. 2020). As an efficient and easy-to-standardize non-invasive monitoring approach (Deiner et al. 2016; Lugg et al., 2018; Ravindran,2019; Seymour, 2019), and with the continuous advancements in DNA sequencing technology, using eDNA metabarcoding to monitor biodiversity is an appropriate method to revolutionize biodiversity monitoring by enabling the census of wide taxonomic species on a high spatio-temporal resolution and large spatio-temporal scale (Deiner et al., 2016; Valentini et al., 2016; Cristescu & Hebert, 2018; Altermatt et al., 2020). Streams and rivers connect upstream and downstream regions, connect land with waterbodies, and transport materials and information through extensive and heterogeneous network systems (Deiner et al., 2016; Shogren et al.,2017; Matsuoka et al., 2019). Riverine water eDNA incorporates biodiversity information across terrestrial and aquatic biomes (Deiner et al., 2016; Matsuoka et al., 2019). Therefore, a sample of riverine water eDNA has 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 by 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 the terrestrial and upstream biodiversity information (Deiner & Altermatt, 2014; Deiner et al.,2016; Jerde et al. 2016; Sansom & Sassoubre, 2017; Pont et al., 2018). The 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 defined the land-to-river and upstream-to-downstream bioinformation transportation, which is driven by watershed ecosystem processes, as the watershed biological information flow (WBIF). WBIF integrates the ecological processes of eDNA, including the origin, state, transport, and fate of eDNA (Barnes & Turner, 2016; Jo et al. 2017; Shogren et al., 2017; Cristescu and Hebert 2018; Tillotson et al. 2018). The transportation effectiveness of WBIF mainly relies on the transport capacity, degradation rate, and environmental filtration of WBIF (Barnes & Turner, 2016; Jo et al. 2017; Shogren et al., 2017; Tillotson et al. 2018). The transport capacity of WBIF mainly depends on erosion and runoff (Shogren et al., 2017; Fremier et al. 2019; Seymour, 2019). Additionally, the degradation rate of WBIF mainly depends on environmental features (Barnes & Turner, 2016; Eichmiller et al. 2016; Nukazawa, et al.,2018;), and the environmental filtration of WBIF mainly depends on the environmental changes of restricting organisms. Collectively, all of these factors are related to the seasons and weather conditions (Nukazawa, et al.,2018). Therefore, we hypothesized that the monitoring effectiveness of riverine water eDNA would vary with the seasons and weather conditions. Moreover, due to taxonomy-specific eDNA degradation rates (Barnes, et al.,2014), species-specific eDNA degradation rates (Deiner & Altermatt, 2014), and form-specific eDNA degradation rates (van Bochove et al., 2020), we hypothesized that the monitoring effectiveness of riverine water eDNA would vary with taxonomic communities.

Herein, we proposed that, in order to identify the effectiveness of using riverine water eDNA to simultaneously monitor the 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 the variation in biodiversity information monitoring effectiveness in different seasons and weather conditions; (2) to identify the variation in the effectiveness for monitoring the biodiversity information of different taxonomic communities; and (3) to test the monitoring effectiveness assessment framework. Our study identified the effectiveness of using riverine water eDNA to simultaneously monitor the biodiversity information in riverine sites and riparian sites on the Qinghai-Tibet Plateau and tested the assessment framework. We encourage more studies on monitoring effectiveness for each taxonomic community’s specific biodiversity information in other watersheds with different environmental conditions. As the bioinformation in WBIF includes the biodiversity information of all taxonomic communities, the information of all taxonomic communities could be monitored by using riverine water eDNA, although variability in monitoring effectiveness exists among different taxonomic communities. In future ecological research, biodiversity conservation, and ecosystem management, riverine water eDNA may be 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 3196 m above sea level on the Qinghai-Tibet Plateau (Fig. 1). The Shaliu River is 106 km long, with a catchment area of 1320 km2. 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 changed by human activity, such as transformation into cultivated land and building land[[1]](#footnote-2). 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.

### Sampling and Sequencing

To identify the seasonal variation of monitoring effectiveness, on April 8 and 9, June 25 and 26, and September 19 and 20 of 2019, we collected eDNA samples (spring group, summer group, and autumn group, respectively), including 27 soil eDNA samples and 27 water eDNA samples. The samples were collected from 9 transects (including riverine sampling sites and riparian sampling sites) of the Shaliu River (Fig. 1). The weather and hydrological conditions of each group are summarized in Table 1. 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 the samples cool can reduce the rate of eDNA decay and is a convenient and efficient method for conserving eDNA samples (Sales et al.,2019), 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 the eDNA of most taxonomic communities (Eichmiller et al.,2016; Li et al.,2018), water samples (with purified water used as a negative control) were filtered by using 0.2-μm membrane filters (JinTeng, Tianjin, PRC) to obtain the eDNA sample in the laboratory (with every step following the general rule of molecular ecology experimentation to control for contamination using bleach to wash the experimental apparatus). Subsequently, the filter membranes of each water sample were placed in a 50-mL sterilized centrifuge tube. The 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. More details are provided in Table 2 and Supplementary Material 1.

To identify the taxonomic variation of monitoring effectiveness, we analyzed three taxonomic communities using the metabarcoding of the 16S rRNA, ITS, and mitochondrial CO1 genes (Collins et al.,2019; Heeger et al.,2019; Giebner et al.,2020). As long DNA fragments show a higher decay rate than short fragments (Jo et al.,2017), short fragments better reflect community richness than long fragments (Wei et al.,2018; Jo et al.,2017). We restricted the amplified fragment length to 300-500 bp and selected the primers 338F/806R, ITS1F/ ITS2R, and mlCOIintF/ jgHCO2198R to detect bacteria, fungi, and metazoan, respectively (Collins et al.,2019; Heeger et al.,2019; Giebner et al.,2020). As the extraction of eDNA (Hermans et al.,2018; Armbrecht et al.,2020), amplification approach, and sequencing (Nichols et al.,2018) can impact the results of eDNA monitoring, a consistent DNA extraction method and amplification approach should be used for comparisons among samples (Dopheide et al.,2019; Giebner et al.,2020; Nicholson et al.,2020). Commercial eDNA labs can help (Ravindran, 2019), in which all approaches (including eDNA extraction, primer synthesis, amplification approach, sequencing, and contamination control, among others) could be standard. In our work, samples were processed by Shanghai Majorbio Bio-pharm Technology Co., Ltd (Shanghai, China). The details are provided in Table 2 and Supplementary Material 1.

On the free online Majorbio Cloud Platform ([www.majorbio.com](http://www.majorbio.com)), we analyzed the raw sequences data, and we obtained the types of operational taxonomic unit (OTU), the sequence number of each OTU, and the taxonomic features of each sample; additionally, we examined the 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 the biodiversity information in riverine sites and riparian sites. In the current WBIF analysis, all of the statistical analyses used the types of OTUs and species in each sample. The processing approach was simply described as follows (indicated by the OTU type).

The transportation effectiveness of 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).

(Eq. 1)

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

As the distance of the land-to-river WBIF was less than 5 m in the present case study, the transportation effectiveness of the land-to-river WBIF was assumed to be constructed by transport capacity and environmental filtration (no degradation rate). 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).

(Eq. 2)

(Eq. 3)

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

WBIF included the effective WBIF (i.e., the flow or migration of living organisms) and noneffective WBIF (i.e., the flow of the bioinformation labeling the biological material that lakced life activity and fertility [dead bioinformation]). The transportation effectiveness of upstream-to-downstream WBIF was determined by the different features of effective WBIF and noneffective WBIF. 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 presuppositions: (1) the 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) the noneffective WBIF degraded over time (i.e., distance) in a logistic manner; and (4) the environmental filtration was consistent in a definite environmental change. These four presuppositions did not exactly describe the complex facts, but they provided a possibility of constructing a model to approximately address the complex facts. The transportation effectiveness of the upstream-to-downstream WBIF could be described by an equation (Eq. 4), in which the transportation effectiveness was the function of runoff distance, and the transport capacity, environmental filtration, and degradation rate were parameters that could be estimated according to the sets of transportation effectiveness and runoff distance. In practice, as WBIF are impacted by varying factors at any site and time, the analytical solution of the parameters in Eq. 4 is impossible. Therefore, we suggested that Eq. 4 could be programming-solved, according to the evolutionary algorithm in Microsoft Excel. As there were only approximate solutions of the parameters in Eq. 4, we suggested obtaining several sets (such as 30 sets) of approximate solutions, after which a statistical analysis could be performed for each parameter.

(Eq. 4)

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

## Results

### WBIF of the Three Seasonal Groups

A total of 10,602, 13,766, and 16,500 bacterial OTU types were detected from the samples (including 9 water samples and 9 soil samples) of the spring, summer, and autumn group, respectively (Fig. 2 and Table S1). The total types of OTUs that were detected from the soil eDNA samples were similar among the seasons (Figs. 2,3). The total types of OTUs that were detected from the water eDNA samples were richest in the autumn (Figs 2,3). The common types of OTUs that were shared between the soil eDNA and water eDNA samples accounted for 36.30%, 71.98%, and 67.58% of the total OTU types that were detected in the soil eDNA samples in the spring, summer, and autumn groups, respectively (Fig. 3).

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 the lowest environmental filtration on the summer rainy day (Tables 3,S2). 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 (Tables 4,S3), among which the transport capacity was more than 99% in all three seasons and the least noneffective WBIF (dead bioinformation) occurred; the longest half-life distance of the noneffective WBIF occurred on the summer rainy day (Table 4).

### WBIF of the Three Taxonomic Groups

A total of 13,766, 7098, and 17,316 kinds of OTUs and 3532, 1032, and 6836 kinds of species were detected among the 18 summer samples, as indicated by the 16S rRNA gene, ITS gene, and CO1 gene, respectively (Fig. 4 and Table S4). The types of OTUs and species detected in the water eDNA samples were generally higher than in the soil eDNA samples for all three taxonomic communities (Fig. 4). The common OTUs and species shared between the soil and water eDNA samples accounted for 71.98% and 87.95%, 60.40% and 76.18%, and 37.93% and 53.52% of the total types of OTUs and species in the bacterial, fungal and eukaryotic group, respectively.

The transportation effectiveness of the 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 the transport capacity and environmental filtration significantly declined with the bacterial, fungal, and eukaryotic communities (Tables 5,S5,S6). 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 with the bacterial, fungal, and eukaryotic communities (Tables 6,S7,S8), 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 (Table 6).

## Discussion

### Seasonal variation of monitoring effectiveness

Driven by the land-to-river and upstream-to-downstream WBIF, biodiversity information across terrestrial and aquatic biomes could be detected in riverine water eDNA (Deiner et al., 2016; Matsuoka et al., 2019), and the monitoring effectiveness of riverine water eDNA relies on the transportation effectiveness of corresponding WBIF (Deiner & Altermatt, 2014; Deiner et al.,2016; Jerde et al. 2016; Sansom & Sassoubre, 2017; Pont et al., 2018). The transportation effectiveness of WBIF mainly relies on the transport capacity, degradation rate, and environmental filtration of WBIF (Barnes & Turner, 2016; Jo et al. 2017; Shogren et al., 2017; Tillotson et al. 2018), which can vary with different seasons and weather conditions (Nukazawa, et al.,2018). We hypothesized that the monitoring effectiveness would vary with the seasons and weather conditions. In the present case, the bacterial community richness in soil did not vary with season, whereas the bacterial community composition in water was richest in the autumn, followed by the summer (Fig. 2,3). The transportation effectiveness of riparian-to-water and upstream-to-downstream WBIF in spring frozen days was significantly lower than in summer rainy days and autumn cloudy days (Tables 3,4,S2,S3). It indicates that the monitoring effectiveness varied with different seasons and weather conditions, and summer and autumn were the optimal seasons, along with rainy days being the optimal weather condition, for using riverine water eDNA to simultaneously monitor the holistic biodiversity information in riverine sites and riparian sites.

The biodiversity information detected by water eDNA could originate from living and dead organisms (Nukazawa et al.,2018; Tillotson et al.,2018). The detection of biodiversity information that originates from a living organism mainly depends on the dispersal of this living organism (Pont, et al.,2018; Ravindran,2019). The detection of biodiversity information that originates from a dead organism mainly depends on its transport capacity and degradation rate (Seymour et al.,2018; Jo et al.,2017; Seymour,2019). In summer and autumn, as driven by active organisms, more eDNA was input into the river system. In particular, the surface runoff caused by rain can input more eDNA from terrestrial soil into the river system and can preserve them in soil aggregates (Wilpiszeski et al. 2019). In the present study, the highest proportion of bacteria in riparian soil was detected in water in summer and autumn, and the rain promoted this phenomenon (Fig. 3 and Tables 3,S2). 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 (Table 4). eDNA (originated from dead organisms) degrades over time in a logistic manner (a half-life time) (Barnes, et al.,2014; Jo et al.,2017; Wei et al.,2018; Seymour,2019), which was described in this study as degrading by half-life distance in a lotic system, which integrates the transport capacity and the degradation rate. In the present work, as driven by runoff discharge and flow velocity (Table 1), the half-life distance of noneffective WBIF was significantly farther in the summer than in autumn and in spring (Table 4).

### Taxonomic variation of monitoring effectiveness

The biodiversity information monitoring effectiveness of riverine water eDNA, as approximated by the transportation effectiveness of WBIF, was impacted by the eDNA degradation rate in WBIF, and there were taxonomy-specific eDNA degradation rates (Barnes, et al.,2014), species-specific eDNA degradation rates (Deiner & Altermatt, 2014), and form-specific eDNA degradation rates (van Bochove et al., 2020). We hypothesized that the monitoring effectiveness of riverine water eDNA would vary with taxonomic communities. In the present case, the results revealed the detection of a significantly higher monitoring effectiveness of water eDNA (both riparian-to-water and downstream-to-upstream) for bacterial communities than for eukaryotic communities (Tables 5,6). A significantly higher monitoring effectiveness of water eDNA was found for micro eukaryotic communities (fungi) than for overall eukaryotic communities (including micro- and macro-organisms) (Tables 5,6). This indicates that the monitoring effectiveness varied with different taxonomic communities, and the effectiveness of monitoring eukaryotic communities was significantly lower than for monitoring bacterial communities; in addition, the effectiveness of monitoring macrobe communities was significantly lower than for monitoring microbe communities.

eDNA surveys that are based on metabarcoding can actually acquire information across the taxonomic tree of life (Deiner et al.,2016; Stat et al.,2017; Ravindran,2019; Djurhuus, et al.,2020; Pawlowski et al.,2020). However, eDNA that originates from different taxonomic groups has a different probability of being left in the environment and input into water (Deiner, et al.,2016; Harper, et al.,2019; Seeber, et al.,2019; Sales, et al.,2020). van Bochove et al. (2020) inferred that the eDNA contained inside of cells is especially resilient against degradation (i.e., intracellular vs. extracellular effects) (van Bochove, et al.,2020). In the present case, more bacteria than eukaryotes and more microorganisms than macroorganisms (both OTU and species levels) in riparian soil could be detected in water (Table 5). The half-life distance of noneffective WBIF for bacteria (detected by the 16s RNA gene) was much farther than that for unicellular eukaryotes (detected by the ITS gene, which is mainly unicellular), than that for multicellular eukaryotes (as detected by the CO1 gene, which is mainly multicellular) (Table 6). We inferred that the eDNA contained inside of bacterial cells was more resilient against degradation than that contained inside of unicellular eukaryotic cells (i.e., prokaryotic cells vs. eukaryotic cells), as well as compared to the eDNA contained inside of multicellular eukaryotic cells or extracellular mitochondria (i.e., unicellular eukaryotic cells vs. multicellular eukaryotic cells or extracellular).

### Monitoring effectiveness assessment framework

In previous studies, the effectiveness of using water eDNA to monitor terrestrial organisms was indicated by the detection probability (Harper, et al.,2019; Seeber, et al.,2019; Sales, et al.,2020), and the effectiveness of using downstream water eDNA to monitor upstream organisms was indicated by the detectable distance (Deiner & Altermatt,2014; Sansom & Sassoubre,2017; Carraro, et al.,2018; Pont, et al.,2018; Seymour,2019; Carraro, et al.,2020). In this study, we approximated the biodiversity information monitoring effectiveness by the WBIF transportation effectiveness and proposed its assessment framework, in which we described the riparian-to-water monitoring effectiveness with the proportion of biodiversity information in riparian soil that was detected by using riverine water eDNA samples. Additionally, we described the downstream-to-upstream monitoring effectiveness with the proportion of biodiversity information in upstream site water eDNA samples that was detected by 1-km downstream site water eDNA samples, and the runoff distance of that 50% of dead bioinformation (i.e., the bioinformation labeling the biological material that lakced life activity and fertility) could be monitored. These indicators provided new usable assessment tools for designing monitoring projects and for 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, by 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 (Table 5), 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 (Table 6). The half-life distance of the noneffective WBIF was respectively 17.82 km, 5.96 km, and 5.02 km for bacteria, fungi, and metazoans at the species level (Table 6). When considering the fact that the monitoring effectiveness of eDNA can not only vary with season, weather, and taxonomic communities, but can also vary with rivers and watersheds with different environmental conditions (Deiner & Altermatt,2014; Sansom & Sassoubre,2017; Tillotson et al.,2018; Seymour,2019), more studies on the 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 (Valentini, et al.,2016; Lugg, et al.,2018), although this is certainly not true in all circumstances (Beng & Corlett,2020). We anticipate that, in future biodiversity research, conservation, and management, we will be able to efficiently monitor and assess the 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 the land-to-river monitoring effectiveness was indicated by detection probability, and the upstream-to-downstream monitoring effectiveness was described by the 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, and on summer rainy days, 43-76% of species information in riparian sites could be detected in 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 for bacteria was approximately 13-19 km and was approximately 4-6 km for eukaryotes. The indicators in the assessment framework that describe the monitoring effectiveness provide usable assessment tools for designing monitoring projects and for evaluating monitoring results.

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## Data Accessibility

The datasets that were 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.

## Author Contributions

HY, HD, and HQ contributed to the conception of the study. HY and LY performed the field sampling. HY and XH conducted the laboratory work. HY, HZ, JL, JW, and CW contributed the acquisition, analysis, and interpretation of the data. QZ administrated the project. HY led the writing of the manuscript, and HZ, JL, JW, and CW contributed critically to the drafts and final version of the manuscript.

## Tables and Figures (with captions)

## Tables (with captions)

**Table 1 The weather and hydrological conditions at each sampling time**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Sampling time | Air temperature | Water temperature | Discharge | Flow velocity | Weather conditions |
| Spring group | 2019.4.8-9 | -6-8°C | -0.5-0.7°C | 1.8-3.9 m³/s | 0.63-1.04 m/s | frozen days (†)  frozen days (‡) |
| Summer group | 2019.6.25-26 | 7-17°C | 4.3-16.4°C | 29.9-45.5 m³/s | 0.84-2.03 m/s | sunny day (†)  light rain day (‡) |
| Autumn group | 2019.9.19-20 | 0-10°C | 0.2-8.8°C | 5.7-12.8 m³/s | 0.57-0.88 m/s | light rain day (†)  cloudy day (‡) |

(†) Collecting 4 samples (2 water samples and 2 soil samples) from transects of SL1 and SL2 (two downstream transects) along the downstream-to-upstream direction.

(‡) Collecting 14 samples (7 water samples and 7 soil samples) from transects of SL3, SL4, SL4b, SL5m, SL5, SL6 and SL6b (seven upstream transects) along the downstream-to-upstream direction.

**Table 2 the steps of sampling and sequencing**

|  |  |  |
| --- | --- | --- |
| **Sample types** | **Soil eDNA sample** | **Water eDNA sample** |
| Sampling site | Riparian area (5 m distance from the river) of each transect | River of each transect |
| Step 1: field sampling | Collecting 5 mL riparian soil using a 5-mL sterilized centrifuge tube | Collecting 1.5 L of river water using a 1.5-L sterilized bottle (rinsed three times with sampling water) |
| Step 2: field samples transport | Transporting to the laboratory of the Rescue and Rehabilitation Center of Naked Carps of Qinghai Lake at 0°C (in an ice bath) | |
| Step 3: samples pretreatment |  | Filtering river water using 0.2-μm membrane filters and placing the filters of each water sample into a 50-mL sterilized centrifuge tube |
| Step 4: samples frozen | Freezing the tubes in a -20°C refrigerator | |
| Step 5: samples transport | Transporting the tubes at -20°C (in a dry ice bath) | |
| Step 6: samples store | Storing the tubes at -80°C (in an ultra-low temperature freezer) until DNA extraction | |
| Step 7: DNA extraction | Extracting DNA using an FastDNA® SPIN Kit for Soil | |
| Step 8: DNA quality testing | Determining the final DNA concentration and purity using a NanoDrop 2000 UV-vis spectrophotometer, checking the DNA quality using 1% agarose gel electrophoresis | |
| Step 9: PCR amplification – primer (with barcode) | 1. Bacterial 16S rRNA gene:  338F (5’- ACTCCTACGGGAGGCAGCAG-3’)  806R (5’-GGACTACHVGGGTWTCTAAT-3’)  2. Fungal ITS gene:  ITS1F (5’-CTTGGTCATTTAGAGGAAGTAA)  ITS2R (5’-GCTGCGTTCTTCATCGATGC)  3. Eukaryotic mitochondrial CO1 gene:  mlCOIintF (5’-GGWACWGGWTGAACWGTWTAYCCYCC)  jgHCO2198R (5’-TANACYTCNGGRTGNCCRAARAAYCA) | |
| Step 9: PCR amplification –reaction system (3 duplicate, with blank controls) | 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, 10 ng of template DNA and ddH2O | |
| Step 10: PCR amplification – program (GeneAmp 9700, ABI, USA) | 1. Bacterial 16S rRNA gene:  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.  2. Fungal ITS gene:  3 min of denaturation at 95 °C; 37 cycles of 30 s at 95 °C, 30 s for annealing at 53 °C, and 45 s for elongation at 72 °C; and a final extension at 72 °C for 10 min.  3. Eukaryotic mitochondrial CO1 gene:  5 min of denaturation at 94 °C; 35 cycles of 60 s at 94 °C, 120 s for annealing at 47 °C, and 60 s for elongation at 72 °C; and a final extension at 72 °C for 5 min. | |
| Step 11: PCR product testing | Testing PCR product quality using 2% agarose gel electrophoresis | |
| Step 12: PCR product extraction and purification | PCR products were extracted from a 2% agarose gel using an AxyPrep DNA Gel Extraction Kit, and then purified using an QIAquick PCR Purification Kit | |
| Step 13: PCR product quantification | PCR products were quantified using QuantiFluor™-ST | |
| Step 14: Miseq library preparation (TruSeq™ DNA Sample Prep Kit) | Adding the standard tags of Illumina to PCR products according another PCR program, extracting, purifying and checking tagged PCR products, preparing single-stranded DNA | |
| Step 15: Miseq sequencing | Purified amplicons were pooled in equimolar amounts and subjected to paired-end sequencing on an Illumina MiSeq platform | |
| Step 16: raw sequence treatment | Raw fastq files were demultiplexed, quality-filtered by Trimmomatic and merged by FLASH. | |
| Step 17: clustering OTU | Operational taxonomic units (OTUs) were clustered with a 97% similarity cutoff using UPARSE, and chimeric sequences were identified and removed using UCHIME. | |
| Step 18: taxonomy identification | The taxonomies of each sequence were analyzed by the RDP Classifier Bayesian algorithm against the corresponding database using a confidence threshold of 70%  Database selection:  1. Bacterial 16S rRNA gene: Silva132/16S\_Bacteria database  2. Fungal ITS gene: Unite8.0/ITS\_Fungi database  3. Eukaryotic mitochondrial CO1 gene: nt database (standard database) | |
| Step 18: communities analysis | The OTU numbers, types and taxonomic features of the samples were analyzed. Community Chao richness at the OTU level was examined using the software of Mothur | |

**Table 3 Seasonal variation of transport capacity, environmental filtration, and transportation effectiveness of watershed biological information flow (WBIF) from the riparian sampling site to adjacent riverine water sampling site in three seasons indicated by bacterial OTUs**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Seasonal group** | **Weather condition** | **Transport capacity** | **Environmental filtration** | **Transportation effectiveness** |
| **Spring group** | Frozen days | 0.268791±0.202388 | 0.385443±0.029320 | 0.166152±0.125394 |
| **Summer group** | Rainy days | 0.684876±0.091302 | 0.083816±0.020574 | 0.627643±0.087327 |
| **Autumn group** | Cloudy days | 0.573579±0.052897 | 0.161800±0.045075 | 0.480933±0.052179 |

The spring group was sampled during April 2019; the summer group was sampled during June 2019; the autumn group was sampled during September 2019. Statistics for the spring group are based on 8 sampling transects except estuary (SL1); statistics for the summer and autumn groups are based on 7 sampling transects except two downstream transects (SL1 and SL2).

**Table 4 Seasonal variation of transport capacity, proportion of noneffective WBIF, half-life distance of the noneffective WBIF, and transportation effectiveness of watershed biological information flow (WBIF) from the upstream to downstream regions indicated by bacterial OTUs**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Seasonal group** | **Weather condition** | **Transport capacity (per km)** | **Proportion of noneffective WBIF** | **Half-life distance of the noneffective WBIF** | **Transportation effectiveness (per km)** | **Environmental filtration from rain point to sunny point** | **Environmental filtration from freshwater to saline-water** |
| **Spring group** | Frozen days | 0.999706±0.000305 | 0.668465±0.003435 | 1.548987±0.126870 | 0.758618±0.000304 | / | 0.160427±0.008244 |
| **Summer group** | Rainy days | 0.994245±0.000941 | 0.434635±0.041681 | 14.52338±1.440539 | 0.974105±0.000926 | 0.005687±0.005450 | 0.544164±0.010042 |
| **Autumn group** | Cloudy days | 0.992250±0.001452 | 0.493504±0.041043 | 10.398112±0.711122 | 0.960671±0.001415 | / | 0.128718±0.017062 |

The spring group was sampled during April 2019; the summer group was sampled during June 2019; the autumn group was sampled during September 2019.

**Table 5 Transport capacity, environmental filtration, and transportation effectiveness of watershed biological information flow (WBIF) from the riparian sampling site to the adjacent riverine water sampling site on summer rainy days, as indicated by three taxonomic groups**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Taxonomic group** | **Taxonomic level** | **Transport capacity** | **Environmental filtration** | **Transportation effectiveness** |
| **Bacteria (detected by the 16S rRNA gene)** | OTU level | 0.684876±0.091302 | 0.083816±0.020574 | 0.627643±0.087327 |
| species level | 0.829912±0.066079 | 0.027020±0.007048 | 0.807461±0.064521 |
| **Fungi (detected by the ITS gene)** | OTU level | 0.600756±0.102865 | 0.258922±0.054794 | 0.447896±0.095670 |
| species level | 0.738975±0.100006 | 0.113469±0.016910 | 0.656191±0.097099 |
| **Metazoan (detected by the CO1 gene)** | OTU level | 0.440871±0.124206 | 0.485954±0.061102 | 0.226403±0.071669 |
| species level | 0.604263±0.092950 | 0.281177±0.028991 | 0.433842±0.066684 |

Bacteria (detected by the 16S rRNA gene), fungi (detected by the ITS gene), and metazoans (detected by the CO1 gene) indicate the groups of bacteria (detected by the 16S rRNA gene), fungi (detected by the ITS gene), and metazoans (detected by the CO1 gene), respectively. Statistics in all groups are based on 7 sampling transects, except for two downstream transects (SL1 and SL2).

**Table 6 Transport capacity, proportion of noneffective WBIF, half-life distance of the noneffective WBIF, and transportation effectiveness of watershed biological information flow (WBIF) from the upstream to downstream regions on summer rainy days, indicated by three taxonomic groups at the OTU and species levels estimated by programming-solved according to the evolutionary algorithm**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Taxonomic group** | **Taxonomic level** | **Transport capacity (per km)** | **Proportion of noneffective WBIF** | **Half-life distance of the noneffective WBIF** | **Transportation effectiveness (per km)** | **Environmental filtration from rain point to sunny point** | **Environmental filtration from freshwater to saline-water** |
| **Bacteria (detected by the 16S rRNA gene)** | **OTU level** | 0.994245±0.000941 | 0.434635±0.041681 | 14.52338±1.440539 | 0.974105±0.000926 | 0.005687±0.005450 | 0.544164±0.010042 |
| **species level** | 0.998188±0.000121 | 0.296484±0.010590 | 17.82057±1.215028 | 0.986898±0.000121 | 0.051209±0.005337 | 0.460245±0.001469 |
| **Fungi (detected by the ITS gene)** | **OTU level** | 0.995550±0.000680 | 0.529290±0.016749 | 4.925445±0.353730 | 0.926377±0.000670 | 0.003482±0.002886 | 0.338354±0.003866 |
| **species level** | 0.999484±0.000244 | 0.386710±0.008333 | 5.961259±0.264864 | 0.957057±0.000242 | 0.000541±0.000258 | 0.224685±0.001239 |
| **Metazoan (detected by the CO1 gene)** | **OTU level** | 0.989275±0.000923 | 0.587740±0.019079 | 4.073058±0.362046 | 0.898288±0.000908 | 0.007897±0.006958 | 0.716408±0.003182 |
| **species level** | 0.992862±0.000724 | 0.537202±0.016816 | 5.018684±0.317762 | 0.924058±0.000713 | 0.005337±0.002702 | 0.607287±0.002642 |

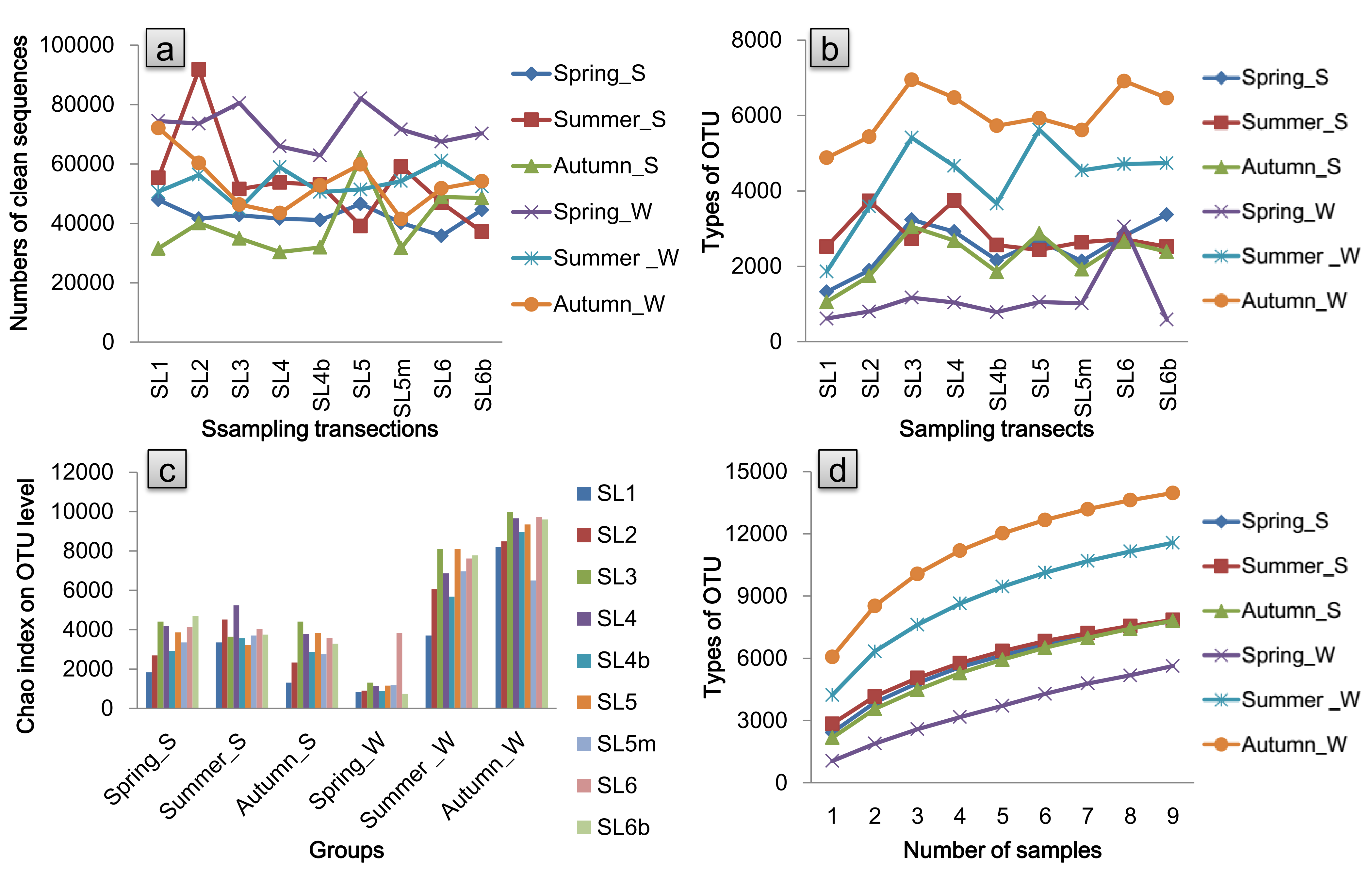
Bacteria (detected by the 16S rRNA gene), fungi (detected by the ITS gene), and metazoans (detected by the CO1 gene) indicate the groups of bacteria (detected by the 16S rRNA gene), fungi (detected by the ITS gene), and metazoans (detected by the CO1 gene), respectively.

## Figures (with captions)



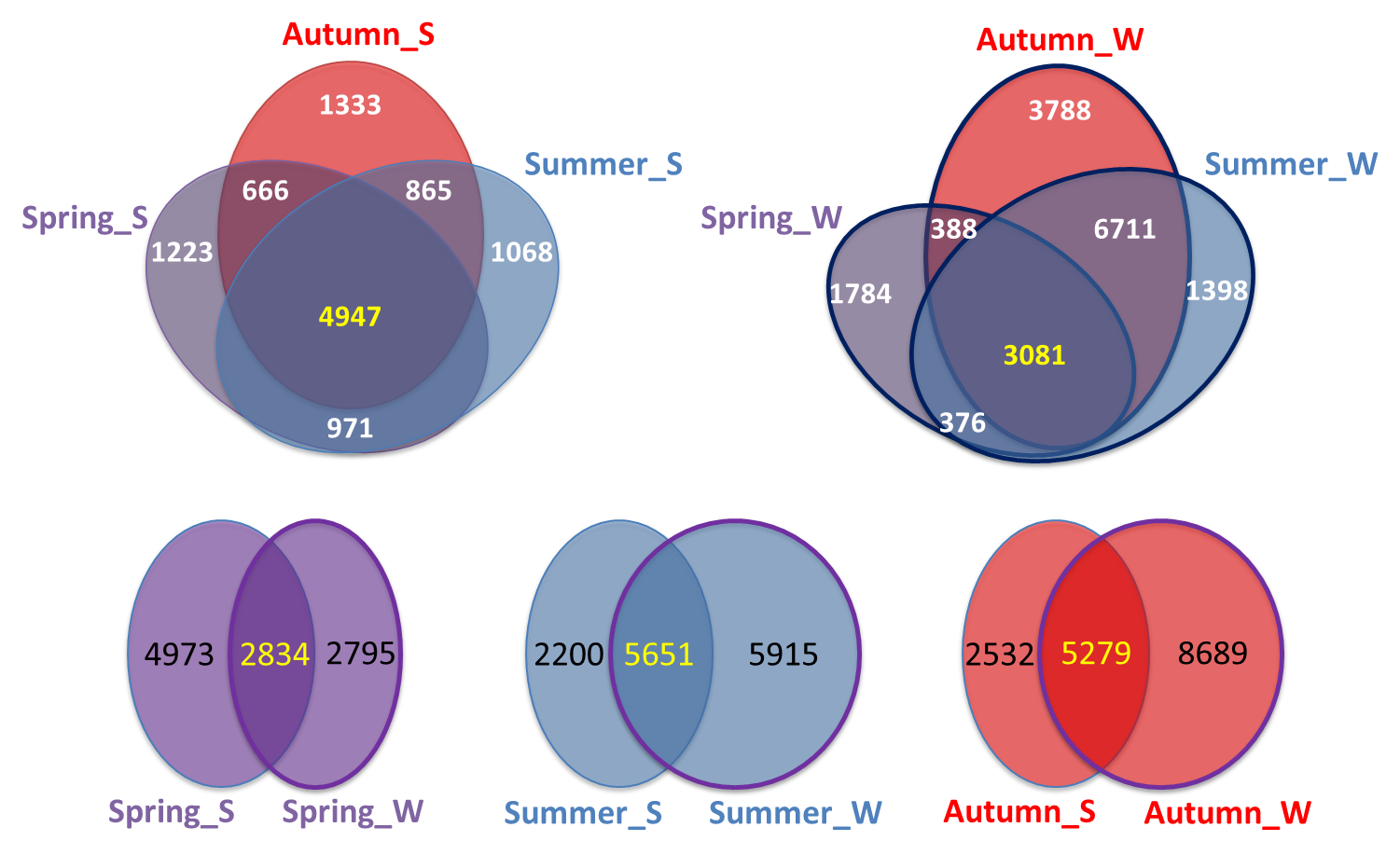
**Fig. 1 Sampling transects**

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.



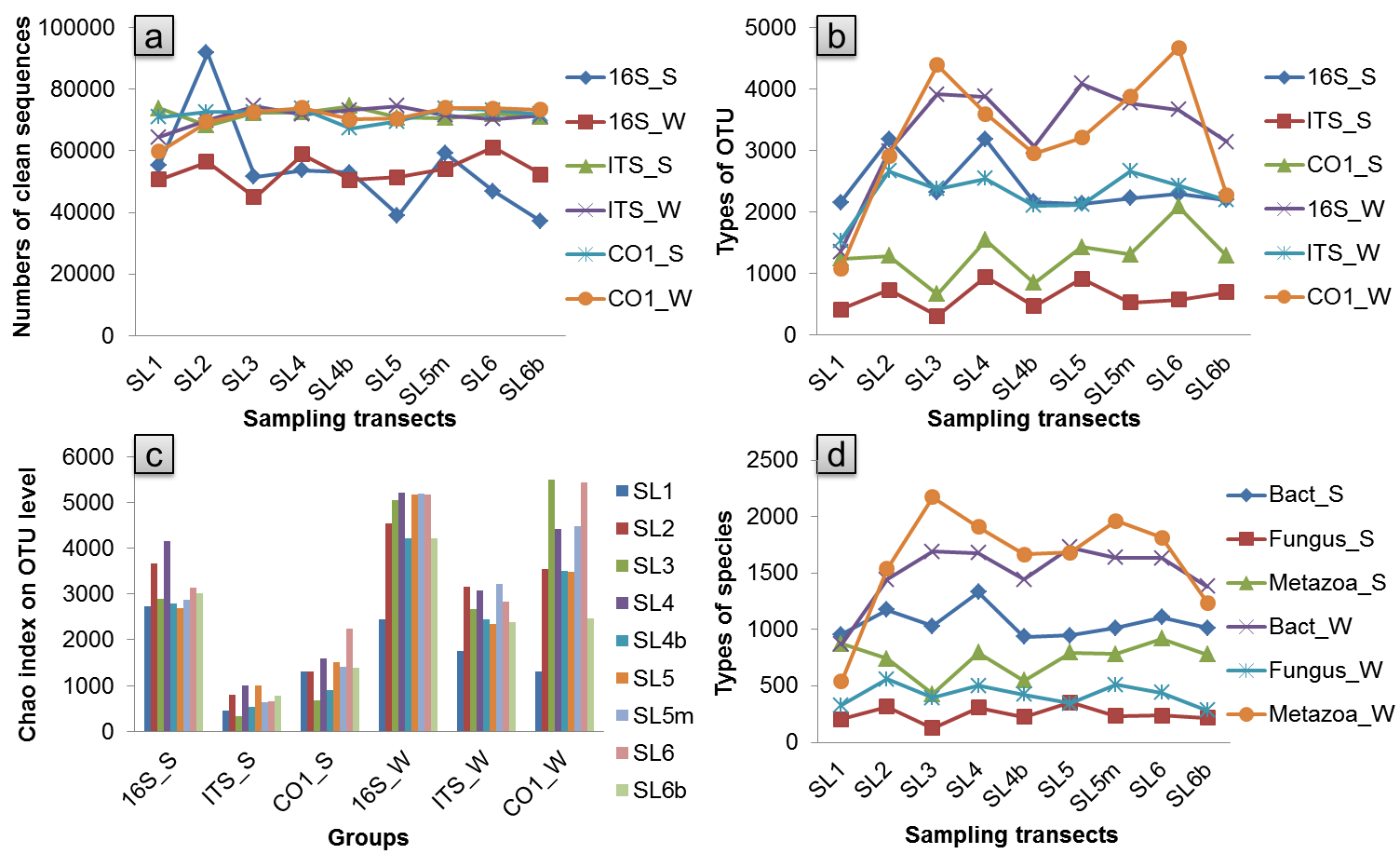
**Fig. 2 Biological information features of the samples: numbers of clean sequences in each sample (a), types of OTUs in each sample (b), community richness of each sample at the OTU level (c) and species accumulation curves at the OTU level (d)**

Spring\_S denotes the soil eDNA samples that were sampled during April 2019; Spring\_W denotes the water eDNA samples that were sampled during April 2019; Summer\_S denotes the soil eDNA samples that were sampled during June 2019; Summer \_W denotes the water eDNA samples that were sampled during June 2019; Autumn\_S denotes the soil eDNA samples that were sampled during September 2019; Autumn\_W denotes the water eDNA samples that were sampled during September 2019.



**Fig. 3 OTU types in riparian soil samples and riverine water samples shared by the three groups**

Spring\_S denotes the soil eDNA samples that were sampled during April 2019; Spring\_W denotes the water eDNA samples that were sampled during April 2019; Summer\_S denotes the soil eDNA samples that were sampled during June 2019; Summer \_W denotes the water eDNA samples that were sampled during June 2019; Autumn\_S denotes the soil eDNA samples that were sampled during September 2019; Autumn\_W denotes the water eDNA samples that were sampled during September 2019.



**Fig. 4 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 types of species in each sample (d)**

16S\_S denotes the soil eDNA samples that were sequenced using the bacterial 16S rRNA gene; ITS\_S denotes the soil eDNA samples that were sequenced using the fungal ITS gene; CO1\_W denotes the water eDNA samples that were sequenced using the eukaryotic mitochondrial CO1 gene. Bac\_S denotes the bacterial group detected in the soil eDNA samples; Fungus\_S denotes the fungal group detected in the soil eDNA samples; and Metazoa\_W denotes the metazoan group detected in the water eDNA samples.

1. http://www.gangcha.gov.cn/html/2125/item.html [↑](#footnote-ref-2)