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CLIMATE ADAPTATION OF INLAND WATERS

A bacteria-based index of biotic integrity assesses aquatic ecosystems effectively in rewetted long-term dry river channel after water replenishment

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Abstract Climate-induced droughts exert a significant influence on the connectivity of river systems. It is estimated that about 25% of the world's rivers ran dry before reaching the ocean due to climate change and human activities. Ecological water replenishment is an effective measure for restoring aquatic ecosystems damaged by drought. It is urgently needed to quantitatively assess the aquatic ecosystems in rewetted dry river channels after water replenishment. This study investigated the variations in phytoplankton, zooplankton, benthic macroinvertebrates, and benthic bacterial communities in the rewetted dry river channel of Yongding River after water replenishment. In comparison with the water column communities, the benthic macroinvertebrates were identified as limiting factors for ecological restoration in rewetted dry river channels. In the absence of a certain recovery time for benthic macroinvertebrates, the benthic bacterial-based index of biological integrity, especially calculated based on their intrinsic properties, can properly assess aquatic ecosystems in rewetted dry river channels.

Keywords Benthic bacterial communities · Benthic macroinvertebrates · Ecological assessment · Plankton · Rewetting dry river · Water replenishment

INTRODUCTION

In the last few decades, climate change and human activities have reduced natural runoff. Many rivers have dried up or become non-perennial. In freshwater ecosystems, intermittent rivers account for more than half of the global river network length (Messager et al. 2021). Approximately 25% of the world's rivers run dry before reaching the ocean due to water withdrawals, diversions, and climate change (Albert et al. 2021). The summer monsoon and an anomalous anticyclone over Mongolia have led to increasing water vapor divergence and decreasing precipitation in North China from 1950 to 2019 (Chen et al. 2023). 23 of the 27 major rivers in the North China Plain have been partly dried and the length of dry river channels exceeding 3600 km, which accounts for 51% of the total length of these 23 rivers (Chen et al. 2021). River drying has caused numerous critical environmental issues, such as declines in groundwater levels, degradation of river morphology, wetland degradation, and the accumulation of pollutants. Moreover, the alternation of drought and rewetting creates unstable habitats, affecting the biodiversity and functionality of aquatic ecosystems (Battin et al. 2016). Water replenishment is an effective measure for the recovery of degraded river ecosystems due to water shortage (Datry et al. 2014). At present, many water diversion projects are operating in China, including the South to North Water Diversion Project, the Diversion of Luan River to Tianjin, the Diversion of Yangtze River to Huai River, and the Diversion of Yellow River to Hebei Province. In addition to guaranteeing anthropogenic water demand, supplementing natural runoff in arid areas improves the aquatic environment of the watershed. Approximately 1448 km of dry river channels in North China have been rewetted through the promotion of water replenishment. The connectivity of the entire river systems of the Yongding River, the Chaobai River, the Hutuo River, and the Daqing River has been temporarily restored (Yang 2022). To date, studies on the ecological benefits of

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water replenishment have focused on lakes or perennial rivers (Bao et al. 2017; Zhou et al. 2018; Chunyu et al. 2019) and have not concentrated on intermittent or dry river ecosystems. The processes, mechanisms, and influencing factors of rewetted river ecosystems after water replenishment remain unclear (Crabot et al. 2021).

Many water replenishment projects operate on specific dates per year, with durations ranging from a few days to several months (Chen and Yu 2021). However, water replenishment can hardly ensure that the rewetted dry river channels become perennial due to the limitations of water availability. The cessation of river flow reduces biodiversity (Miliša et al. 2022). The variability between wet and dry conditions and the fragmentation of river habitats lead to the succession of local communities in river ecosystems. Species with poor drought adaptability will gradually disappear (Phillipsen and Lytle 2013), while those with greater migration capabilities or stronger reproductive abilities are less affected (Bohonak and Jenkins 2003). Another issue with water replenishment is that the replenishment period may not coincide with the natural wet season, which can disrupt local biological rhythms and ultimately impact aquatic ecological health. Some aquatic communities, such as fish and plankton, have difficulty to survive or complete their life cycle during the interval between river replenishment. Nonetheless, some aquatic macroinvertebrates have the ability to resist the impacts of temporary river drying (Lake 2000). To date, research on intermittent rivers has mainly focused on the impacts on invertebrates (Arias-Real et al. 2022; Fournier et al. 2023; Chanut et al. 2022; Di Sabatino et al. 2023; Reich et al. 2023) and benthic microbial communities (Mora Gómez et al. 2018; Li et al. 2023). Only few studies have concentrated on the assessment of ecological variations in rewetted dry river channels.

The index of biological integrity (IBI) is a valuable tool for aquatic ecological assessments. The IBI is calculated based on variations in aquatic communities rather than environmental factors. Fish (Mercado-Silva et al. 2002), macroinvertebrates (Klemm et al. 2003), and plankton (Wu et al. 2012) are common indicators used in the calculation of the IBI. However, a single indicator may hardly reflect the recovery of local aquatic ecosystems in the ecological assessments of rewetted dry river channels due to the varying recovery rates among different communities. In comparison with common indicators, benthic microbial community is characterized by strong adaptability and high sensitivity to environmental variations. These characteristics make benthic microbial communities potential indicators in the development of a new IBI. Moreover, in biological integrity assessments, each assessment must define its specific reference area and select and filter candidate metrics manually due to the spatiotemporal specificity of ecosystems. The definition of reference areas usually considers local water quality or anthropogenic intensity but assessments for the rewetting process are lacking. In the calculation of the IBI, the selection and filtration of candidate metrics may also reduce the accuracy and universality in the application to rewetted dry river channels due to the lack of unified standards. A biological integrity index developed based on the intrinsic properties of benthic microbial communities has the potential to reduce the need for artificial interventions. The intrinsic properties of benthic bacterial communities, such as composition, diversity, and function, can be obtained directly from high-throughput sequencing data and are sensitive to environmental variations. This advantage allows for the comparison of biological integrity between different aquatic ecosystems, providing greater universality in ecological assessments of rewetted dry river channels.

Water replenishment projects are generally designed based on water availability or the calculation of ecological flow requirements (Jiang et al. 2019). In practical applications, accurate and convenient methods for ecological assessment after water replenishment are required, especially in the rewetted long-term dry river channels. This study aims to understand the ecological variations and attampt to identify aquatic communities that can effectively assess aquatic ecosystems in rewetted dry river channels after water replenishment. Because of the indeterminacy of the period and length of the rewetted dry river channels, a sampling campaign was conducted within one week after the river channel had been fully rewetted. This sampling campaign was designed based on different periods of submersion in different river channel sections to obtain the variations in different aquatic communities. Common aquatic communities, such as phytoplankton, zooplankton, benthic macroinvertebrates, and benthic bacterial communities were investigated. Because benthic bacterial communities are directly linked to biogeochemical processes and are characterized by high diversity and high sensitivity to environmental variations (Yergeau et al. 2007), We hypothesized that benthic bacterial communities may be better indicators than other communities in the assessment of ecological variations in rewetted dry river channels. The aquatic communities in rewetted dry river channels were assessed using the IBI. Furthermore, a new IBI method was developed based on the intrinsic properties of benthic bacterial communites to extend the accessibility and the universality of the IBI in the application to rewetted dry river channels. This study provides a reference for aquatic ecological restoration under water replenishment against long-term dry climate in arid area. Additionally, this study offers an applicable ecological assessment method for water management and governance, contributing to the development of an ecosystem monitoring network in the Beijing-Tianjin-Hebei urban agglomeration (northern China) (Kattel et al. 2020).

MATERIALS AND METHODS

Study area and sampling campaign

The Yongding River is one of the major river systems in northern China with a total length of 747 km and a drainage area of 47 016 km^2. It originates from Inner Mongolia and flows into the Bohai Sea. In the upper reaches of the Yongding River, there are two tributaries: the Sanggan River and Yanghe River. These two rivers converge and flow into the Guanting Reservoir (GTSK). Subsequently, the Yongding River traverses the Guanting Gorge and enters the North China Plain at Sanjiadian (SJD). The length of mountainous river section below GTSK is 108.5 km, and the plain river section is approximately 200 km long (Fig. 1). The annual average temperature of the Yongding River system is 11.6 °C. The annual average precipitation is 556 mm and mainly concentrated from July to August.

The Yongding River has been largely dry for decades due to excessive water consumption and a reduction in natural inflow. The plain river section has been completely dry since 1996 (Huang et al. 2023). In spring 2019, a trial water replenishment was conducted in the Yongding River. Further water replenishment was conducted in spring 2020 (164 million m^3), autumn 2020 (67 million m^3), spring 2021 (112 million m^3), and autumn 2021 (104 million m^3). The water replenishment between spring 2020 and spring 2021 rewetted approximately 70 km of dry river channels but did not recover the entire connectivity of the Yongding River. In autumn 2021, the Yongding River was replenished with water from the Guanting Reservoir and the Qingshui River (QSH). The water source of the Qingshui River is from the South to North Water Diversion. On September 27th, 2021, the replenished water reached the Bohai Sea for the first time since 1996, indicating that the entire dry river channel had been rewetted.

The sampling campaign was conducted from October 4th to 6th, 2021. Water and sediment were sampled along the Yongding River from the estuary (LCZ_down) to the Guanting Reservoir (GTSK), including one tributaries (the Qingshui River). SJD_down marks the boundary between the mountainous river section and the plain river section. The altitudes of the sampling locations in the mountainous river section range from 456.2 to 86.7 m (from GTSK to SJD_up), and the altitudes of the sampling locations in the plain river section range from 53.4 to 10.5 m. The water depth at the sampling locations is 2–3 m, except at GTSK_down and QSH, where the depth is less than 1 m. TTH, CBH, and QJD_down are newly rewetted dry river channels (Fig. 1).

Temperature, pH, conductivity (Cond.), dissolved oxygen (DO), and concentrations of chlorophyll a and phycocyanin were measured directly using an EXO2 Multiparameter Sonde (YSI, Inc., USA). The detectors of temperature, pH, conductivity, chlorophyll a, and phycocyanin were calibrated before the sampling campaign, and the DO detector was calibrated at each sampling location. Water samples were collected 0.5 m below the river surface at each sampling site using a water sampler. The collected water samples were stored in 1-L polyethylene bottles, and the pH was adjusted to 2 with concentrated sulfuric acid for subsequent chemical parameter measurements. 20 L of collected water were filtered through a plankton net to concentrate plankton, and then 1.5 mL of Lugol's solution was added for fixation. Because of the low accessibility of sediment samples in the Guanting Gorge, sediment sampling focused on the plain river section. Sediment samples were collected using a grab sampler (20 × 15 cm). Benthic macroinvertebrates were sorted after sample collection. Most benthic macroinvertebrates can be identified using a dissecting microscope. For the identification of Chironomid larvae, the heads were separated and identified together with the bodies under an optical microscope. Approximately 500 g of sediment samples for physical and chemical parameter measurements and high-throughput sequencing were stored in sterilized plastic bags, transported to the laboratory in an icebox, and stored at − 20 °C.

Determination of physical and chemical parameters

The total nitrogen (TN), nitrate (NO_3^-), ammonium (NH_4^+), total phosphorus (TP), and phosphate (PO_4^{3-}) in the collected water samples were analyzed using a continuous segmented flow analyzer (SEAL Analytical, Inc., Germany). The sediment samples were freeze-dried and then filtered to remove stones and plant debris. 5 mL of HCl (1 mol L^{-1}) was added to 2 g of the filtered sediment samples. The mixture was heated to remove inorganic carbon for the measurement of total organic carbon (TOC), total nitrogen (TN), and total sulfur (TS) using an elemental analyzer (VARIO EL, Germany). The other part of the sediment samples was digested with a mixture of nitric acid, hydrofluoric acid, and hydrochloric acid for the measurement of heavy metals (As, Cd, Co, Cr, Cu, Ni, Pb, and Zn) using an inductively coupled plasma mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA).

Identification of phytoplankton and zooplankton

Fixed plankton were left to stand in brown glass bottle at 22 °C for 48 h. Then, the supernatant was discarded, and the plankton were concentrated to a volume of 30 mL. A

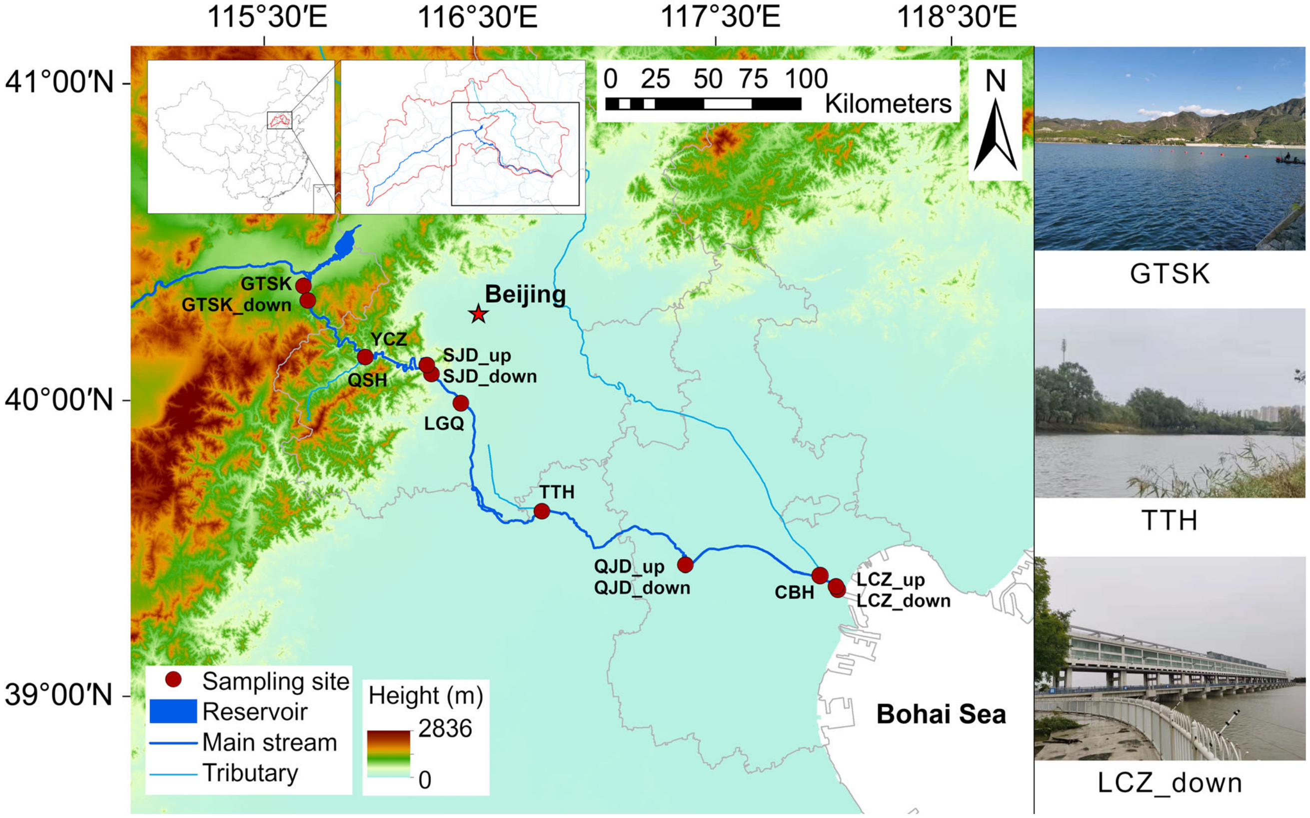

Fig. 1 The topographic map of the Yongding River extends from the Guanting Reservoir to its estuary. The sampling sites are named according to their respective locations: GTSK for the Guanting Reservoir; GTSK_down for the area downstream of the Guanting Reservoir's outlet; QSH for the Qingshui River; YCZ for Yanchizhen; SJD_up for the area upstream of the Sanjiadian Dam; SJD_down for the area downstream of the Sanjiadian Dam; LGQ for Lugouqiao; TTH for the area near the Tiantang River; QJD_up for the area upstream of the Qujiadian Sluice; QJD_down for downstream of Qujiadian sluice; CBH for the area near the Chaobaixin River; LCZ_up for the area upstream of the tidal gate; and LCZ_down for the area downstream of the tidal gate

20 mm × 20 mm counting box and a 10 × 20 microscope were used to identify and count phytoplankton and zooplankton based on *Flora of freshwater algae in China* and *Fauna Sinica*. After the identification of plankton and benthos, the Shannon–Wiener diversity index and the Margalef richness index were used to calculate species diversity.

DNA extraction, illumina MiSeq amplicon sequencing, and bioinformatics analysis

The genomic DNA was isolated from filters and sediment samples using the E.Z.N.A.$^{\circledR}$ Soil DNA Kit (Omega Bio-Tek, Norcross, GA, USA). Then, the extracted DNA was checked on a 1% agarose gel. The concentration and purity of the extracted DNA were determined using a NanoDrop 2000 UV spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The primer pair 338F and 806R were used to amplify the V3-V4 hypervariable region of the bacterial 16S rRNA gene from the extracted genomic DNA. Technical triplicates were set for PCR. The 20 μL PCR solution consisted of 4 μL 5 × TransStart FastPfu buffer, 2 μL 2.5 mM dNTPs, 0.8 μL forward primer (5 μM), 0.8 μL reverse primer (5 μM), 0.4 μL TransStart FastPfu DNA Polymerase, and 10 ng template DNA. PCRs were performed in triplicate on an ABI GeneAmp® 9700 PCR thermocycler (ABI, Carlsbad, CA, USA) according to the following protocol: 3 min at 95 °C; 27 cycles of 30 s at 95 °C; 30 s at 55 °C; and 45 s at 72 °C; and a final extension step of 10 min at 72 °C. The PCR product was checked on a 2% agarose gel and purified using an AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA), and quantified using a Quantus$^{\text{TM}}$ Fluorometer (Promega, Madison, Wisconsin, USA). The purified PCR products were pooled in equimolar amounts. Sequencing was performed by Majorbio Bio-Pharm Technology Co., Ltd. (Majorbio, Shanghai, China) on an Illumina MiSeq PE300 platform (Illumina, San Diego, USA).

The triplicate reads were merged for downstream analyses. Alpha diversity was calculated using the Shannon–Wiener index and the Chao1 index with the phyloseq package in R. Beta diversity was determined by nonmetric multidimensional scaling (NMDS) based on the calculation of the Bray–Curtis similarity index using PAST3. CANOCO 5 was used for multivariate statistics. The functions of bacterial communities, such as nitrogen- and sulfur-related metabolism, were predicted using PICRUSt2 in R against the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (Douglas et al. 2020). Sequencing data were submitted to the NCBI with BioProject ID: PRJNA850031.

Development of the IBIs

The calculation of the IBI followed the general protocols established by Wu et al. (2012) with modifications in the selection of candidate metrics. The 25th percentiles of water quality parameters (DO, TN, NH^{4+},and TP) were used as thresholds for distinguishing reference sites from impaired sites. According to the results in "Determination of physical and chemical parameters" section, the reference sites had $DO > 10.14\ mg\ L^{-1}$, $TN < 0.98\ mg\ L^{-1}$, $NH^{4+} < 0.14\ mg\ L^{-1}$, $TP < 0.09\ mg\ L^{-1}$. Therefore, sites GTSK, GTSK_down, YCZ, and SJD_up were defined as reference sites. The IBIs based on phytoplankton (P-IBI), zooplankton (Z-IBI), and benthic macroinvertebrates (B-IBI) were developed based on candidate metrics that included four categories: composition metrics, density metrics, biomass metrics, and diversity metrics (Tables S1, S2, and S3). The IBI based on benthic bacterial communities (Ba-IBI) was developed based on candidate metrics that included three categories: composition metrics, function metrics, and diversity metrics (Table S4). Metrics with significant discrimination power were filtered using Mann–Whitney tests and box-plot tests. The discrimination power of the metrics was demonstrated by the degrees of interquartile (IQ) overlap in box-plot tests. Spearman's correlation analysis was performed to identify redundant metrics. Metrics with a significant correlation ($r > 0.75$, $p < 0.01$) were considered redundant, and one of the redundant metrics was selected as a representative based on its applicability. Mann–Whitney U tests were conducted using PAST3.

The "5th and 95th percentile" scaling system was applied to calculate the score of each metric.

For metrics that decreased with restoration:

$$S_i = \frac{Q_{\max} - Q_i}{Q_{\max} - Q_5}$$

where S_i is the index scoring of metric i, $Q_{\max}$ is the maximum value of the biological index, Q_i is the biological index i, Q_5 is the 5th percentile of the biological index.

For metrics that increased with restoration:

$$S_i = \frac{Q_i}{Q_{95}}$$

where S_i is the index scoring of metric i, Q_i is the biological index i, Q_{95} is the 95th percentile of the biological index.

The final IBI score of each site was the average of scores of all metrics.

To reduce artificial interventions, a new IBI based on benthic bacterial communities (nBa-IBI) was developed to account for the variations in the intrinsic properties of these communities. Compared to the Ba-IBI, the new method has two main differences:

1. The sampling sites were categorized as perennial river sites and rewetted river sites, rather than reference sites and impaired sites. This distinction is due to the fact that the intrinsic properties of benthic bacterial communities, such as diversity, composition, and function, vary between perennial and rewetted river channels. The nBa-IBI was developed based on candidate metrics that included three categories: diversity metrics, composition metrics, and function metrics, as shown in Table S4.
2. The score of each metric was calculate using the "5th and 95th percentile" scaling system. However, no test was conducted to screen for metrics with high discrimination power.

RESULTS AND DISCUSSION

Improvement of the aquatic environment

The physical and chemical parameters of both water and sediment samples are presented in supplementary information (Figures S1 and S2). Generally, aquatic environmental factors including conductivity, TN, and TP, were influenced by upstream inflows from two distinct water sources: GTSK and QSH. Additionally, significant differences ($p < 0.05$ in ANOVA tests) were observed in NO_3^-, NH_4^+, DO, pH, chlorophyll a levels, and phycocyanin levels between the mountainous and plain river sections (Table S6). The significant correlation ($p < 0.05$) between phytoplankton indicators (chlorophyll a and phycocyanin) and chemical indicators (e.g., NH^{4+} and TP) indicated that phytoplankton communities in the rewetted dry river channels were influenced by local environmental conditions (Table S5). Meanwhile, the contents of TN, TOC, TS, and heavy metals in sediment samples from SJD_up, SJD_down, LGQ, and QJD_down, which are close to areas with intensive human activity, were higher than those at other sampling sites (Figure S2). The principal water quality parameters assessed in this study (Figure S1), including TN, NO_3^-, NH_4^+, TP, and chlorophyll a, were lower than those reported in a previous study of the Beijing River section of the Yongding River before 2020 (Li et al. 2022). These results indicated that recent water replenishment and environmental improvement initiatives have effectively improved the aquatic environment of the Yongding River (Huang et al. 2023).

Ecological variations in rewetted dry river channels

The density and biomass of plankton and benthos are shown in Fig. 2. The diversities of plankton and benthic macroinvertebrates are displayed in supplementary information Figure S3. Four classes and 11 species of benthic macroinvertebrates were identified. The average density and average biomass of benthic macroinvertebrates were 230 ind. m^{-2} and 30.78 g m^{-2}, respectively. Benthic macroinvertebrates were identified only in the main stream between SJD_up and QJD_up. The highest density was observed at LGQ (860 ind. m^{-2}) and SJD_down had the highest biomass (77.41 g m^{-2}). Gastropoda at QJD_up had the lowest density (15 ind. m^{-2}) and biomass (2.46 g m^{-2}) compared to the other sampling sites at SJD_up, SJD_down, and LGQ. ANOVA results indicated that phytoplankton and zooplankton had significant regional characteristics between mountainous river sections and plain river sections ($p < 0.05$) but there was no significant difference between perennial river sections and rewetted dry river sections ($p > 0.05$; Table S7). For rewetted dry river channels in our study (sampling sites at TTH, QJD_down, and CBH), the plankton population recovered rapidly before the sampling campaign. In contrast, the benthic macroinvertebrate community did not recover at the same sites. During the restoration process, the benthic macroinvertebrate community recovered and migrated downstream at a rate of 250 m per day after rewetting (Fournier et al. 2023). However, Di Sabatino et al. (2023) indicated the benthic macroinvertebrate community did not recover to the normal state after 5 months of rewetting. The results indicated that the slow recovery of benthic macroinvertebrates is a limiting factor for the ecological recovery in rewetted dry river channels. Unfortunately, the full recovery of dry river aquatic ecosystems may require more time than the duration of water replenishment. The different recovery time of aquatic communities indicated that assessments based on water communities may overestimate the ecological recovery.

A total of 656 371 sequences were obtained after demultiplexing, merging, and excluding chimeric sequences. The read numbers ranged from 20 796 to 49 108, with an average of 31 256. The alpha diversity, indicated by the Shannon–Wiener index and the Chao1 index, is displayed in supplementary information Figure S4. The alpha diversity in the rewetted dry river channels was higher than that in the perennial river channels. The relative abundance of dominant genera is displayed as a percentage of total sequence reads. The benthic bacterial communities in newly rewetted river channels (TTH, CBH, and QJD_down) were different from those in perennial river channels (Fig. 2G). This difference was indicated by the beta diversity (Fig. 3A). Specifically, *Thiobacillus* and JG30-KF-CM45 had relatively high abundance in the perennial and newly rewetted river channels, respectively (Fig. 2G). *Thiobacillus* is a strictly chemoautotrophic sulfur-oxidizing bacterium under anaerobic conditions and is widely distributed in freshwater environments. The family JG30-KF-CM45, which is widely distributed in soil (Zhang et al. 2021; Shen et al. 2021), indicated that the transformation from soil bacterial communities to benthic bacterial communities have not yet been completed. Sulfur-related and nitrogen-related metabolisms were predicted by PICRUSt2 based on the sequencing results (Fig. 4). In general, nitrogen-related metabolisms were higher in newly rewetted river channels than that in perennial river channels. Assimilatory sulfate reduction and sulfate-sulfur assimilation were higher in newly rewetted river channels, while dissimilatory sulfate reduction and thiosulfate oxidation were higher in perennial river channels. Redundancy analysis (RDA) was used to determine community-shaping environmental factors (Fig. 3B). However, no measured physical and chemical parameter showed a significant influence ($p < 0.05$), indicating the impacts of rewetting on the benthic bacterial communities. Studies have shown that benthic microbial communities change their structures and functions after prolonged drying due to a shift from benthic communities to terrestrial communities, such as the increased relative abundance of Actinobacteria and Firmicutes (Schimel et al. 2007; Barnard et al. 2013; Aslam et al. 2016), which is consistent with the results in our study (Fig. 2G). In rewetted river channels, the terrestrial community began to revert to benthic communities. This ongoing transformation was indicated by the increased alpha diversity.

Assessment of aquatic communities in rewetted dry river channels

The P-IBIs, Ba-IBIs and nBa-IBIs are displayed in Fig. 5. In the calculation of the P-IBI, the Mann–Whitney tests, the box-plot tests, and the Spearman correlation tests indicated that the biomass of Bacillariophyta was the metric with significant discrimination (Table S1; Figure S5). However, the Mann–Whitney test indicated that the P-IBI scores had no significant discrimination ($p > 0.05$) between perennial river channels and rewetted dry river channels (Fig. 5A and B). The Mann–Whitney test indicated no metric with significant discrimination that

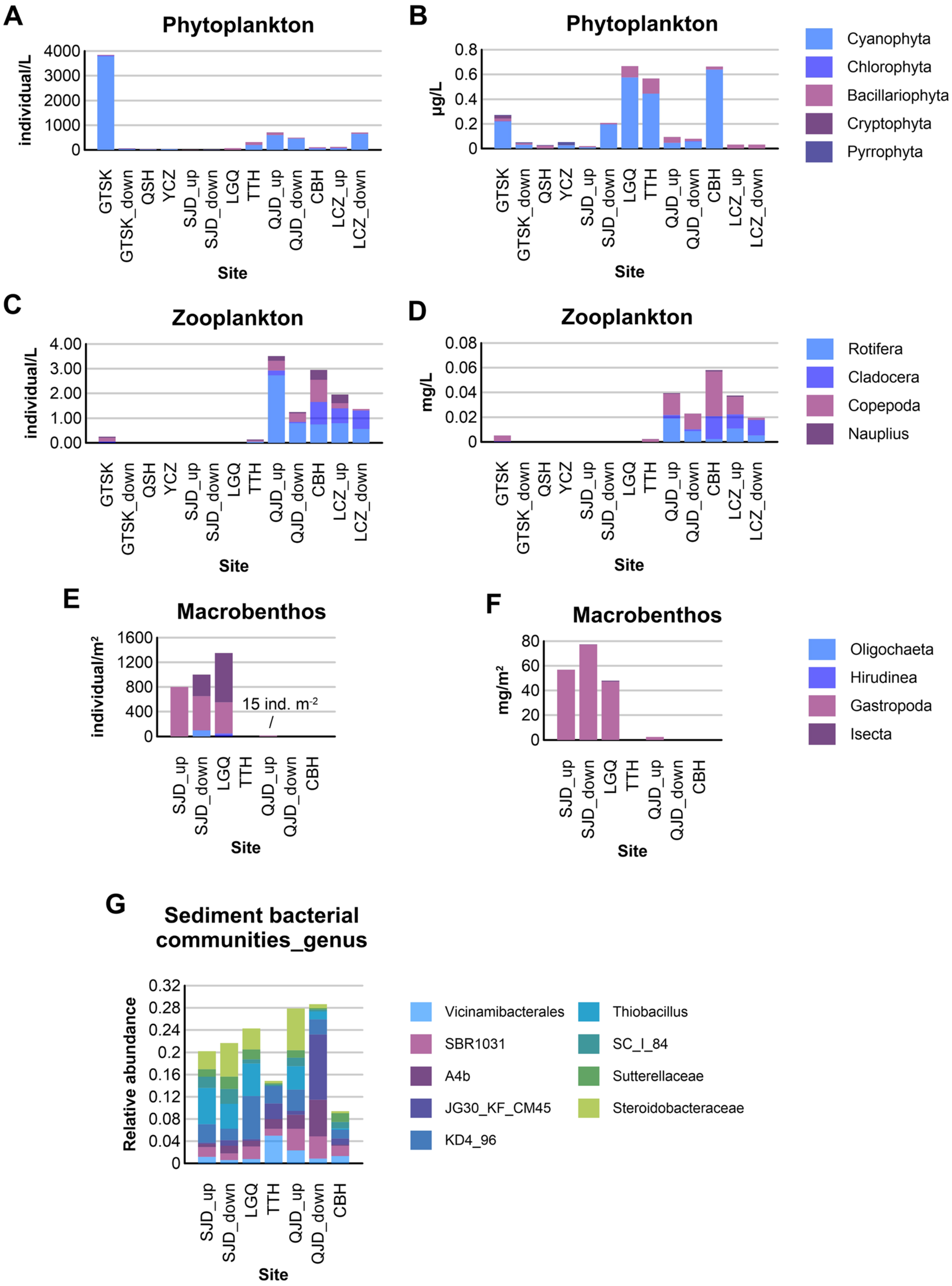

Fig. 2 Density (left) and biomass (right) of **A** and **B** phytoplankton, **C** and **D** zooplankton, and **E** and **F** macrobenthos. **G** Benthic bacterial community structures at the genus level, showing genera with relative abundance greater than 1.5%

could evaluate the ecosystems in the calculation of the Z-IBI and B-IBI (Tables S2 and S3). These results indicated that although phytoplankton and zooplankton had recovered rapidly before sampling, the P-IBI and Z-IBI cannot distinguish ecological differences between perennial river channels and dry river channels. Therefore, the IBI calculated based on phytoplankton and zooplankton can hardly determine the ecological recovery of rewetted

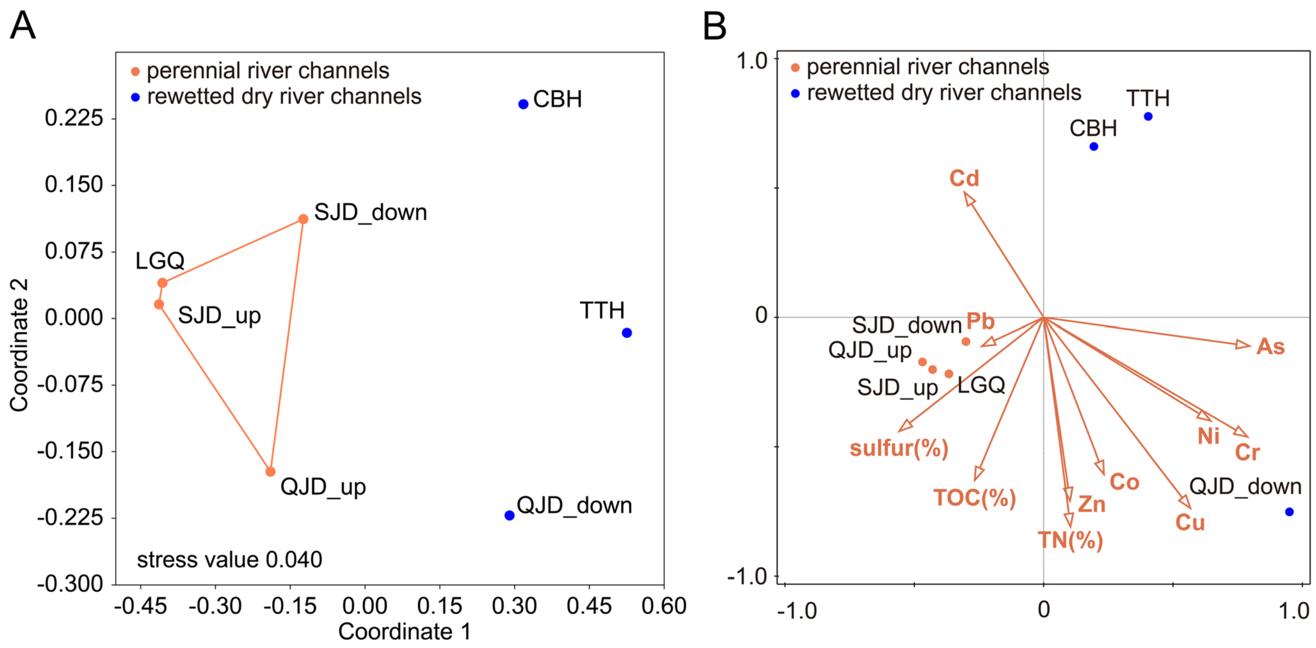

Fig. 3 **A** Beta diversity of benthic bacterial communities in the Yongding River obtained by NMDS plots. **B** RDA of the environmental influence on benthic bacterial communities

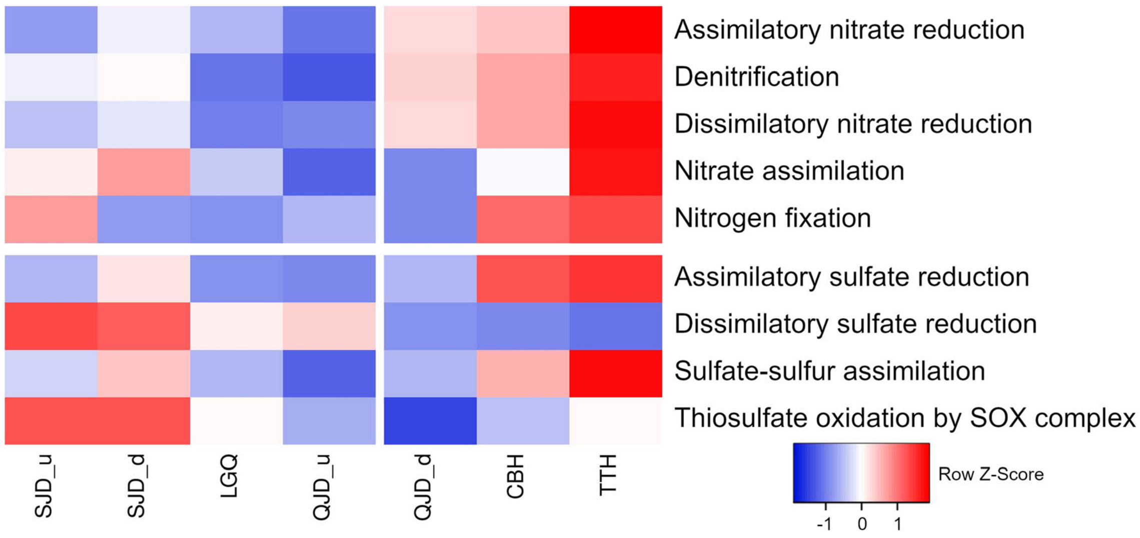

Fig. 4 Heatmap of nitrogen- and sulfur-cycle related metabolism predicted by PICRUSt2

dry river channels. It seems that an assessment based on benthic communities would be more appropriate. Utilizing benthic macroinvertebrates for long-term ecosystem assessment is a well-established method. However, for short-term assessments, especially when the duration of water availability is insufficient for the recovery of benthic macroinvertebrates, a data gap resulting from the slow recovery of benthic macroinvertebrates may hinder the applicability.

Due to the large community members, high adaptability and sensitivity to environmental variations, benthic bacterial communities have potential in ecological assessment. In the calculaion of the Ba-IBI, the Chao1 index in the diversity metrics, nitrogen-related metabolism in the function metrics, and sulfur-related groups (e.g., *Desulfobacterota* and *Sulfuritalea*) in the composition metrics had significant discrimination (Table S4 and Figure S5), indicating that nitrogen- and sulfur-related taxa were sensitive to the impacts of rewetting. Both the Ba-IBI scores and the nBa-IBI scores indicated low biological integrity in rewetted dry river channels (Fig. 5C and E). The Mann–Whitney test indicated that the Ba-IBI scores had

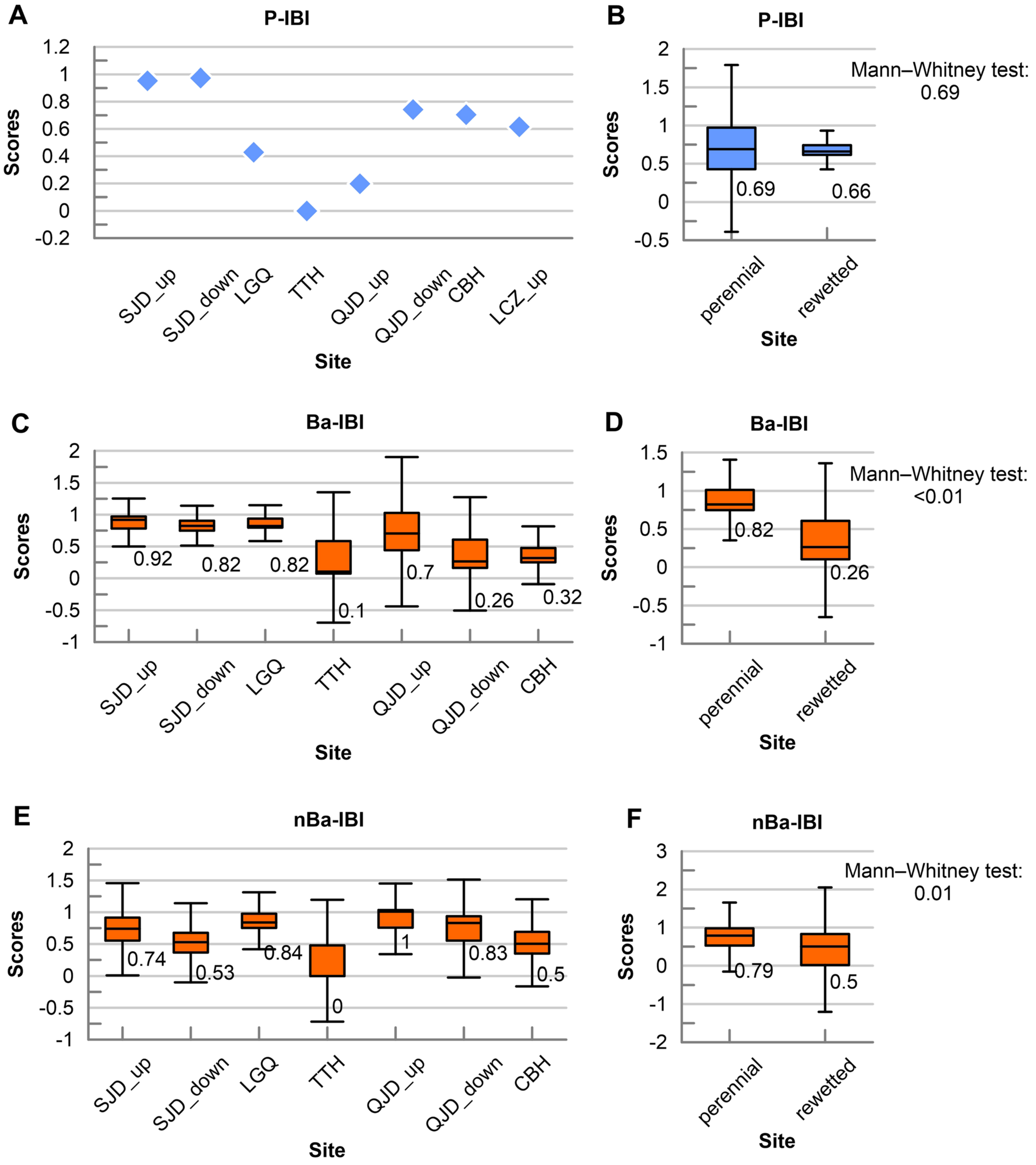

Fig. 5 The IBIs of **A** phytoplankton, **C** benthic bacterial communities, and **E** benthic bacterial communities with reduced artificial interventions, and the comparison in IBI scores between those in plant perennial river channels and in rewetted dry river channels, respectively (**B**, **D**, and **F**). Median numbers were indicated in each box plot

significant discrimination between perennial river channels and rewetted dry river channels (Fig. 5D), as well as the nBa-IBI (Fibure 5F). The p-value of the Mann–Whitney test for the nBa-IBI was higher than that for the Ba-IBI score because no filtration methods for higher discrimination power were applied in the calculation of the nBa-IBI. Compared to other indicators, the development of the Ba-IBI and nBa-IBI determined that benthic bacterial communities could be applied to environmental assessment according to the significant discrimination between perennial river channels and rewetted dry river channels (Fig. 5D and E). Both the Ba-IBI scores and nBa-IBI scores were significantly lower in the rewetted dry river channels than those in the perennial river channels, indicating relatively incomplete ecosystems in the rewetted dry river channels. These results were consistent with the reality of the slow-restored benthic macroinvertebrates.

Bacterial-based IBIs were applied in the Taihu Lake and the Three Gorges Reservoir due to the local extinction of traditional indicator macroorganisms (Niu et al. 2018) and the special environmental conditions caused by reservoir operations (Li et al. 2018), indicating their potential application in different aquatic ecosystems. However, the metrics in the IBI calculation differed among these studies. Due to artificial interventions, such as the definition of reference areas and the selection of candidate metrics, the ecological assessments by the IBI often exhibited regional specificity (Lunde and Resh 2012), which limited the application across different aquatic ecosystems. In our study, two statistical tests were applied in the calculation of the P-IBI, Z-IBI, and Ba-IBI to filter a large number of candidate metrics. These two tests made the IBIs more discriminating but it is almost impossible to obtain the same indicators across different studies. Therefore, we established a new IBI based on the intrinsic properties of benthic bacterial communities to reduce artificial interventions. The nBa-IBI had a similar trend to the Ba-IBI, demonstrating the effectiveness of the new IBI method in ecological assessment in the rewetted dry river channels (Fig. 5C and E). However, bacterial communities are highly sensitive to antibiotics, which means that human activities may considerably limit the application of the Ba-IBI. In contrast, microeukaryotes, such as fungi, are less affected by antibiotics but have similar advantages to bacteria, making microeukaryotes promising indicators for ecological assessment.

CONCLUSION

This study investigated the aquatic ecosystems in a newly rewetted, long-term dried river channel of the Yongding River after water replenishment. The phytoplankton and zooplankton were mainly influenced by local environmental factors rather than the influence of the rewetting process. Benthic macroinvertebrates and benthic bacterial communities showed significant differences between the perennial and newly rewetted river channels. Benthic macroinvertebrates recovered slowly compared to phytoplankton and zooplankton, indicating that benthic macroinvertebrates are the limiting factors for ecological restoration in rewetted dry river channels. The diversity, compositon, and function of benthic bacterial communities were affected by the rewetting process, with variations observed from soil to benthic communities. Before the uncertain recovery of benthic macroinvertebrates, benthic bacterial communities serve as better indicators in rewetted dry river channels after water replenishment. The benthic bacterial-based index of biological integrity, especially when calculated based on the intrinsic properties of benthic bacterial communities, can effectively assess aquatic ecosystems.

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