

Enhancing the engineering design of diversion routes to safeguard water quality: New insights from a carbon cycling perspective

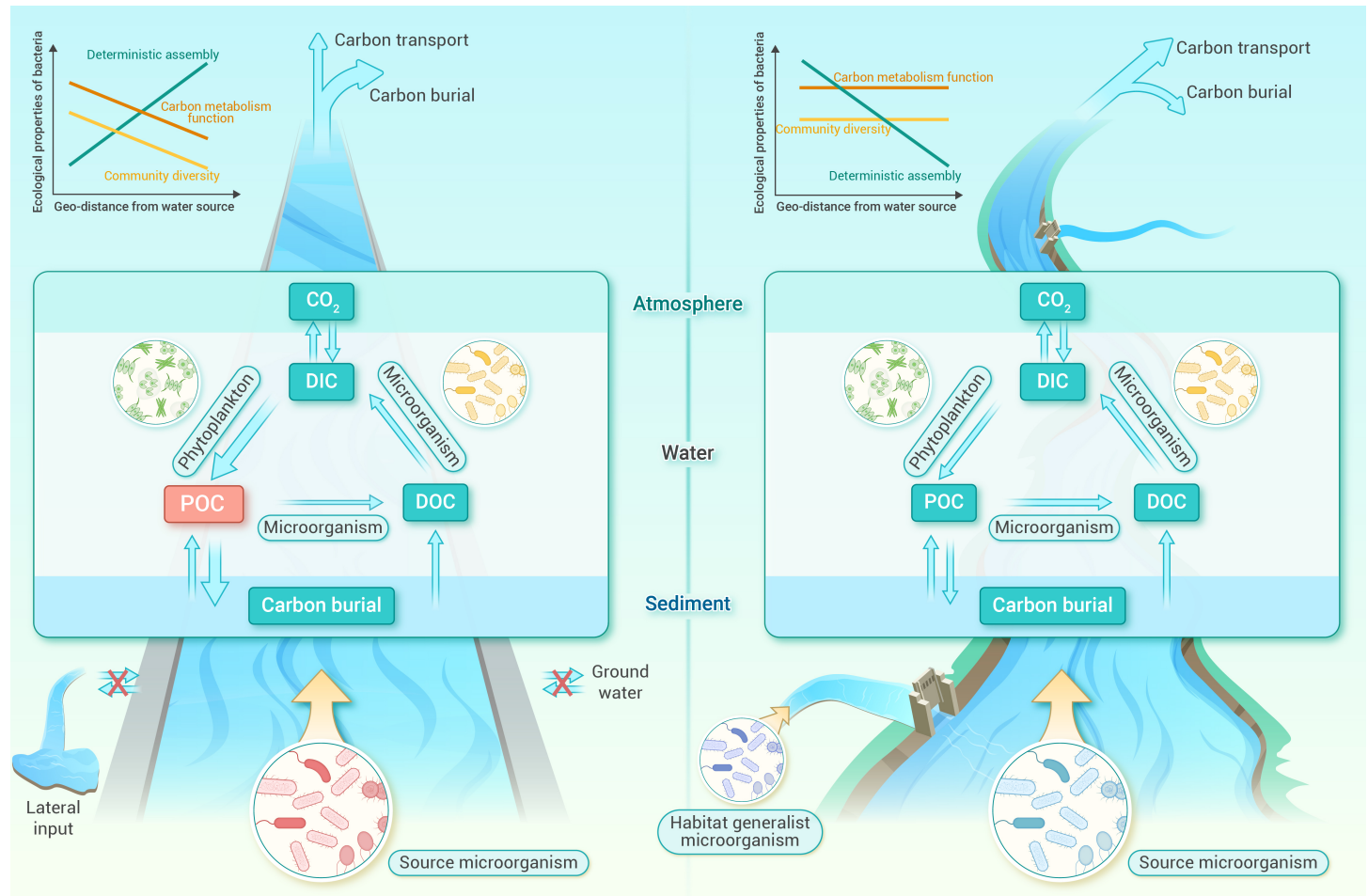
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GRAPHICAL ABSTRACT



PUBLIC SUMMARY

- Water diversion channel type and route layout markedly shape the assembly of aquatic microbial communities.
- Isolated and concrete channels lead to vulnerable bacterial community with reduced carbon metabolism ability.
- Emphasize algae-microbe interaction in water diversion channels to prevent detrital organic matter buildup.

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Conserving the quality of transferred water ensures the success and sustainability of interbasin water diversion works, which are increasingly crucial for global water security. Despite intensive scientific and engineering efforts, carbon dynamics in these artificial rivers have been overlooked. The accumulation of organic mud in some water transfer canals, for example, in the Middle Route of the South-to-North Water Diversion (SNWT) in China, poses multifaceted risks, including jeopardized water quality, disrupted water supply, and surging mud removal expenses. In contrast, natural channels for water delivery, such as the parallel East Route of the SNWT, do not experience mud accumulation challenges. Our study reveals that the open concrete canal of the Middle Route, devoid of connections to local water bodies, compromises hydrological connectivity, contrasting with the East Route, which enhances it. This, in turn, undermines microbial stochastic assembly processes, impedes the introduction of habitat-generalist microbial species, and facilitates detrital carbon accumulation within the ecosystem, presumably due to constraints on bacterial community functions. These findings can improve future engineering designs of water transfer schemes, including water diversion approaches and channel construction, for preserving transferred water quality.

INTRODUCTION

The global demand for an adequate supply of high-quality water across diverse regions conflicts with the uneven distribution of water resources. In response, extensive engineering solutions, including the construction of dams and the implementation of large-scale water transfer projects, have been devised to effectively manage these resources and satisfy human demands.¹ Interbasin water transfer schemes accounted for 14% of global water withdrawals in 2005, and this percentage is expected to increase to 25% by 2025.^{2,3} It is undeniable that interbasin water transfer is steadily evolving into a vital element of global water security. In the past, engineering designs for such projects primarily focused on water resource allocation,^{4,5} operation stability,^{6,7} pollution prevention,^{8,9} and, of course, cost. However, apart from water, diversion channels also transport vast amounts of substances, such as organic matter. In natural rivers, carbon (C) in different forms is deposited in sediment, is transported to the sea or undergoes complex transformations, thereby influencing aquatic environments.^{10,11} However, far less is known about carbon dynamics in water diversion channels, particularly in concrete canals that cannot store carbon in bed sediment or convey carbon to the sea. The fate of organic matter is closely linked to the quality of transferred water and maintenance of the channel. For instance, the Middle Route of the South-to-North Water Diversion (SNWD) project in China, which uses an isolated concrete canal to deliver water without hydrological connectivity to soil or tributaries, was found to experience challenges associated with increasing algal organic matter accumulation in the canal.^{12,13} In contrast, the parallel East Route of the SNWD project, which uses a natural channel to deliver water, does not exhibit a similar problem. The presence of organic matter-rich mud can obstruct pumps, disrupt the water supply,¹⁴ give rise to odor issues,¹⁵ and subsequently result in significant maintenance expenses for either the removal of organic mud or the modification of the purification process. This presents a potential threat to the long-term sustainability of the

project's operation. Similarly, excessive growth of filamentous algae and accumulated algal debris were reported in lined transfer canals in Russia, leading to water quality deterioration.¹⁶ Worldwide, several water transfer projects have encountered problems associated with water quality deterioration (Figure S1),^{17–20} mostly linked to the blocked biogeochemical cycling of organic matter, and should be a cause for concern.

Microbes serve as ubiquitous scavengers in ecosystems, assimilating organic carbon generated by autotrophs.^{21–23} The coupling or mutualistic relationships between phytoplankton and microbes may distort under certain levels of environmental stress, thereby affecting carbon flow in transferred water bodies.²⁴ Nevertheless, the role and importance of microbial taxa in water diversion systems, which are highly engineered and equipped with a series of hydraulic constructions, have been inadequately addressed. Contradictory theories exist on the roles of deterministic and stochastic processes in microbial assemblies present in water transfer channels. Zhang *et al.* proposed that deterministic processes predominantly drive the formation of bacterial assemblages, while stochastic processes play a more important role in microeukaryotic community assemblies in water diversion canals.¹³ However, Gweon *et al.* claimed that stochastic processes were more dominant in the free-living and particle-associated bacterial community assemblies of lotic habitats along a river continuum.²⁵ The predominant factor influencing the balance between stochastic and deterministic processes in microbial assemblies within water diversion systems remains unclear.²⁶ There are three major forms of water transfer approaches, including pipelines, canals, and existing river channels.¹⁷ A water transfer route may employ one or a combination of these methods, leading to varied hydrological connectivity to the surrounding water bodies. The extent to which and how the chosen water transfer approach influences microbe-mediated carbon dynamics within the diverted water constitutes a significant knowledge gap.

Here, we hypothesized that the water transfer approach might exert a substantial influence on microbial assembly and functions, thereby impacting the carbon flow within the channels. We analyzed the planktonic microbial communities in the Middle and East Routes of the SNWD megaproject. Our objectives were to understand 1) how microbial communities, assembly processes, and functions evolve within the two types of water diversion channels, particularly within the context of differing hydrological connectivity, and 2) how these variations in microbial communities contribute to alterations in carbon flow and water quality within the aquatic ecosystems of these water transfers. The results will provide new insights into the microbial ecology of long water transfer channels, the interactions between microorganisms and aquatic ecosystems in water diversion, as well as valuable suggestions for designing sustainable water transfer schemes.

RESULTS

Differences in carbon dynamics between the Middle and East Routes

The Middle and East Routes of the SNWD project concurrently transport water from the Yangtze River to North China, effectively alleviating water shortages in the northern region.^{4,27} They have comparable capacities but use differently designed water transfer channels (Figure 1). Researchers simulated the Middle and East Route ecosystems using Ecopath with Ecosim. The



Figure 1. The two water transfer routes and locations of sampling sites The green line represents the Middle Route, and the yellow line represents the East Route. The locations of the 17 sampling sites in the summer (MS) and the 24 sampling sites in the autumn (MA) along the Middle Route are annotated. The Yellow River Crossing Tunnel divides the Middle Route into upstream and downstream. The locations of the 20 sampling sites for summer and autumn along the East Route are annotated. The Huaian Sluice the East Route into upstream and downstream. The site photos taken in different sections of the two water transfer lines are presented on the right side of the figure.

in both routes originates from autogenic sources. In contrast, the source analysis of particulate organic matter (POM) in the delivered water of the two water diversion routes based on stable isotopes revealed that *in situ* plankton production mostly contributed to POM in the Middle Route, whereas soil organic matter was a significant source of POM in the East Route, as indicated by the empirical carbon and nitrogen isotopic composition range.³⁷ The scanning electron microscopy (SEM) images of aggregates (Figure S3) collected from the Middle Route canal showed various diatom species and confirmed that algal debris is the dominant component of the POM in the Middle Route.

Furthermore, the composition and quantity changes of DOM in the transferred water along the two routes during summer were thoroughly evaluated (Figure 2A, detailed results listed in Figures S4–S6). The assessed DOM content in water samples collected from the reservoir and upstream of the Middle Route remained consistently low and stable (Figure 2A). However, a notable spike in DOM concentration occurred approximately 380 km from the source water, coinciding with the location of the Yellow River Crossing Tunnel. This increase suggests amplified production of dissolved organic carbon at this point. Subsequently, the concentration of autochthonous DOM induced by microbial activity decreased to a lower level and exhibited fluctuations in the downstream section. Water in the East Route generally had higher fluores-

cence intensity scores than that in the Middle Route. The content of fulvic and humic acid-like substances noticeably increased from the sampling site ~350 km from the headwaters (Figure 2A), where the *Cyanophyta* bloom ended in Luoma Lake according to *in situ* observations. In addition, we quantitatively monitored the dominant phytoplankton taxa along the two routes. The results in Figure S7 show that the biomass of *Bacillariophyta* and *Chlorophyta* gradually increased with geographical distance from the source water in the Middle Route, wherein the *Bacillariophyta* biomass showed almost linear growth with distance ($R^2 = 0.77$, Figure S7). In particular, the abundance of *Cyclotella* (diatom) cells increased markedly from the headwaters to the end of the canal (Figure S8). In contrast, the phytoplankton biomass along the East Route (Figure S7) did not exhibit a consistent variation pattern. Furthermore, the deposition of algal debris in the Middle Route has had a detrimental effect on water quality by generating odorous compounds and disinfection byproducts.^{15,38} In contrast, water quality in the East Route is predominantly influenced by pollution originating from the original river channels and nonpoint sources (Table 1).^{39,40} Structural equation modeling was employed to identify the key factors influencing changes in total organic carbon (TOC) levels in the Middle Route (see Figure 2B). The

results revealed that the amount of organic matter per unit area flowing into the detritus pool in the Middle Route was significantly higher than that in the East Route (Table 1). This difference was primarily attributed to the lower ecotrophic efficiency of the Middle Route (< 0.1),²⁸ which quantifies the proportion of production utilized by the subsequent trophic level,²⁹ in contrast to the value of the East Route (~ 0.846).³⁰ Additionally, the Middle Route exhibited higher primary productivity of phytoplankton, especially during the summer (Figure 2A). This aligns with the reported phenomenon of increasing quantities of algal debris accumulated in the Middle Route canal and partly deposited in front of the sluice gates (Figure S2),^{12,31,32} which has rarely been observed in the East Route. The other food web properties listed in Table 1 further revealed that the Middle Route ecosystem has low utilization efficiency of primary productivity, whereas the East Route represents a relatively mature ecosystem. The comparison of carbon dynamic data between the two water diversion lines in Table 1^{33–36} indicates that the dissolved organic carbon contents in the East Route are slightly higher than those measured in the Middle Route during the same period, whereas the fluorescence index (FI), biological index (BIX), and humification index (HIX) of the dissolved organic matter (DOM) in the two routes are at similar levels, suggesting that the DOM

Table 1. Summary of the key parameters for the aquatic ecosystems in the Middle and East Routes

		The Middle Route	The East Route	References
Project overview	Length of water diversion route (km)	1432.0	1467.0	
	Designed water diversion capacity (billion m ³ /per year)	13.0	14.8	
	Hydraulic buildings	32 aqueducts, 117 inverted siphons and 15 culverts	22 hydraulic infrastructures and 34 pump stations	
	Start year of water diversion	2014	2013	
Ecosystem attributes based on Ecopath simulation ^a	Sum of all flows into detritus (TD) [t/(km ² ·year)]	9260.33	5473.20	28, 30
	Total primary production/total respiration (TPP/TR)	87.86	1.14	28, 30
	Total primary production/total biomass (TPP/TB)	91.60	6.92	28, 30
	Finn's cycling index (FCI) % of total throughput	2.87	6.77	28, 30
	Finn's mean path length (FML)	2.18	2.85	28, 30
	Content of dissolved organic carbon (mg/L)	3.39±0.14 (04/2019) 2.21±0.33 (09/2019) *	3.73±0.41 (04/2019) 4.19±0.43 (09/2019) *	33, 34
Reported data on carbon dynamics	Fluorescence index (FI) *	2.30±0.37	2.34±0.12	33, 34
	Biological index (BIX)	0.99±0.15	1.04±0.09	33, 34
	Humification index (HIX)	2.39±0.45	2.10±1.06	33, 34
	δ ¹³ C _{POM} for particular organic matter	-29.6‰~-29.1‰	-28.8‰~-23.5‰	35, 36
	δ ¹⁵ N _{POM} for particular organic matter	-0.88‰~0.99‰	2.33‰~9.16‰	35, 36
	C/N ratios for particular organic matter	5.0~7.0	6.3~8.6	35, 36
Major water quality risks	Pollutants of particular concern in routine monitoring	Chlorophyll-a (summer: 8.49±6.88 µg/L; winter: 1.09±0.77 µg/L)	Sulfate (106±80 mg/L) Fluoride (0.62±0.26 mg/L)	38, 39
	New pollutants of particular concern	Odorous compounds (2-methylisoborneol: 7.43±9.63 ng/L)	Antibiotics, herbicides	15, 40

a. The simulation of the East Route was based on the largest impounded lake - Hongze Lake.³⁰

b. * indicates the data from the Middle Route and East Route were significantly different according to the Kruskal-Wallis test ($P < 0.05$)

physicochemical characteristics of water had a strong direct effect on TOC, with both algal and microbial communities exerting indirect influences. Notably, the microbial factor exhibited higher path coefficients than phytoplankton, underscoring the need for a more in-depth investigation.

Microbial communities and their relation to environmental variables

We revealed the temporal and spatial changes in the dominant microbial phyla and genera of the microbial communities in the Middle (Figure S9) and East Routes (Figure S10), as described in detail in Text S1. The bacterial samples of the Middle Route separated along the first axis (29.1%) for different seasons, while the East Route samples from different seasons separated along the second axis (14.0%) (Figure S11). The microbial community similarity between the two routes consistently decayed with geographical distance for both bacterial (Figure 3A) and microeukaryotic groups (Figure S12). The slopes of the distance-decay curves for the microbial communities of the Middle Route (bacteria: 0.180, 0.165; microeukaryotes: 0.211) were all steeper than those for the East Route (bacteria: 0.119, 0.130; microeukaryotes: 0.103), indicating that geographical distance may play a more important role in determining the spatial similarity and in shaping the microbial community compositions in the Middle Route than in the East Route, particularly for the microeukaryotic plankton community.

We also compared the diversity of the bacterial and microeukaryotic plankton communities in the two routes. The results in Figure 3B show that the diversity indices, including the richness index (Chao1), diversity index (Shannon), uniformity index (Pielou's evenness, Pielou's e) and evolutionary diversity index (Faith's PD), of the bacterial community of the Middle Route were significantly lower than those of the East Route ($p < 0.001$). In contrast, this trend was not observed in the microeukaryotic plankton community in the two water transfer routes (Figure S13), whose diversity indices showed no significant differences. In addition, the Shannon diversity slightly decreased along the Middle Route channel, while it showed a general increasing trend for the East Route at similar diversion lengths (Figure S14). Regarding the

influence of environmental factors on microbial communities, the bacterial group in the Middle Route exhibited a correlation only with water pH among the tested water characteristics (Mantel test; $p < 0.01$, $r \geq 0.2$ in summer, $r < 0.2$ in autumn) in Figure S15. In contrast, the bacterial community in the East Route displayed a higher degree of correlation with various water characteristics. It was significantly correlated with total nitrogen (TN), total phosphorus (TP), and chemical oxygen demand (COD) in summer ($p < 0.01$, $r \geq 0.2$) and correlated with COD and electrical conductivity (EC) in autumn ($p < 0.01$, $r \geq 0.2$) (Figure S15).

Changes in community structure and assembly patterns along the diversion routes

The spatial divergence of the bacterial community along the two water transfer lines was analyzed by dividing the diversion routes into two sections, upstream and downstream, according to key hydraulic facilities, which showed different impacts on the taxonomic distribution and dominant population of bacterial communities in the Middle and East Routes. The results in Figure S16 reveal that the bacterial community of the Middle Route can be classified into two groups based on the season. The second axis indicates that the samples obtained from the two sides of the Yellow River Crossing Tunnel form two clusters. In contrast, the samples along the East Route can be clustered by season but not by hydraulic facilities.

The relationship between the occurrence frequency of operational taxonomic units (OTUs) and their relative abundances was described by the neutral community model, elucidating the assembly process for microbial communities in the two water diversion routes (Figure 3D). For the bacterial community, the relative contribution of stochastic processes explained 58.0% of the community variance for the Middle Route, while it explained 78.2% of the community variance for the East Route. Specifically, the low explainability of the neutral community model for middle-line bacterial assembly was more obvious in summer than in autumn, while the bacterial community in the East Route showed a similar goodness of fit for the

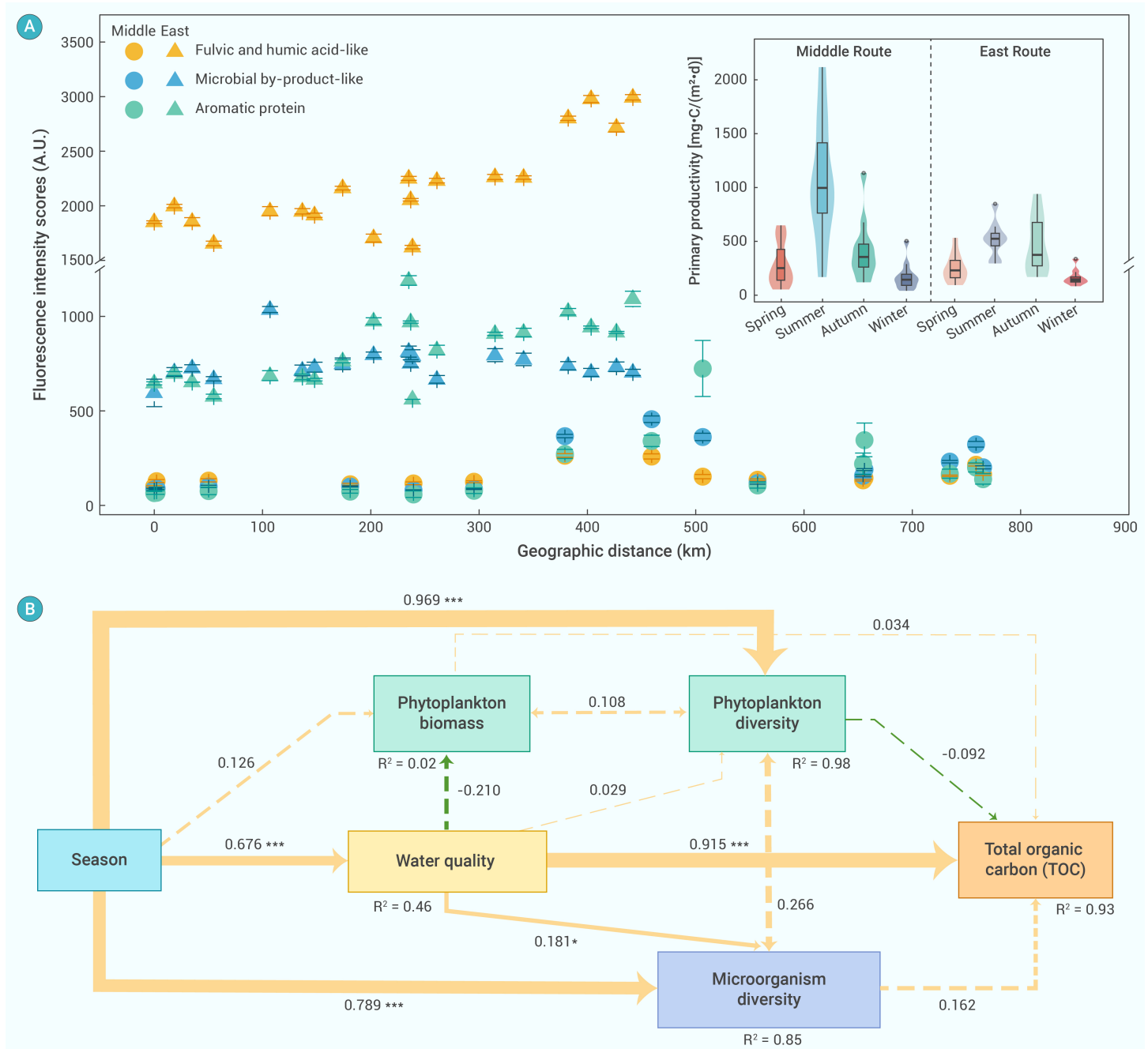


Figure 2. Analysis of key processes in the carbon cycle for the Middle and East Routes Variations in the levels of different types of dissolved organic matter, including fulvic and humic acid-like substances (yellow), microbial byproduct-like substances (blue) and aromatic proteins (green), based on a comparison of the relative intensities of three-dimensional excitation and emission matrix fluorescence of each sample along the Middle Route represented by circle shapes and the East Route represented by triangle shapes (A). Fulvic and humic acid-like substances were grouped together due to the low response value in the humic acid-like peaks. The seasonal variation in primary productivity along the Middle and East Routes was presented in the upper right corner (A). Graphical illustration of the structural equation model depicting the effects of investigated factors on total organic carbon in the Middle Route (B). A yellow arrow indicates a positive standardized path coefficient, while a green arrow indicates a negative standardized path coefficient. All arrows are scaled to the magnitude of their respective path coefficients.

model in the two studied seasons (Figure S17). For the microeukaryotes, the relative contribution of stochastic processes estimated by the neutral community model to community assembly was lower overall than that of the bacterial community (Figure S18). Checkerboard score (C-score) analysis based on the null model showed that the Middle Route displayed a much higher standardized effect size (SES) than the East Route (Figure S19), indicating the enhanced importance of deterministic processes for the bacterial assemblage of the Middle Route. Niche breadth analysis also showed that the bacterial community of the East Route exhibited wider niche breadth than that of the Middle Route (Figure S20). The average niche breadth of the East Route (18.2) was significantly higher than that of the Middle Route (14.1) according to the Wilcoxon test ($p < 0.05$). Further-

more, the bacterial assembly processes in the Middle and East water transfer routes were analyzed by the iCAMP approach, quantitatively identifying the relative importance of selection, dispersal, diversification and drift for community assembly. The results in Figure 3D show that deterministic processes, including heterogeneous selection and homogeneous selection, played more important roles in the bacterial assembly process of the Middle Route than in that of the East Route, which is consistent with the analysis results of the neutral model and null model. Additionally, Figure 3D reveals that the relative importance of drift for bacterial assembly in the Middle Route (44.1%) was much higher than that in the East Route (32.0%), while the dispersal limitation in the Middle Route (13.0%) showed lower relative importance than that in the East Route (31.0%).



Figure 3. Diversity of bacterial communities and their correlation to geographic distance. Assembly patterns of bacterial communities in two studied water diversion routes Relationship between geographic distance and Bray–Curtis similarity of bacterial communities in the Middle Route (A left) and East Route (A right) samples. Their relation was examined by Pearson's correlation analysis, and Pearson's r and p are annotated in the lower left corner of the figures. The Chao1, Shannon, Pielou's evenness (Pielou's e) and Faith's PD indexes of bacterial community diversity of the Middle and East Routes (B). The corresponding diversity indices for microeukaryote communities are presented in Figure S13. The predicted occurrence frequencies of bacterial communities from the Middle Route (C left) and the East Route (C right). The solid line is the best fit to the neutral community model (NCM), and the dashed line indicates the 95% confidence intervals around the NCM prediction. The fitting by the NCM of bacterial communities for different seasons is presented in Figure S17, and that of microeukaryotic communities is presented in Figure S18. R^2 represents the fit to this model. Relative contributions of different ecological processes to the bacterial assembly in the Middle (D left) and East Routes (D right) estimated by iCAMP.

Predictive functional analysis and shifts in carbon metabolic pathways

The significant differences among the PICRUSt-predicted Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways at level 2 of the different

sections of one water transfer route during the summer were examined using STAMP software, with $p < 0.05$ considered significant. For the Middle Route, Figure S21 shows that metabolic functions, including the metabolism of

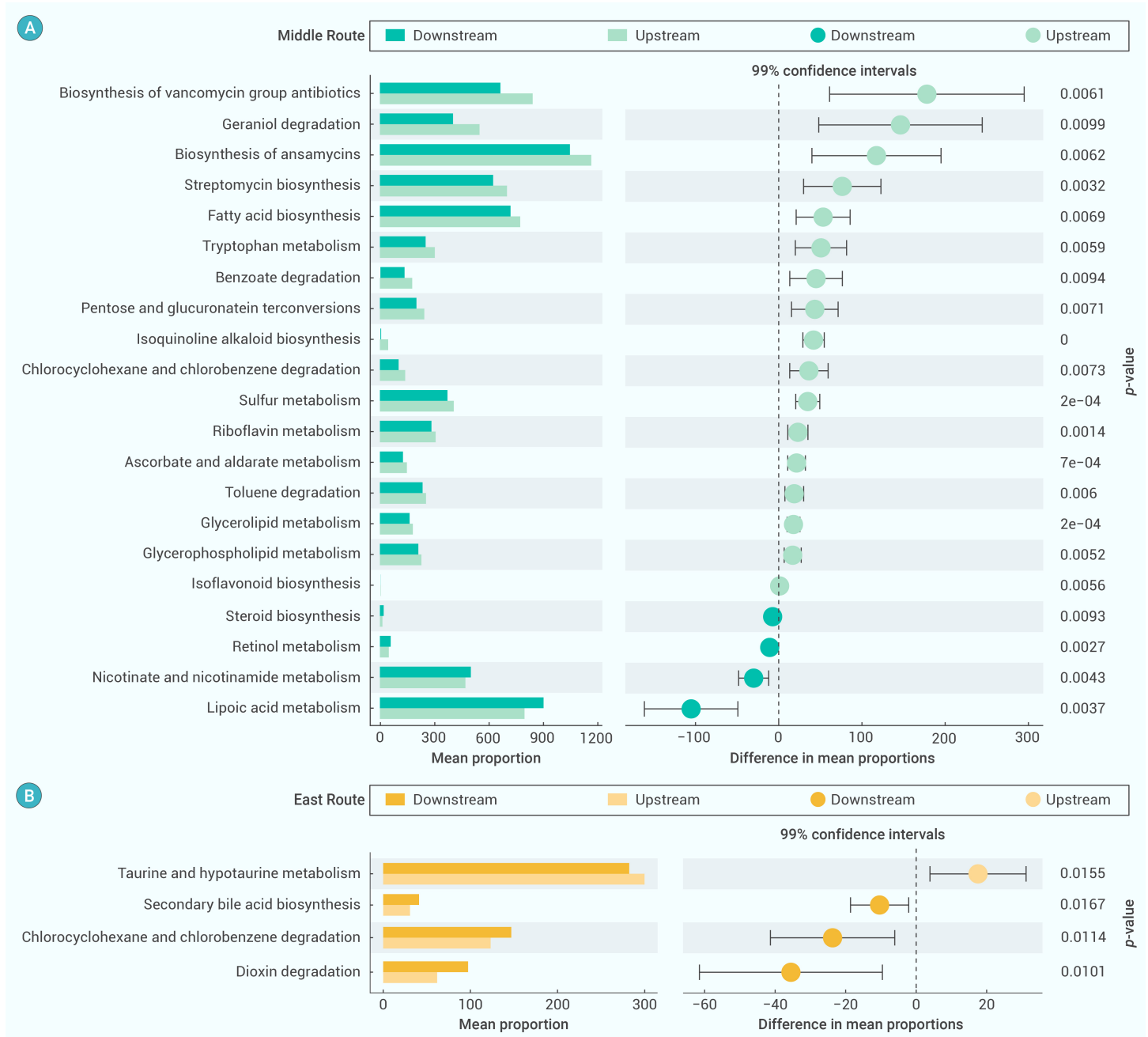


Figure 4. Significant differences in PICRUSt-predicted KEGG pathways between upstream and downstream groups in two studied water diversion routes The significant differences in the PICRUSt-predicted KEGG pathways at level 3 of the different sections of the Middle Route (A) and East Route (B) examined via STAMP software, with $P < 0.01$ considered significant. For the Middle Route, the upstream and downstream sections were divided by the Yellow River Crossing Tunnel, and for the East Route, the two sections were divided by the Huaian Sluice.

terpenoids and polyketides, lipids, carbohydrates and xenobiotics, were the most differentially expressed metabolic pathways in the upstream section, whereas cell growth and death were the most distinct functional pathways in the downstream segment. In contrast, the two sections in the East Route divided by the Huaian Sluice showed few significant differences in the KEGG pathways, except those of xenobiotic biodegradation and metabolism (Figure S22). Detailed shifts in microbial metabolism based on the KEGG pathways at level 3 were analyzed using STAMP software with $p < 0.01$. The results in Figure 4A revealed that metabolic pathways in the middle-route bacterial community weakened significantly from the upstream to downstream areas, particularly for the biosynthesis of vancomycin group antibiotics, geraniol degradation and biosynthesis of ansamycins derived from terpenoid and polyketide metabolic pathways. The shift in metabolic functions in the East Route bacteria (Figure 4B) showed the opposite pattern, in which xenobiotic biodegradation and metabolic pathways, mainly for dioxin,

chlorocyclohexane and chlorobenzene degradation, were enhanced downstream. To further explore the related microbial populations that caused the functional differences in the Middle Route, a heatmap of changes in the dominant microbial abundance is presented in Figure S23. The relative abundance of *Flavobacteria* decreased in the downstream section of the Middle Route. In contrast, filamentous anoxygenic phototrophic bacteria, mainly affiliated with *Chloroflexi* and its subgroups listed in Table S1, progressively gained a competitive advantage with the flow direction.

DISCUSSION

More deterministic assembly of bacteria in the concrete canal with reduced hydrological connectivity

The design of the water diversion route, including channel type and system openness, influences the assembly of microbial communities. The East and Middle Routes were developed with two different and typical diversion

measures. The East Route connects existing river channels with tributaries and lakes, while the Middle Route uses a newly excavated and constructed canal without any lateral input. In addition, the Middle Route canal is completely lined with concrete to prevent pollutant inputs and interaction with ground water.²⁴ Correspondingly, the microbial communities of the two water transfer lines were driven by different assembly processes. Specifically, the deterministic process of selection and the stochastic process of drift showed almost equal significance in influencing the community assemblies of bacteria in the Middle Route, while the stochastic processes of dispersal limit and drift dominantly drove the assembly of the East Route bacterial community. Therefore, the Middle Route bacteria tend to be controlled by niche assembly, while the East Route bacteria belong to a neutral community.²⁵ Here, we classified the main differences between the two water diversion methods primarily based on their hydrological connectivity, including river-stream connectivity and surface water-groundwater connectivity.⁴¹ The Middle Route exhibited a particularly low hydrological connectivity with no water exchange occurring with tributaries, floodplain wetland or groundwater, whereas the East Route increased the hydrological connectivity of the original channel.⁴² Normally, water diversion increased connectivity between isolated water bodies, and thus enhancing dispersal process⁴² as well as reshaping diversity of microbial community.⁴³ This work revealed that water transfer through an open concrete canal resulted in low hydrological connectivity, which increased the role of deterministic process in bacterial assembly. However, the observed community assembly trends of the bacteria were not applicable to the microeukaryotes present in the two studied water transfer lines; the microeukaryotes were probably more strongly controlled by the deterministic process of environmental filtering due to their larger body sizes,⁴⁴ as confirmed by the weak prediction of the neutral community model in Figure S18. In addition, the microeukaryotes in the Middle and East Routes also showed limited differences in diversity; thus, the high protection measure in water diversion design might have more impacts on the assembly and function of bacterial communities than those of the microeukaryotes.

Microbial assembly processes further affect the biogeographic patterns of communities. Although the community assembly in the Middle Route was less influenced by dispersal limitation than that in the East Route, the dominance of environmental selection and isolation from local structures that prevented "environmental generalist" microbes from being able to cross highly heterogeneous habitats²⁸⁻³⁰ resulted in a sharp decline in species similarity and a slight decrease in species diversity with geographic distance. The assembly of the East Route was significantly controlled by dispersal limitation; however, the dominance of stochastic processes and continuous introduction of environmental generalist microbial species resulted in weak distance decay of species similarity and an increase in species diversity. Additionally, a niche-based community (Middle Route) is theoretically sensitive to environmental filtering.^{31,32} Nevertheless, the Middle Route bacteria weakly correlated with the tested environmental factors, excluding pH, as shown in Figure S15. Here, we propose other potential unmeasured environmental filtering conditions that may affect the environmental selection of bacteria in the Middle Route. First, the smooth surface of the concrete canal in the Middle Route could serve as a filter that affects environmental selection. The roughness coefficient of the concrete surface of the Middle Route canal was 0.0147-0.015,⁴⁵ whereas the roughness coefficient of a natural river with a soil surface ranges from 0.025 to 0.040. The properties of the solid-liquid interface significantly affect microbial colonization, and low surface roughness results in low shear stress and hence weakens the adhesion of microorganisms to revetment surfaces.^{16,46,47} Second, the Middle Route withdraws water from the upper layer of the Danjiangkou Reservoir to the canal, which has low turbidity values generally less than two nephelometric turbidity units (NTU),^{48,49} thus, the suspended sediment content in the diverted water is low. This can result in inadequate habitat spaces for microbial reproduction and biofilm formation for microorganisms that utilize suspended aggregates as carriers.¹⁷ Moreover, low turbidity enhances light penetration and thus benefits the growth of specific bacterial groups and algae, as confirmed by the microbial analysis results in Figure S23 and the phytoplankton analysis results in Figs. S7 and S8. Third, the Middle Route has strict seepage control measures to prevent groundwater inflow and has wide isolation zones to prevent pollution from runoff, which compromises hydro-

logical connectivity and prevents an influx of local species from tributaries. Thus, the bacterial assembly process becomes more deterministic with reduced biodiversity and niche breadth due to enhanced environmental selection (Figures S24-S25). This change may, in turn, constrain community functions and diminish ecosystem resilience.^{50,51} This is exemplified by the significant modification of the microbial group in the Middle Route by the Yellow River Crossing Tunnel, whereas the group in the East Route appears to be less sensitive to the hydraulic facilities.

Community assembly pattern influences carbon cycling and water quality

The change in microbial functions significantly affects material flow within an aquatic system. The differentially abundant KEGG pathways clearly revealed dissimilar patterns of shifts in the metabolic pathways of the bacterial communities in the Middle and East Routes. A wide range of metabolic pathways associated with the biogeochemical cycles of carbon and nutrients declined from upstream to downstream in the Middle Route, while the biomass of dominant phytoplankton taxa continuously increased in the summer season. Specifically, the most differentially abundant pathways upstream of the Middle Route for terpenoid and polyketide metabolism, such as the pathways for geraniol degradation and the biosynthesis of ansamycins, have been reported to exhibit high abundance in wastewater treatment systems,³⁸⁻⁴⁰ indicating that they may be closely related to organic pollutant degradation. In contrast, limited differences were observed between the metabolic pathways upstream and downstream in the east line. The enrichment of xenobiotic biodegradation and metabolic pathways in the downstream section is probably due to increased contamination by persistent organic pollutants in the Suqian and Xuzhou segments of the East Route, which were assigned to the downstream section in this work.⁵² Currently, limited knowledge is available to clarify the mechanisms by which community assembly history influences ecosystem functions.⁵³ This work may provide some new insights. Closed systems with rigorous protection measures, such as the Middle Route, shift the balance between stochastic and deterministic assembly processes toward determinism when subjected to high selection pressures.⁵⁴ These intense selection processes might cause opportunistic species that have good adaptation mechanisms to the unique habitat to prevail in the community and impair community metabolic functions,⁴⁴ impeding the previously consistent biological cycles of substances. Correspondingly, environmental generalist microbes, such as *Flavobacteria* and *CL500-29-marine-group*, which are pioneers in organic matter mineralization and capable of utilizing nearly all types of carbon substrates,^{55,56} achieved limited growth in the system (Figures S16 & S23). In contrast, an open and diverse metacommunity that is driven by stochastic processes and continuously supplemented with local species can achieve stable metabolic functions.⁵⁷ The DOM analysis also implied a carbon dynamics problem in the Middle Route canal. In the East Route, the microbial community responded quickly to algal blooms; thus, the fulvic and humic acid-like substances increased after a high abundance of *Cyanophyta* occurred. However, the DOM content did not align with the trends observed in the continuous growth of phytoplankton in the Middle Route (Figure 2A and Figure S7). This implies that the organic matter derived from primary productivity mainly accumulates as detrital carbon with limited effective microbial assimilation, whereas the primary productivity of phytoplankton in the Middle Route attains the level observed in eutrophic rivers and lakes.^{58,59}

Despite the recognized importance of microbial metabolism in riverine carbon flux,^{60,61} there is little information on bacterial carbon metabolism in water diversion systems, particularly for highly protected and "closed" channels, such as the Middle Route. Carbon fluxes in floodplain rivers are thought to be governed by a balance between the longitudinal transport of carbon from catchments and the lateral transport of carbon between floodplains and river channels.⁶² However, this dynamic may not be directly applicable to the Middle Route, as it receives minimal carbon inputs from the catchment, and there are no carbon sinks in the floodplains or the ocean. In fact, both the Middle and East Route channels have relatively small allochthonous carbon inputs compared to those of natural streams, owing to the pollution control efforts of the Chinese government. Nonetheless, a question arises: why did organic matter not accumulate in the East Route while the Middle Route faced this challenge? Several previous studies have explored this issue by

examining algal proliferation.^{12,31,32} In contrast, our work contributes to understanding carbon dynamics in the water diversion channel through microbial ecology. We acknowledge the limitations, including incomplete carbon cycling data such as the absence of carbon dioxide emissions (pCO_2) and the quantification of enzyme activity. Nonetheless, we emphasize the urgency of exploring carbon cycling processes and efficiency within water diversion systems and advocate for the inclusion of this aspect during the design stage, as it is closely related to water quality. The East Route initially encountered greater challenges in water pollution control, primarily attributable to elevated sulfate and fluoride levels within the original river channel,³⁹ along with the presence of antibiotic and herbicide pollutants stemming from local breeding and agricultural activities (Table 1).⁴⁰ Water diversion helped alleviate this issue to some degree by facilitating water exchange.^{63,64} In contrast, the Middle Route transported water from the Danjiangkou Reservoir, characterized by lower concentrations of nutrients and pollutants (Table S2). Unexpectedly, the undecomposed algae in the canal have posed an increased risk of odor problems and the generation of disinfection byproducts. During the summer, the average concentration of 2-methylisoborneol exceeds the olfactory threshold.¹⁵

Highly protected water transfer canal compromises organic matter metabolism

For a long time, material engineers have directed substantial efforts into reducing canal surface roughness to reduce water head loss and to control water evaporation. However, this approach may need to be modified from the perspective of preserving resilient microbes in water diversion systems. The extremely smooth surfaces of concrete canals may increase selection pressures or enhance the environmental filtering of microbial taxa. Likewise, a highly protected and isolated water transfer system aimed at preventing external pollution likely disrupts hydrological connections and excludes the introduction of "environmental generalist" microbes. Additionally, it weakens the influence of the dispersal limit and drives a deterministic shift in the assembly of the bacterial community. Consequently, the engineering design of water diversion reshapes microbial communities and affects biogeochemical processes associated with organic matter decomposition, potentially jeopardizing ecosystem sustainability. Although this effect is difficult to predict due to the complex network of interactions that occur among microbes, such efforts are pivotal for enhancing the ecological and operational resilience of these ambitious water transfer schemes and ensuring their sustainable contribution to global water security.

MATERIALS AND METHODS

See supplemental information for details.

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AUTHOR CONTRIBUTIONS

H.L. Y., J.Y. Z. and Q.W. C. conceived the study. H.L. Y., Y.X. Z. and S.F. H. contributed to the field sampling data. C. C. Z.Y. W, Y.Q. L., and H.L. Y. collected the data. H.L. Y., T. F and Y.C. C performed the analysis. H.L. Y., and Q.W. C. wrote the manuscript. H.L. Y., Y.Q. L., and Q.W. C. reviewed and edited the manuscript. All authors read and approved the final manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

DATA AND CODE AVAILABILITY

All data are available in the main text and/or the supplementary materials.

SUPPLEMENTAL INFORMATION

It can be found online at <https://doi.org/10.59717/j.xinn-geo.2024.100075>