Original Research

Microeukaryotic plankton community dynamics under ecological water replenishment: Insights from eDNA metabarcoding

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ABSTRACT

Ecological water replenishment (EWR) is an important strategy for river restoration globally, but timely evaluation of its ecological effects at a large spatiotemporal scale to further adjust the EWR schemes is of great challenge. Here, we examine the impact of EWR on microeukaryotic plankton communities in three distinct river ecosystems through environmental DNA (eDNA) metabarcoding. The three ecosystems include a long-term cut-off river, a short-term connected river after EWR, and long-term connected rivers. We analyzed community stability by investigating species composition, stochastic and deterministic dynamics interplay, and ecological network robustness. We found that EWR markedly reduced the diversity and complexity of microeukaryotic plankton, altered their community dynamics, and lessened the variation within the community. Moreover, EWR disrupted the deterministic patterns of community organization, favoring dispersal constraints, and aligning with trends observed in naturally connected rivers. The shift from an isolated to a temporarily connected river appeared to transition community structuring mechanisms from deterministic to stochastic dominance, whereas, in permanently connected rivers, both forces concurrently influenced community assembly. The ecological network in temporarily connected rivers post-EWR demonstrated significantly greater stability and intricacy compared to other river systems. This shift markedly bolstered the resilience of the ecological network. The eDNA metabarcoding insights offer a novel understanding of ecosystem resilience under EWR interventions, which could be critical in assessing the effects of river restoration projects throughout their life cycle.

1. Introduction

Ecological water replenishment (EWR) is an approach focused on restoring the ecological balance of aquatic ecosystems, and it has gained significant attention in recent years. EWR entails artificially replenishing water bodies to enhance their ecological conditions and preserve their diverse biota. The fundamental objective of EWR is to restore, maintain, and enhance the ecological integrity of water-dependent ecosystems. This approach is pivotal in addressing water scarcity, habitat degradation, and loss of biodiversity [1,2]. Given the aforementioned, EWR has surfaced as a critical scheme to bolster ecosystem restoration [3,4]. A variety of EWR projects, including the EWR of Yongding River, the South-to-North Water Diversion Project, the Tarim River ecological water replenishment restoration project, the Kissimmee River restoration project of natural hydrological conditions, and the Transaqua project to refill Lake Chad, have been executed in Asia, North America, and Africa [5,6].

Although research on the interrelationship of "flow-biota-ecosystem processes" is still nascent, it constitutes the current research focus for river ecological restoration [7–13], primarily due to the significant interaction between water flow, biota, and ecosystem processes and its substantial influence on river health and biodiversity. For example, varied flow regimes may influence the spatial distribution of species, biodiversity, and trophic dynamics within the river ecosystem [14,15]. Variations in flow have the potential to affect habitat availability, subsequently influencing the composition and distribution of aquatic communities [14]. Numerous studies aim to unravel the mechanisms behind how flow regimes affect biota and ecosystem processes [11,16,17]. Extensive research has demonstrated that interconnected river networks nurture diverse and adaptable biota communities, enabling them to flourish in various flow regimes [17]. Fluctuations in water levels and connectivity with the main river markedly influence the productivity and composition of various biota groups within these ecosystems [18,19].

Microeukaryotic plankton in rivers often represent the pioneer species most affected by the process of EWR [20]. Furthermore, they are considered reliable biological indicators of environmental change due to their rapid and pronounced responses to anthropogenic disturbances [21]. It is well-established that EWR can significantly affect biological communities and ecological functions by influencing various elements of aquatic ecosystems, such as eco-habitats, hydrological conditions, and water quality [22,23]. During water replenishment, as connectivity is enhanced and nutrients are reallocated, microeukaryotic plankton is compelled to respond significantly to the changing conditions. However, there is still a lack of systematic assessment of their response variations and the community stability under such large-scale artificial intervention in aquatic ecosystems. Environmental DNA (eDNA) metabarcoding represents a potent approach that could overcome the limitations of traditional methods in identifying microeukaryotic plankton and characterizing its community composition [24]. Consequently, the eDNA approach can expedite our understanding of community stability variations in microeukaryotic plankton. This study primarily aims to evaluate the ecological effects and sustainability. Consequently, the eDNA approach can expedite our understanding of community stability variations in microeukaryotic plankton. This study primarily aims to evaluate the ecological effects and sustainability of large-scale artificial interventions on river ecosystems, specifically focusing on the stability and successional mechanisms of microeukaryotic plankton communities.

Post-ecological restoration, the river ecosystem is anticipated to achieve stability and self-sustenance, characterized by its biological components' resistance to stress or disturbance [25]. Ecosystem stability can be elucidated by analyzing the variability of α, β, and γ [26]. The average variation degree (AVD) index has been deemed suitable for assessing community stability [27]. However, this index still fails to explain the underlying causes of variations in community assembly. Influencing the biogeographic variation of communities, factors such as determinism (encompassing environmental filtering and biotic interactions) and stochasticity (including dispersal, diversification, and ecological drift) are significant [28,29]. One study illustrated that environmental selection predominated in shaping the biogeographic pattern of planktonic and benthic diatom communities in the Yangtze River [30], whereas another study indicated a predominant role of stochasticity in the distribution of microeukaryotic planktonic communities in the Ting River [31]. While a multitude of factors, such as changes in pH, nutrient sources, and ecosystem productivity, can impact the interplay of deterministic and stochastic processes, the equilibrium between these processes is critical in defining the stability of microeukaryotic plankton communities in natural aquatic ecosystems [32,33].

Ecological networks, showcasing biotic interactions, serve as a potent instrument to assess ecosystem stability by evaluating species network stability (e.g., network robustness, vulnerability, composition stability, and node and link constancy and persistence) [34,35]. Co-occurrence networks are frequently employed within microbial ecology to infer potential biotic interactions. However, these networks are influenced not solely by biotic interactions but also by factors like environmental filtering and dispersal limitation [29,34]. The recent development of the iDIRECT (Inference of Direct and Indirect Relationships with Effective Copula-based Transitivity) method enables the differentiation of direct interactions in co-occurrence networks [36]. Integrating iDIRECT with the established ecological network analysis (MENA) [37] facilitates the inference of authentic interactions within a microbial community, thus offering considerable benefits for network analysis. Environmental changes consequent to water replenishment significantly impacts the ecological network and its stability. Therefore, the construction and analysis of ecological networks are highly promising and practical in assessing the ecological impacts of water replenishment.

In this study, we focused on the ecological effects of EWR on the Yongding River in North China, a long-term cut-off river subsequently replenished by ecological water. The Beiyun River (BYR) adjacent to the Yongding River, as well as the Ting River (TR) in Southeast China, and the Jinsha (JSR) and Lancang rivers (LCR) in Southwest China, were selected as reference river ecosystems. These rivers, which flow continuously year-round, were selected for their representativeness and typical characteristics, offering insightful comparisons for understanding the ecological shifts induced by water replenishment efforts. We conducted measurements to assess the ecological effects of water replenishment. These measurements included changes in water quality, microeukaryotic plankton diversity, distribution patterns, community assembly mechanisms, and ecological network resistance. The main objectives of our study include (i) assessing the ecological stability under EWR conditions through the planktonic community and (ii) elucidating the successional mechanism of microeukaryotic planktonic community stability from a cut-off river to a long-term connected river.

2. Material and methods

2.1. Study design and sampling

Landscape features along the Yongding River course displayed obvious changes, with sampling sites YD1–YD12 located in a mountainous section (Fig. 1a and b; Fig. S1a and d), while YD13–YD26 were located in an urban and plain section (Fig. 1a and b; Fig. S1b, c, e, f). All sites of the referenced BYR belong to an urban and plain section (Fig. 1c; Fig. S1g and h). The Yongding River was connected by water replenishment from sites YD15 to YD19 in 2020, making these sites only sampled in AEWR (after EWR) (Fig. 1b). Conversely, all other sites were sampled for both BEWR (before EWR) (2019) and AEWR (2020) (Fig. 1a and b). The water discharge in April 2020 increased the water level (Fig. S2a) and enhanced river connectivity for Yongding River (Fig. S2b).

Water samples were collected before and after the EWR of the Yongding River, which was named BEWR and AEWR, respectively. The BEWR samples were collected during September 2019 (wet season) from 21 stations (denoted by YD1 to YD14, YD20 to YD26) along a northwest-southeast orientation from the Yongding River (Fig. 1a, 40°19′–39°13′ N, 115°36′–114°24′ E). During June 2020 (wet season, one month after EWR), the AEWR samples were collected from 26 stations (denoted by YD1 to YD26) along the
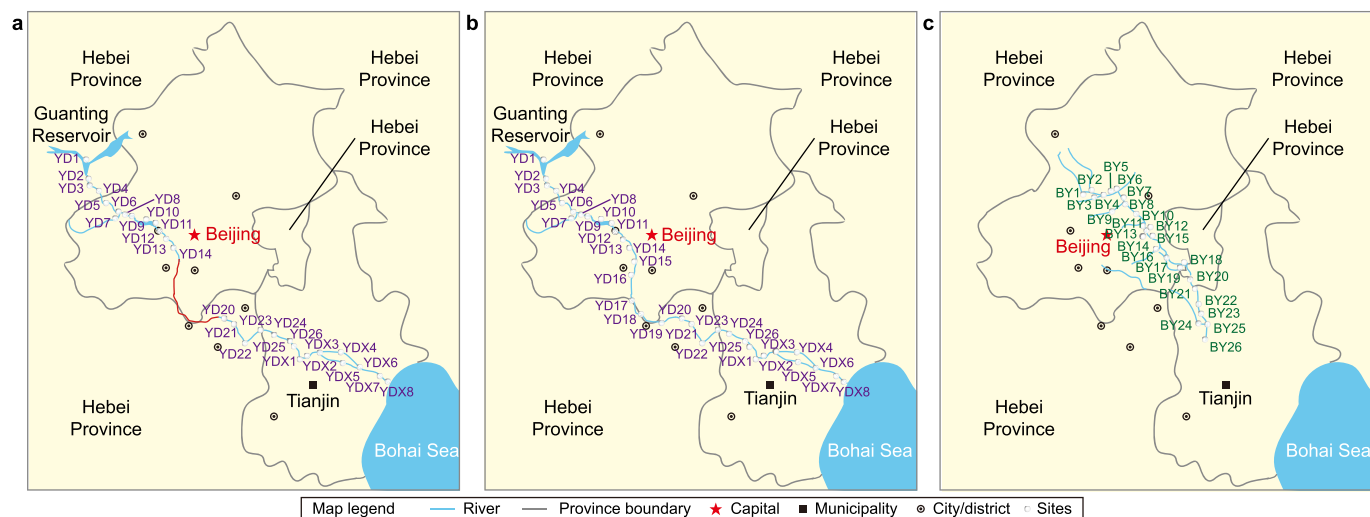
Fig. 1. Study area and sampling sites. **a,** The 21 sampling stations of BEWR (before ecological water replenishment) were distributed along the Yongding River, a long-term cut-off between YD14 and YD20 (the red line). The eight sampling stations of YDXR_September (Yongdingxin River in September). **b,** The 26 sampling stations of AEWR (after ecological water replenishment). The eight sampling stations of YDXR_June (Yongdingxin River in June). **c,** The 26 sampling stations of BYR (Beiyun River).

Yongding River (Fig. 1b). During September 2019 and June 2020, samples were collected from eight stations (denoted by YDX1 to YDX8) along the Yongdingxin River (Fig. 1a and b). During July 2020 (wet season), sampling was carried out at 26 stations (denoted by BY1 to BY26) along the BYR (Fig. 1c, $40°9'-38°60'$ N, $117°40'-116°12'$ E). Water samples were collected at a depth of 0.5 m below the water's surface and divided into two subsamples. One subsample (1 L) was used for the microeukaryotic plankton community analyses, and another subsample (2.5 L) was used for water chemistry analyses. Three replicates were collected for each station. In addition, this study utilized data from 30 stations along the Ting River (TR) ($116°19'-116°37'$ E, $24°59'-25°24'$ N) referred to Chen et al. [31], data from 35 stations along the Lancang River (LCR) referred to Chen et al. [38], and data from 16 stations along the Jinsha River (JSR) referred to Lu et al. [39] (Fig. S3).

2.2. Environmental conditions

From upstream to downstream, the Yongding River mainly flows through three types of terrain: a mountainous section (MS) (Fig. S1a and d), an urban and plain section (UPS) (including urban suburbs (Fig. S1b and e), and farmland (Fig. S1c and f)). The BYR mainly flows through urban and plain terrain (Fig. S1g and h). The average distance between sampling points was calculated based on the distance matrix of the distance between pairs of sampling sites. Water temperature (WT), pH, dissolved oxygen (DO), electric conductivity (EC), oxidation-reduction potential (ORP), and turbidity were measured in the field using a Manta + multi-parameter water quality analyzer (Eureka, CA, USA). The water quality parameters, including total nitrogen (TN), ammonia nitrogen (NH_4-N), total phosphorus (TP), biochemical oxygen demand (BOD_5), permanganate index (COD_{Mn}), and chemical oxygen demand (COD_{Cr}), were quantified using specific analytical methods. Total nitrogen (TN) was quantified using the alkaline potassium persulfate digestion UV spectrophotometric method, following established protocols widely utilized in environmental research. Ammonia nitrogen (NH_4-N) was determined through Nessler's reagent spectrophotometry, a commonly adopted approach for quantifying ammonia in water samples. Total phosphorus (TP) was quantified using the ammonium molybdate spectrophotometric method, a widely

recognized technique for TP analysis in water samples. Biochemical oxygen demand (BOD_5) was measured using the dilution and inoculation method, a standard procedure for estimating the level of biodegradable organic matter in water. The permanganate index (COD_{Mn}) was determined using the permanganate index method, a well-established approach for assessing the content of organic compounds in water samples. Finally, chemical oxygen demand (COD_{Cr}) was quantified using the dichromate method, a commonly employed technique for estimating the amount of organic and inorganic substances in water samples. Metal ion concentrations, including As and Se, were determined using an atomic fluorescence spectrometer (AFS, HGF-V, Haiguang instrument, China). The collected water samples were subjected to specific pretreatment procedures to prepare the samples for measurement. An appropriate volume of concentrated HCl was added to the water samples for As and Se analyses. As for Pb, Cu, Zn, Cr, and Cd analyses, an adequate amount of HNO_3 was added, and the pH was adjusted to the range of $1-2$ using acidification. Subsequently, the samples underwent acid digestion to ensure proper solubilization of the metal ions. After these pretreatment steps, the samples were then subjected to measurement using the designated instruments. Non-metric multidimensional scaling (NMDS) ordinations based on Bray-Curtis distance were performed to explore the differences in the environmental variables among the different sampling regions, and an analysis of similarity (ANOSIM) was performed.

2.3. DNA extraction, PCR, and illumina high-throughput sequencing

All water samples were immediately pre-filtered through a 200-μm mesh to remove larger particles and then filtered through a 0.22-μm pore size polycarbonate membrane (142-mm diameter, Millipore). After sampling, the membranes were stored at -80 °C until further analysis. Total DNA was extracted using a two-step procedure. First, the CTAB (Cetyl/Hexadecyl Trimethyl Ammonium Bromide) method was employed for initial DNA extraction [40]. Briefly, this process involves the lysis of cells using a CTAB buffer solution under high salt conditions to liberate DNA. The released DNA is separated from other cellular components through chloroform:isoamyl alcohol extraction. The DNA is then precipitated using isopropanol and washed with ethanol before

resuspension in TE buffer. Second, the extracted DNA was cleaned and concentrated using the Zymo DNA Clean & Concentrator kit (Zymo Research Corp, Irvine, USA) per the manufacturer's instructions [41]. DNA quality was checked by agarose gel electrophoresis. DNA concentrations were determined using a NanoDrop One spectrophotometer (Thermo Fisher Scientific, Carlsbad, USA). The DNA was used as a template for polymerase chain reaction (PCR) amplification using the 18S rRNA gene primers 18S V9F (5′-CCCTGCCHTTTGTACACAC-3′) and 18S V9FR (5′-CCTTCYGCAGGTT-CACCTAC-3′) [42]. The 30-μL PCR reaction included 15-μL of Hief-f®Robust PCR Master Mix (Yeasen Biotech Co., Ltd., Shanghai, China), 0.2 μM of each primer, and 10 ng of DNA.

The PCR cycling parameters included an initial denaturation at 98 °C for 1 min, followed by 30 cycles of 10 s at 98 °C (denaturation), 50 °C for 30 s (annealing), and 72 °C for 30 s (extension). At the end of the amplification, the amplicons were subjected to a final 10-min extension at 72 °C. The PCR products from triplicate reactions per sample were pooled and gel-purified. High-throughput sequencing was conducted on the Illumina NovaSeq 6000 PE250 platform (Illumina, San Diego, USA) in Novogene Co., Ltd. The sequencing parameters were as follows: paired-end reads of 250 base pairs in length, minimum Phred quality score of 30, and a sequencing depth of 50,000 reads per sample.

2.4. Bioinformatic analyses on sequencing data

The raw sequencing data were sorted into individual samples based on the unique sequence tags. Low-quality raw reads with adaptors, ambiguous bases, and low complexity, and those with average quality scores of less than 20, were discarded using the UPARSE pipeline [43]. Operational taxonomic units (OTUs) were identified based on an identity threshold of ≥97%. For a single sample, the number of sequences obtained ranged from 49,113 to 86,934. Taxonomic annotation analysis was conducted using the QIIME2 pipeline [44] against the SILVA-119 reference database. Each OTU table was normalized to 40,000 sequences per sample.

2.5. Alpha-, beta-diversity, and statical analysis

Alpha diversity indices, including the observed richness, Shannon, and phylogenetic diversity (PD) metrics, were calculated with QIIME and visualized with R software. Principal component analysis (PCA) was conducted to evaluate the differences in microeukaryotic plankton community compositions for all samples in the R package *vegan*.

To assess the dissimilarity in water quality among different sampling conditions, we analyzed the similarity (ANOSIM) dissimilarity test. ANOSIM is a non-parametric statistical method commonly employed in ecological studies to compare the dissimilarity between two or more groups based on their multivariate data. In our study, ANOSIM was applied to evaluate the variations in water quality parameters across the different sampling conditions. The ANOSIM test generates a global R statistic that quantifies the degree of dissimilarity between groups, with a higher *R*-value indicating a greater dissimilarity. Additionally, permutation tests were performed to assess the significance of the observed dissimilarity, yielding a corresponding *p*-value.

2.6. Stability analysis of species composition in communities

The stability of species composition in a community was characterized by the average variation degree (AVD). AVD was evaluated using the deviation degree from the mean of the normally distributed OTU relative abundance [27]. AVD was calculated using equation (1):

$$AVD = \frac{\sum_{i=1}^{n} \frac{|x_i - \overline{x_i}|}{\delta i}}{k \times n} \tag{1}$$

where x_i represents the rarefied abundance of the OTU in one sample, $\overline{x_i}$ denotes the average rarefied abundance of the OTU in one sample group, δi is the standard deviation of the OTU in one sample group, k is the number of samples in one sample group, n is the number of OTUs in each sample group. A lower AVD value indicates a higher level of constancy and potentially greater resistance to perturbations of species composition in a community, as it reflects the degree of consistency or uniformity among the species present.

2.7. The contribution analysis of environmental and spatial factors in the community assembly processes

To assess the significance of both environmental and spatial factors in shaping the communities, we conducted a variation partitioning analysis (VPA). This allowed us to quantify the respective influences of spatial factors and environmental selection on community assembly. Concurrently, we performed partial redundancy analysis (pRDA) to further explore these relationships. The relative contributions of different components, including spatial variables (S), environmental variables (E), pure spatial variables (S\E), pure environmental variables (E\S), and the combined effects of both spatial and environmental variables (S∩E), were assessed to understand their roles in the assembly of communities. The Mantel test revealed the significance of Spearman's rank correlations between environmental variables and community structures, visualized using the *vegan* package in R.

2.8. The neutral community model and null model

Sloan's community model was used to assess the potential importance of neutral processes [45]. Regarding this model, the null hypothesis underlying this approach is that the observed variation in community composition is solely driven by neutral processes, where species dispersal and ecological drift dominate, and species fitness or environmental factors have no impact on community assembly. By testing this null hypothesis, we can determine whether deterministic factors, such as environmental filtering and biotic interactions, significantly shape community composition. To evaluate the influences of stochastic and deterministic processes, we used the analytical framework proposed by Stegen et al. (2013) [46]. Null models in which the tips of the phylogenetic tree are randomized ($n = 999$) were applied to calculate the null mean nearest taxon distance (βMNTD) using the function *comdistnt* (abundance.weighted = true) from the package *picante* in R.

We performed several calculations using the β-nearest taxon index (βNTI) and the Raup-Crick metric based on Bray-Curtis dissimilarity (RCbray) to distinguish the relative roles of deterministic and stochastic processes. The βNTI measures the phylogenetic relatedness of co-occurring species compared to the expected phylogenetic relatedness under neutral processes. Positive βNTI values ($>+2$) indicate heterogeneous selection, implying the influence of deterministic processes, while negative βNTI values (<-2) indicate homogeneous selection, suggesting the dominance of deterministic processes. When |βNTI| values are less than 2, it

indicates that stochastic processes are responsible for the observed differences in phylogenetic community composition. Furthermore, we utilized the RCbray metric to determine the relative contribution of stochastic processes. Pairwise comparisons with RCbray > +0.95 and |βNTI| < 2 correspond to dispersal limitation, indicating that limited dispersal drives community composition differences. RCbray < −0.95 and |βNTI| < 2 correspond to homogenizing dispersal, similar to mass effects and source-sink dynamics. Pairwise comparisons with |RCbray| < 0.95 correspond to undominated processes, which suggest weak selection, weak dispersal, diversification, and drift processes.

2.9. MENs construction and characterization, quantification of the contribution of community assembly processes to MENs, and network stability analysis

Molecular ecological network analysis (MENA) (http://ieg4.rccc.ou.edu) was constructed based on Spearman correlations of log-transformed OTU abundances, followed by the random matrix theory (RMT)-based method to automatically define a threshold [37]. iDIRECT was integrated into the MANA to disentangle direct and indirect relationships [36]. Furthermore, the link test for environmental filtering or dispersal limitation (LTED) approach was used to determine the relative contribution of environmental filtering and/or dispersal limitation to the MENs (molecular ecological networks) [34]. We tested all links in the MENs for BEWR, AEWR, BYR, TR, JSR, and LCR with spatial and environmental variables at either $|r| \geq 0.6$ or $|r| \geq$ the network correlation cutoff. We calculated the correlation between dA, representing the dissimilarity of species A's abundance among samples, and spatial distance D to assess the degree of influence of dispersal limitation on species A. We applied analogous procedures to nodes connected to species A. If both linked nodes demonstrated a significant response to dispersal limitation, the connection between these nodes probably originated due to dispersal limitation. This framework was applied to all the links using either $r \geq 0$ or $r \geq 0.5$ (Pearson correlation) with $p < 0.05$. To evaluate whether the constructed networks were random, a permutation-based null model analysis with 100 permutations was employed, with the number of nodes and links kept constant. Networks were visualized with the NetworkAnalyzer plugin of the Cytoscape 3.1.1 [47].

Network stability was calculated by evaluating the network robustness, defined as the proportion of species remaining in the network after random or targeted node removal [37]. The robustness was calculated by the following two steps. Firstly, we used equation (2) to calculate the abundance-weighted mean interaction strength of node i ($wMIS_i$):

$$wMIS_i = \frac{\sum\limits_{j \neq i} b_j s_{ij}}{\sum\limits_{j \neq i} b_j} \tag{2}$$

where b_j is the relative abundance of species j and s_{ij} is the association strength between species i and j, measured by the Pearson correlation coefficient. Second, we removed nodes with $wMIS_i$ value ≤ 0 from the network, and finally, the proportion of the remaining nodes was reported as the network robustness.

Positive and negative cohesion derived from pairwise correlations could reflect the degree of cooperative or competitive behaviors in a sample among community members [34,48]. Positive correlations can be caused by facilitation/mutualism among taxa reflecting ecological or functional similarity, and negative correlations can result from competition reflecting divergent niche

requirements among taxa. Two cohesion values (positive and negative) were calculated using the following equations (3) and (4):

$$\text{Positive cohesion}: c_j^{\text{pos}} = \sum_{i=1}^{n} a_i \times \bar{r}_{i,r} > 0 \tag{3}$$

$$\text{Negative cohesion}: c_j^{\text{neg}} = \sum_{i=1}^{n} a_i \times \bar{r}_{i,r} < 0 \tag{4}$$

where a_i is the abundance of OTU i in the sample j and $\bar{r}_{i,r}$ is the connectedness.

2.10. Explanations of key terms and variables

Our study employs several key terms and variables, each with specific abbreviations for clarity and brevity. The average variation degree (AVD) quantifies community stability based on species composition variance. Variation partitioning analysis (VPA) assesses the roles of spatial factors and environmental selection in community assembly. The β-nearest taxon index (βNTI) evaluates phylogenetic relatedness in communities to discern deterministic and stochastic processes. The Raup-Crick metric based on Bray-Curtis dissimilarity (RCbray) is utilized to gauge stochastic processes in community assembly. Molecular ecological network analysis (MENA) constructs ecological networks to represent biotic interactions in micro-eukaryotic plankton communities. Integrated into MENA, iDIRECT differentiates direct and indirect relationships within these networks. Lastly, the link test for environmental filtering or dispersal limitation (LTED) determines the influence of environmental filtering and dispersal limitation on ecological networks.

3. Results

3.1. Water quality changes

BEWR, AEWR, and BYR exhibited significant heterogeneities in their water quality characteristics along the direction of water flow according to NMDS analyses (stress = 0.11 for BEWR, Fig. S4a; stress = 0.12 for AEWR, Fig. S4b; stress = 0.09 for BYR, Fig. S4c). According to the ANOSIM dissimilarity test, significant variations in water quality were observed, indicated by the global R values of 0.288 ($p = 0.001$) for BEWR, 0.314 ($p = 0.001$) for AEWR, and 0.346 ($p = 0.001$) for BYR, respectively (Fig. S4a−c). These values reflect the degree of dissimilarity in water quality between the respective sampling conditions. To be more specific, a significant variation was observed in the water quality between the mountain section (YD1−12), and the urban and plain section (YD13−26) for BEWR (Fig. S4a). Similarly, a clear variation was observed in the water quality between the mountain and urban sections for AEWR (Fig. S4b). The water quality of BYR exhibited obvious variation in different areas of the river, including upstream (BY1−13) and downstream (BY14−26) (Fig. S4c). Our analysis using non-metric multidimensional scaling (NMDS), analysis of similarities (ANOSIM), and paired sample t-test revealed a significant difference in water quality before and after ecological water replenishment (BEWR and AEWR). Specifically, the NMDS analysis showed a stress value of 0.17, ANOSIM reported an R-value of 0.262 with a p-value of 0.001 (Fig. 2a), and the paired sample t-test indicated a significant difference with a p-value of 0.012. In contrast, our analyses indicated no significant difference when comparing water quality between YDXR_9 (Yongdingxin River in September) and YDXR_6
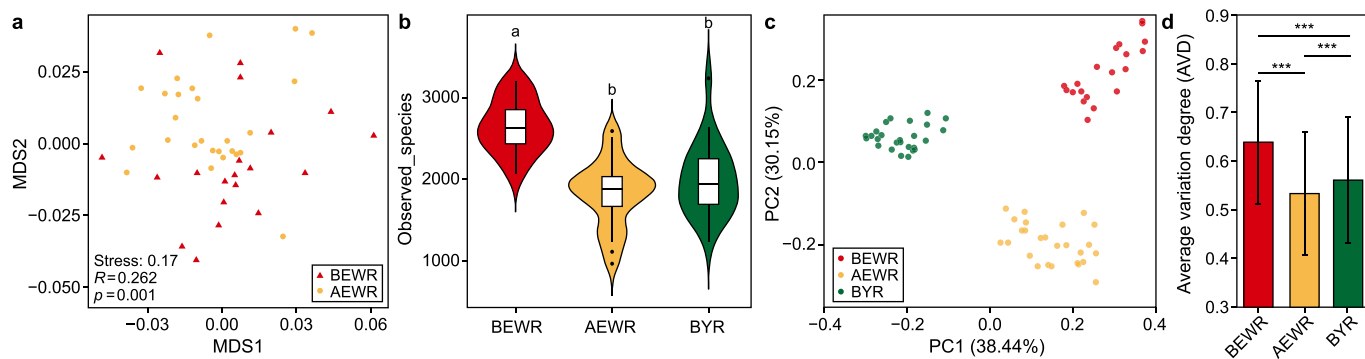
Fig. 2. Comparison of environmental factors, community composition, and stability among BEWR, AEWR, and BYR. **a**, NMDS ordination based on Bray-Curtis dissimilarity shows the variation of environmental factors between BEWR and AEWR. **b**, Observed species of micro-eukaryotic plankton in different rivers (BEWR, AEWR, and BYR). The difference was calculated by ANOVA analysis; different letters indicate significant differences (p value < 0.05). **c**, The β-diversity is based on the weighted distance of micro-eukaryotic plankton communities among BEWR, AEWR, and BYR. Each circle represents one sample color-coded according to BEWR, AEWR, and BYR, respectively. **d**, The AVD (average variation degree) values for BEWR, AEWR, and BYR, respectively. *** represents p value < 0.001.

(Yongdingxin River in June). This was evidenced by an NMDS stress value of 0.10 and an ANOSIM result of $R = 0.079$ with a p-value of 0.152 (Fig. S5a).

3.2. The diversity and variations of microeukaryotic planktonic community

The rarefaction curves tended to approach a saturation plateau, indicating that the sequencing depths were adequate for subsequent analysis (Fig. S6). From upstream to downstream, for both the YDR and BYR, all α-diversity indices, including average observed species number, Shannon index, and PD index, displayed a declining trend (Fig. S7). Biodiversity indices in the mountain section of the Yongding River were higher than in the urban and plain sections (Fig. S7a−b, d−e, g−h). For the BYR, biodiversity indices were higher in the upstream section (Fig. S7c, f, i).

To illustrate the community shifts, we present detailed analyses of the microeukaryotic plankton community before and after water replenishment. Our analysis results indicate that water replenishment in the Yongding River led to notable changes in the microeukaryotic community. The PCA plot (Fig. 2c) illustrates that the microeukaryotic plankton community composition of the Yongding River before replenishment (BEWR) is distinctly different from that after replenishment (AEWR). Both differ significantly from the BYR, with the principal components accounting for 38.44% (PC1) and 30.15% (PC2) of the variation. Species richness increased post-replenishment, as shown in the violin plot (Fig. 2b; Fig. S9), suggesting a diversification of the microeukaryotic species in AEWR compared to BEWR. Furthermore, the bar chart (Fig. 2d) depicts a decrease in the average Bray-Curtis distance within the AEWR group, indicating a more homogenized microeukaryotic plankton community following the replenishment event. It should be noted that the Yongdingxin River (YDXR) course reaches the sea and was minimally affected by water replenishment. Therefore, YDXR was used to support that different wet season months (June and September) affected the community composition only to a small degree (PC1 = 18.04%, PC2 = 14.61%) (Fig. S10). BEWR samples were clustered into two groups: one group included sites 1−12 (mountain section), and the other group included sites 13, 14, 20−26 (urban and plain section), with the first two principal components explaining 48.30% (PC1) and 10.02% (PC2) of the variation in community composition (Fig. S8a). However, AEWR samples (PC1 = 18.19%, PC2 = 11.96%) and BYR samples (PC1 = 23.50%,

PC2 = 16.03%) did not show significant differences in spatial distribution (Fig. S8b and c).

All OTUs were classified into 710 genera and 22 phyla. The dominant phyla (relative sequence abundance >1%) across all samples were Ciliophora (14.166%), Diatomea (11.316%), Chlorophyta (7.972%), Cryptophyceae (4.973%), Rotifera (4.889%), Cercozoa (3.085%), Euglenophyceae (2.098%), Arthropoda (1.378%), Gastrotricha (1.212%), Protalveolata (1.186%), and unclassified (41.670%), accounting for 93.945% of the microeukaryotic sequences (Fig. 3). The phylogenetic analysis reveals a dynamic shift in the Yongding River's microeukaryotic plankton community following the replenishment process (Fig. 3). Genera such as *Ebria*, unidentified *Spirotrichea*, *Cocconeis*, *Gonyostomum*, and *Prymnesium* were prevalent in the river's ecosystem before the intervention (BEWR) but experienced a decline afterward (AEWR). This trend suggests these organisms were either more suited to the original river conditions or could not compete effectively with other microeukaryotic plankton after the environmental shift. In contrast, groups like unidentified *Eukaryota*, *Teleaulax*, *Ochromonas*, and unidentified *Mediophyceae* saw a rise in abundance after the water was replenished. This pattern could indicate either an introduction of new species through the replenishment or an advantageous adjustment of existing species to the altered river conditions. Overall, the replenishment has restructured the microeukaryotic plankton ecosystem of the Yongding River, with some species diminishing in number while others flourish, pointing to an evolving ecological state within the river habitat. The variation of species composition in communities was assessed by AVD. The AVD values of BEWR, AEWR, and BYR were 0.637, 0.533, and 0.560, respectively (Fig. 2d), indicating that AEWR and BYR had lower community stability than BEWR. This result suggested that the distribution of microeukaryotic planktonic community before water replenishment had the highest community stability in species composition compared to the river after water replenishment and the adjacent reference river.

3.3. Variation partitioning of the spatial and environmental drivers on microeukaryotic planktonic community

To dissect the relative roles of environmental selection and dispersal in shaping the communities, environmental variables (16 environmental variables in BEWR, 18 environmental variables in AEWR, and 19 environmental variables in BYR) and spatial variables
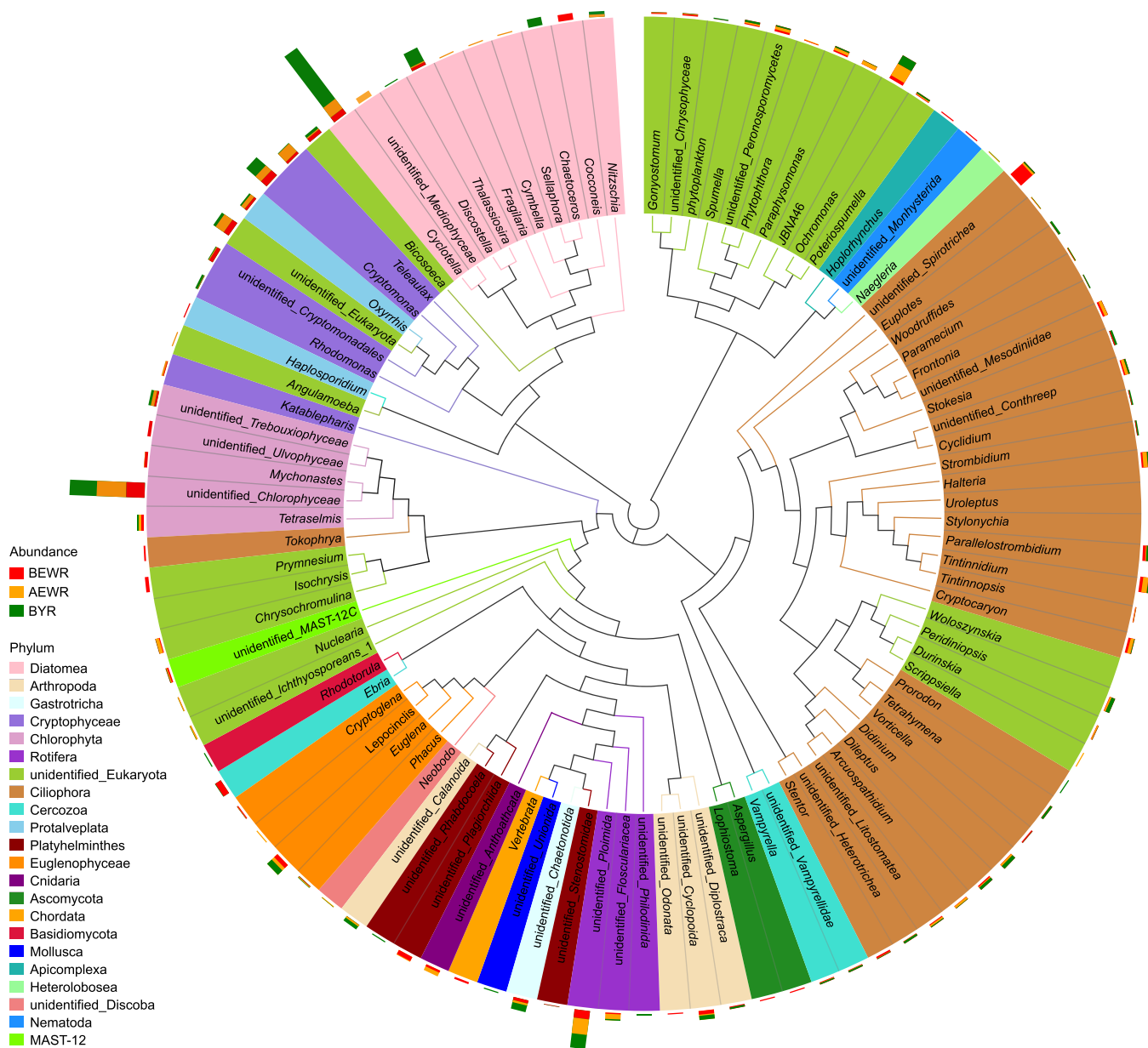
Fig. 3. The phylogenetic tree at the genus level. The phylogenetic tree was constructed based on the representative sequences of 100 genera. The colors of the branches and fan-shaped sections indicate the corresponding phylum. The stacked bar diagram outside the fan ring represents the abundance distribution information of the genus among BEWR, AEWR, and BYR.

(four spatial variables in BEWR, AEWR, and BYR) were used for variance partitioning. VPA revealed that the distribution pattern of microeukaryotic plankton communities in BEWR was primarily driven by the spatial difference of environmental factors (E: 52.5%; E/S: 43.54%) (Fig. 4a). In AEWR and BYR, 61.03% and 62.78% of the changes in community composition could not be explained by the environmental and spatial variables, respectively (Fig. 4b and c). Mantel test revealed that TN, WT, NH_4–N, EC, ORP, As, Cr, Cu, and pH were the most relevant environmental variables for BEWR community variation (Fig. S11a). Compared with BEWR, fewer environmental factors correlated with AEWR and BYR's community composition. WT, COD_{Mn}, and COD_{Cr} were the environmental variables that most correlated with AEWR community variation, while WT, DO, pH, Se, and Cu were the most correlated to the changes in the BYR community (Fig. S11b and c).

3.4. Shifts in community assembly processes and mechanisms

The neutral community model (NCM) was used to estimate the relationship between the occurrence frequency of OTUs and their relative abundance variations. The results suggested a strong influence of stochastic processes in shaping microeukaryotic planktonic community assembly for AEWR, BYR, TR, JSR, and LCR, as indicated by the goodness of fit to a neutral community model (Fig. 4e−i). In contrast, stochastic processes had a low explanation for BEWR's community assembly (Fig. 4d). This indicated the stochastic processes had relatively higher contributions to the assembly of microeukaryotic planktonic communities in the rivers which were connected year-round but contributed less to the cut-off river before water replenishment.

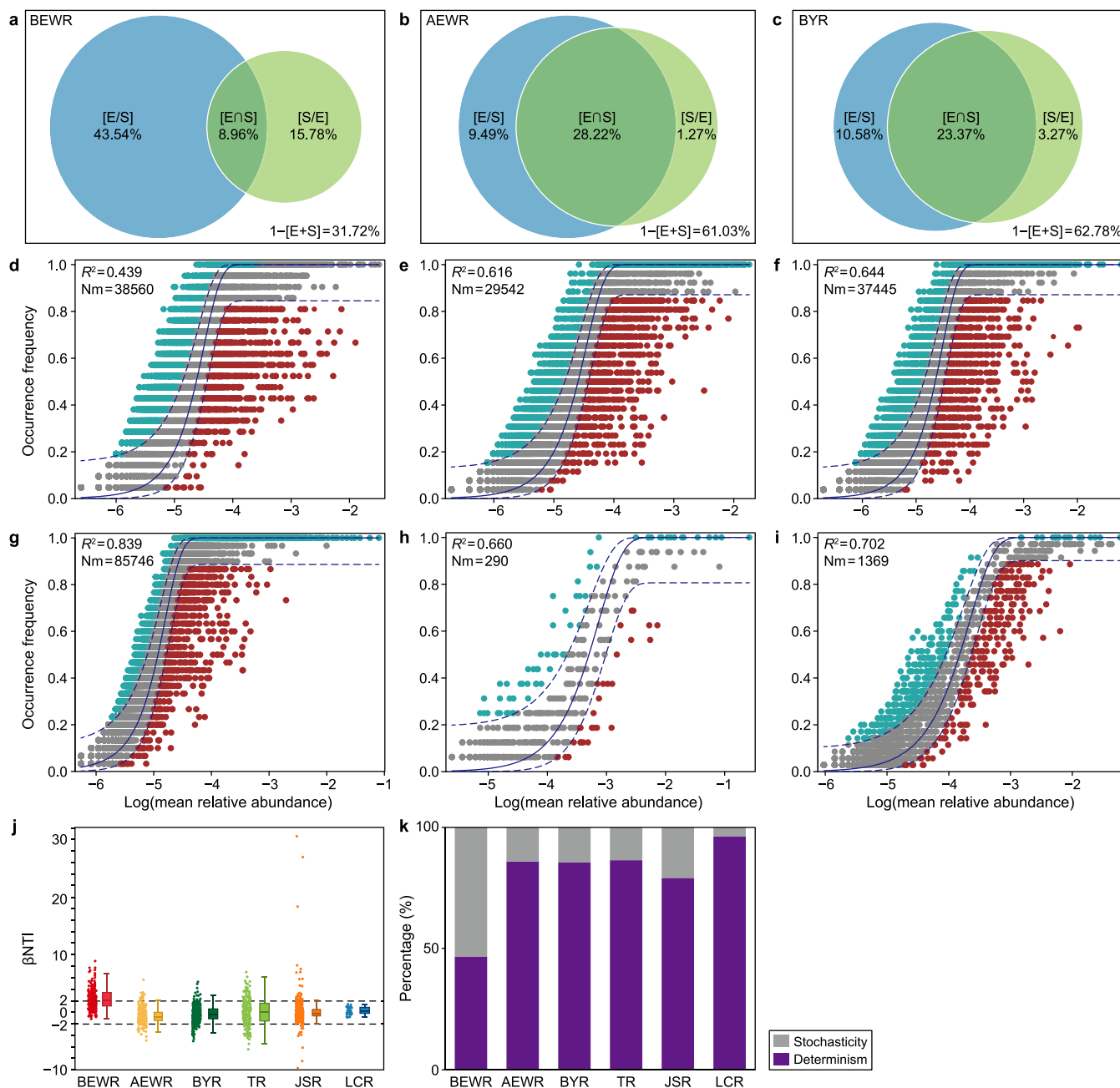The null model analysis further revealed that the relative

Fig. 4. Results of assessing the relative contribution for community assembly of deterministic and stochastic roles. **a**−**c**, The VPA results show the effects of geographic distance and local environmental conditions on community compositions of eukaryotic plankton in BEWR (**a**), AEWR (**b**), and BYR (**c**). The explanatory power of the pure environmental factors (E/S), pure spatial factors (S/E), and the joint environmental and spatial factors (E∩S). **d**−**i**, Fit of the neutral community model (NCM) of community assembly. The predicted occurrence frequencies for BEWR (**d**), AEWR (**e**), BYR (**f**), TR (Ting River) (**g**), JSR (Jinsha River) (**h**), and LCR (Lancang River) (**i**) representing micro-eukaryotic plankton communities from the respective sample sets. The solid blue lines indicate the best fit to the NCM, as in Sloan et al. (2006). The dashed blue lines represent the 95% confidence intervals around the model prediction. OTUs that occurred at a higher or lower frequency than predicted by the NCM are shown in different colors. The cyan color indicates frequencies higher than the NCM prediction, gray indicates agreement with the NCM prediction, and red indicates frequencies lower than the NCM prediction. Nm indicates the metacommunity size times immigration, and R² indicates the fit to this model. **j**, The βNTI patterns for BEWR, AEWR, BYR, TR, JSR, and LCR. **k**, Partitioning of deterministic and stochastic processes for the micro-eukaryotic plankton communities.

contributions of community assembly processes differed among BEWR, AEWR, BYR, TR, JSR, and LCR (Fig. 4j and k; Fig. S12). Heterogeneous selection, a deterministic process, had a much larger effect on the community assembly of BEWR (βNTI > +2), explaining 53% of community turnover (Fig. S12a), while stochastic processes, including dispersal limitation and drift, had greater impacts on the community assembly of AEWR, BYR, TR, JSR and LCR (|βNTI| < 2) (Fig. 4j and k). In addition, stochastic processes exhibited relatively

larger effects on the community assemblies of YDXR_9 and YDXR_6, accounting for 68% and 64% of community turnover, respectively (Fig. S13).

3.5. Variations and stability of the ecological network

To determine changes in species interactions within the planktonic communities during the transition from cut-off to connected
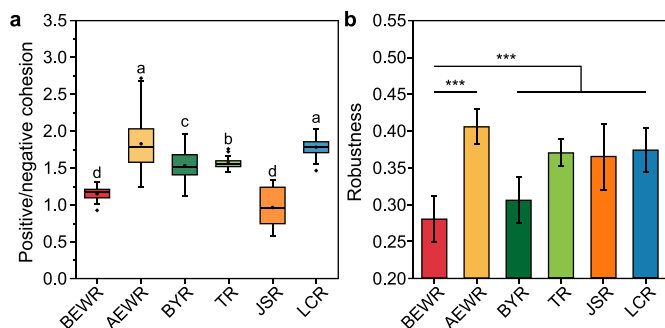
Fig. 5. The topological stability of the ecological network. **a**, Positive/negative cohesion indices for BEWR, AEWR, BYR, TR, JSR, and LCR. The difference was calculated by ANOVA analysis; different letters indicate significant differences (*p* value < 0.05). **b**, Robustness analysis for BEWR, AEWR, BYR, TR, JSR, and LCR communities. *** represents *p* value < 0.001.

river states, an independent molecular ecological network (MEN) integrated with iDIRECT was used for each river (Fig. S14). The corresponding topological features are summarized in Supplementary Tables S6 and S7. The overall topology indices revealed that all network connectivity distribution curves fitted well with the power-law model (R^2 values from 0.645 to 0.938) (Table S6). The MENs of BEWR, AEWR, BYR, TR, JSR, and LCR were composed of 202 nodes with 357 edges, 105 nodes with 208 edges, 127 nodes with 127 edges, 304 nodes with 489 edges, 57 nodes with 123 edges, and 106 nodes with 194 edges, respectively (Fig. S14). Based on the within-module connectivity (Zi) and among-module connectivity (Pi), a total of 8, 2, 0, 6, 0, and 1 module hubs were detected across the MEN of BEWR, AEWR, BYR, TR, JSR, and LCR, respectively. Only the MEN of TR possessed connectors (Fig. S15). These hubs and connectors could be regarded as keystone species that play key roles in shaping network structure.

Except for JSR, all river systems showed a significantly higher cohesion index, the ratios of positive to negative cohesion, than that of BEWR (Fig. 5a). Network robustness analysis showed that the robustness of BEWR, AEWR, BYR, TR, JSR, and LCR were 0.281 ± 0.031, 0.406 ± 0.024, 0.307 ± 0.031, 0.371 ± 0.019, 0.365 ± 0.045 and 0.375 ± 0.029, respectively (Fig. 5b). The robustness of AEWR was significantly higher than that of BEWR (Fig. 5b). This indicated the robustness after water replenishment was the highest of all rivers, whereas the opposite (lowest robustness) had been true before water replenishment. In addition, the robustness of rivers in southern China (TR, JSR, and LCR) was significantly higher than that of rivers in northern China (BEWR, BYR).

3.6. Major drivers of changes in co-occurrence networks

The LTED method was employed to examine the association of co-occurrence networks with either environmental selection or dispersal limitation. Suppose the link between two taxa arises from their co-variation with environmental conditions. In that case, a robust correlation between the two taxa and the relevant environmental variables should be discernible. We assessed all links within the MENs of BEWR, AEWR, BYR, and TR, using either $|r| \geq 0.6$ or $|r| \geq$ the network correlation cutoff and their corresponding variables. The examination revealed that less than 1% of the links represented taxon-taxon-environment covariates. This suggests species co-variation with the observed environmental conditions was of limited significance. Consequently, the influence of environmental selections attributable to these variables on shaping the MENs for BEWR, AEWR, BYR, and TR was likely minimal (Fig. S16a).

If a specific portion of connections within the networks met the conditions of both $r > 0$ and $r > 0.5$ with a significance level of $p < 0.05$ (Pearson correlation), it indicated that dispersal limitation played a prominent role in shaping the MENs (Fig. S16b). In this study, 70.21% of links in the AEWR network fulfilled the criteria of $r \geq 0$ with $p \leq 0.05$, while 0.47% of the links fulfilled the criteria that $r \geq 0.5$ with $p \leq 0.05$, implying that dispersal limitation was important for shaping AEWR's MEN (Fig. S16b). However, none of the links in BEWR, BYR, TR, JSR, and LCR met the criteria $r \geq 0.5$, implying dispersal limitation was not the major driver in shaping their MENs (Fig. S16b).

The above results suggested that dispersal limitation was the driving factor that shaped the formation of AEWR's co-occurrence network, while biotic interactions were the decisive factor in shaping the co-occurrence networks of BEWR, BYR, TR, JSR, and LCR (Table S8).

4. Discussion

With the construction of large water conservation projects (e.g., canals, dam constructions, water diversion, water replenishment, etc.), more and more artificial interventions occur in natural rivers. There is an urgent need to fully assess the effects and sustainability of these projects on the ecology of these aquatic systems, but how to evaluate the ecological effects of these large-scale artificial interventions or restorations on river ecosystems is highly challenging, especially in their early stages [11,49]. Microeukaryotic plankton are important components of freshwater ecosystems [20,50] and form an important part of the aquatic food web by constructing and maintaining substance and energy fluxes in river ecosystems [51]. Therefore, their assembly process and stability mechanism might reflect the health of the river ecosystem as a whole and determine its future sustainability. However, due to the high fluidity and variability, a small local survey cannot achieve the goal of reflecting the entire river ecosystem. However, large-scale surveys are still scarce, especially during artificial interventions.

In this study, we systematically surveyed the aquatic microeukaryotic plankton in the Yongding River before and after a large water replenishment (BEWR and AEWR). The BYR served as an optimal reference system for comparison. We observed significant shifts in the microeukaryotic plankton community post-water replenishment. These shifts are crucial for understanding the ecological stability of the river ecosystem. We discuss how these changes impact the river's ecological balance and the underlying mechanisms driving these shifts. We also compared our current observations with previously published data from the Ting (TR), Jinsha (JSR), and Lancang (LCR) rivers, which serve as flowing river comparisons. The data in this study were generated using the eDNA metabarcoding approach based on 18S rRNA genes and were analyzed using a standardized pipeline. The application of eDNA metabarcoding offers valuable insights into the potential mechanisms driving community responses in highly dynamic ecosystems, enabling the timely and effective optimization of remediation strategies. Utilizing this large-scale data, we proposed a framework that included three aspects: variation pattern in species composition, community assembly mechanism, and resistance mechanism of the ecological network to fully reflect the stability of the aquatic planktonic community. Through this framework, we assessed the ecological effects of water replenishment in the Yongding River.

4.1. Water replenishment reshaped the composition stability of the microeukaryotic planktonic community

The field observations demonstrated that the EWR of Yongding River not only raised the surface water level but also reconnected

the long-term cut-off river section (Fig. 1; Fig. S2). Environmental heterogeneity is an important determinant in shaping species distribution patterns and affecting abundance in aquatic ecosystems [52,53]. In our study, we found that the water quality in the Yongding River exhibited a significant "mountain section - urban and plain section" distribution pattern both before and after the large water replenishment (BEWR and AEWR) (Fig. S4). Additionally, the water quality in the adjacent BYR also showed a significant "upstream-downstream" distribution pattern (Fig. S4). Alpha diversity was higher in the cut-off period, which might be attributed to eukaryotic microorganisms being stimulated to differentiate under greater external nutrients and environmental pressure [54]. The introduction of water replenishment had a notable impact on the α-diversity of the planktonic community in the Yongding River, leading to a significant decrease. This decline in α-diversity was comparable to the levels observed in the adjacent BYR (Fig. 2b; Fig. S9). Ecological systems tend to be finely tuned to their existing conditions [55]. However, abrupt changes like the introduction of water replenishment can disrupt this delicate balance. Rapid shifts in water levels, flow patterns, and sediment distribution can trigger physical disturbances within habitats, potentially resulting in the displacement or mortality of specific species, especially those less adaptable to such fluctuations [56]. Consequently, these disruptions can contribute to an overall reduction in community diversity. The β diversity of microeukaryotic plankton significantly differed between BEWR and AEWR (Fig. 2c). We quantified the degree of community composition stability using average species variation (AVD). We found lower AVD values in AEWR and BYR than in BEWR, indicating the water replenishment increased the stability of community composition in the reconnected Yongding River (Fig. 2d). Therefore, species composition stability in the community might be improved at the river scale after EWR.

4.2. A "seesaw" model of dynamic balance between stochasticity and determinism reflects the assembly stability of microeukaryotic planktonic community

River ecosystems exhibit remarkably high levels of biodiversity, which are influenced by a combination of deterministic factors such as environmental filtering and biotic interactions, as well as stochastic processes, including dispersal, diversification, and ecological drift [57]. Previous research has shown that deterministic and stochastic processes play important roles in assembling microeukaryotic plankton communities. This is crucial for understanding and predicting the ecological effects of different water replenishment strategies [58]. For instance, the dominance of deterministic processes might indicate that environmental filtering and biotic interactions play a key role in community dynamics post-water replenishment, while an increase in stochastic processes could reflect enhanced biodiversity and community resilience [59]. In aquatic environments, it is important to note that homogenizing dispersal could play a significant role, potentially contributing to a more uniform distribution of species [31,60]. In this study, we also found the microeukaryotic planktonic communities of BYR, TR, LCR, and JSR were mainly controlled by the stochastic processes of either dispersal limitation or undominated process (Fig. 4). Together, these results indicated stochasticity played a greater role in microeukaryotic planktonic distributions along large-scale rivers than determinism, which could be a natural characteristic of river ecosystems. In the Yongding River, the community of BEWR was mainly controlled by the deterministic process of environmental selection, where water quality acted as the deterministic filtering factor (Fig. 4a; Fig. S11a). Similarly, a previous study reported that a prominent shortage in the water storage of Danjiangkou Reservoir, related to seasonal flood control, possibly imposed a higher

environmental selection effect on the microbiota in the reservoir and across the water diversion canal [61]. After water replenishment, the community of AEWR was more weakly impacted by environmental filtering through water quality (Fig. 4b; Fig. S11b), while the stronger impacts of dispersal limitation and stochastic assembly were likely to overrule deterministic processes (Fig. 4; Fig. S12). Thus, water replenishment for ecological restoration drove the community assembly of aquatic plankton from strong environmental filtering to the natural stochastic state. Our research highlights the deterministic and stochastic influences on microeukaryotic plankton communities post-ecological water replenishment. However, we recognize the lack of data on water retention time and hydrological load, key factors in community assembly, as a limitation. Future studies incorporating these aspects could yield a more nuanced understanding of ecological impacts [62].

Theoretically, the species co-occurrence networks (e.g., MENs) were also driven by both deterministic (biotic interaction and environmental filtering) and stochastic (dispersal limitation) processes [63,64]. The link test for environmental filtering or dispersal limitation (LTED) analysis illustrated that the MENs for natural rivers (BYR, TR, JSR, and LCR), as well as the Yongding River before water replenishment, were driven by biotic interactions (Fig. S16; Table S8). Similar to the latest views, 'biotic interactions' should be treated as 'putative interactions' but are used here for simplicity [34]. However, after the water replenishment, the MEN of AEWR was more likely driven by the dispersal limitation (Fig. S16b; Table S8). Therefore, we found a simple rule during water replenishment: dispersal limitation is associated with forming a co-occurrence network, and the "rise and fall" of biotic interactions and dispersal limitation regulate species co-occurrence patterns before and after water replenishment.

In our study, we propose a conceptual model called the "Seesaw model" to describe the balance between deterministic and stochastic processes in the assembly of microbial communities within river ecosystems, particularly during ecological restoration after water replenishment (Fig. 6). The Seesaw model is inspired by the observation that the relative importance of deterministic and stochastic processes in community assembly can shift as ecological conditions change. In cut-off rivers (e.g., BEWR), where
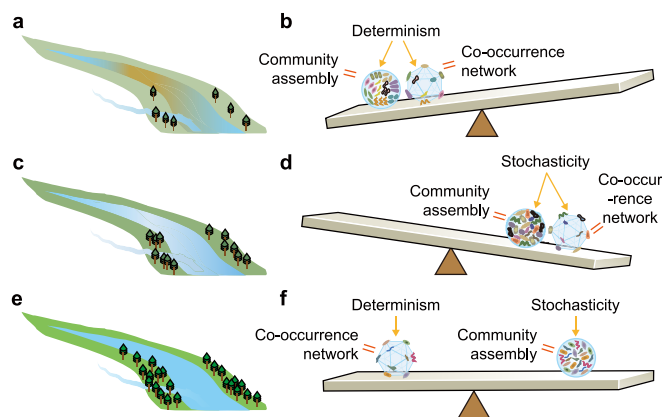

Fig. 6. Conceptual models reveal the ecological processes underlying the trade-off of the micro-eukaryotic community in BEWR (**a**, **b**), AEWR (**c**, **d**), BYR, TR, JSR, and LCR (**e**, **f**). **a**, Diagram of the long-term cut-off river. **b**, The deterministic roles dominate community assembly and co-occurrence networks, including environmental filtering and biotic interactions. **c**, Diagram of short-term river reconnection by ecological water replenishment. **d**, Dispersal limitation (stochasticity) simultaneously dominates community assembly and co-occurrence networks. **e**, Diagram of long-term connected rivers that flow year-round. **f**, Deterministic biotic interactions dominate the ecological networks, and the stochastic processes dominate community assembly.

connectivity is limited and environmental filtering strongly influences community composition, deterministic processes play a dominant role. This is represented as one end of the Seesaw, where deterministic forces are "up" and stochastic forces are "down." Biotic interactions also contribute to the co-occurrence network in this stage but are still part of the deterministic processes (Fig. 6a and b). However, after water replenishment and the restoration of connectivity (e.g., AEWR), the dynamics of community assembly shift. Dispersal limitation becomes more pronounced due to the increased movement of organisms between different parts of the river, leading to greater stochasticity in the community assembly process. This is represented as the other end of the Seesaw, where deterministic forces are "down" and stochastic forces are "up" (Fig. 6c and d). Additionally, the co-occurrence network becomes more influenced by dispersal limitation rather than deterministic biotic interactions. In contrast, in natural, continuously flowing rivers (e.g., BYR, TR, JSR, and LCR), we propose that the ecosystem achieves a balanced state, where deterministic biotic interactions largely govern the co-occurrence network, while stochastic processes dominate the community assembly. This balanced state is represented by the middle position of the Seesaw, where deterministic and stochastic forces are in equilibrium, creating a stable and sustainable ecosystem (Fig. 6e and f).

The Seesaw model highlights the dynamic nature of community assembly in river ecosystems and the potential shifts in the importance of deterministic and stochastic processes during ecological restoration. By understanding and quantifying these processes, we can gain valuable insights into the ecological responses of microbial communities to environmental changes and management interventions. This model provides a useful framework for interpreting and predicting community dynamics in river ecosystems, guiding effective restoration strategies, and aiming for a balanced and healthy ecological state similar to that of natural, continuously flowing rivers.

4.3. Water replenishment increased the ecological network stability of the microeukaryotic planktonic community

The third stability assessment of this study was based on the biotic interaction networks. It is well known that species interactions are one of the foundations of ecological stability [35,65,66]. The more complex and robust an ecological network of species interactions, the higher the resistance to environmental changes and the better the sustainability of the ecosystem [67]. Here, the biotic interactions among microeukaryotic planktonic species were identified using molecular ecological network analysis (MENA) integrated with iDIRECT [36,37], which has been verified to infer the true interactions within a microbial community. Here, we found the robustness of MEN for all natural rivers (BYR, TR, JSR, and LCR) and AEWR were significantly higher than that of BEWR (Fig. 5b), indicating the water replenishment enhanced the stability of ecological networks in the Yongding River. In addition, this study also clarified that the higher diversity of microeukaryotic plankton in BEWR (Fig. 2b) did not result in higher stability of the ecological network.

Positive and negative cohesion can represent the intensity of cooperative and competitive behaviors among community species, respectively [34]. The ratio of positive to negative cohesion in BYR, TR, LCR, and the Yongding River after water replenishment was significantly higher than that in the Yongding River before water replenishment (Fig. 5a), indicating there was more cooperative behavior among planktonic species in natural rivers and after water replenishment. These enhanced cooperations may also contribute to the resilience of the microeukaryotic community to a changing environment [68]. Overall, these results demonstrated that water replenishment could increase the network stability of microeukaryotic communities to levels similar to or higher than that of natural rivers.

5. Conclusion

In conclusion, there are many mechanisms to maintain the stability of microeukaryotic planktonic communities in rivers. Large flow and EWR at the river scale significantly changed the diversity, community species composition, and stability of microeukaryotic plankton. There was a succession of control mechanisms for micro-eukaryotic community assembly in the evolution from a cut-off river to a temporarily connected river after EWR, and then to the long-term connected river. This progression is characterized by a shift "from dominated by deterministic role to dominated by stochastic role, and then to dominated by both deterministic and stochastic roles." After EWR, the robustness and cohesion of the ecological network of the micro-eukaryotic community in the Yongding River were significantly improved. By comparing the robustness and cohesion levels of five rivers in the south and north of China, we anticipate a decline in these attributes for the Yongding River after long-term connectivity. However, they are still higher than the stability level of the ecological network of microeukaryotic communities before EWR. Our findings highlight the importance of understanding community shifts in microeukaryotic plankton and their impact on ecological stability. The insights gained from our study on microeukaryotic plankton community dynamics in specific ecological water replenishment contexts offer a universally applicable understanding for broader aquatic ecosystem management and conservation efforts globally. Future research should further explore the effects of different water replenishment strategies on the stability of these communities.

CRediT authorship contribution statement

Shuping Wang: Conceptualization, Methodology, Software, Validation, Formal Analysis, Investigation, Resources, Data Curation, Writing - Original Draft, Writing - Review & Editing, Visualization, Supervision, Project Administration, Funding Acquisition. **Songsong Gu:** Methodology, Software, Formal Analysis, Data Curation, Writing - Original Draft, Writing - Review & Editing. **Yaqun Zhang:** Methodology, Software, Formal Analysis, Data Curation. **Ye Deng:** Conceptualization, Methodology, Formal Analysis, Writing - Original Draft, Writing - Review & Editing, Supervision, Project Administration, Funding Acquisition. **Wenhui Qiu:** Writing - Review & Editing. **Qianhang Sun:** Investigation. **Tianxu Zhang:** Investigation. **Pengyuan Wang:** Investigation. **Zhenguang Yan:** Supervision, Project Administration, Funding Acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Raw sequences for samples before and after the EWR of the Yongding River have been stored in the public NCBI Sequence Read Archive (SRA) database under the BioProject numbers PRJNA1004980 and PRJNA1005206. Raw sequences for samples of BYR have been stored in the NCBI SRA database under the BioProject number PRJNA1005052. Raw sequences for samples of Yongdingxin River have been stored in the NCBI SRA database under the BioProject number PRJNA1005490.

Acknowledgments

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ese.2024.100409.

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