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Abstract

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Full Text

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Diversity, structure, and network relationships of microbial communities in different areas of the upper reaches of the Jinghe River, China

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Abstract: The Jinghe River, as one of the rivers flowing into the Yellow River, China, exhibits ecosystem vulnerability to agricultural activities and urbanization. However, the impact of agricultural activities and urbanization process on the physical-chemical properties and microbial community structure in its upper reaches has not been fully documented. This study selected three distinct sections along the upper Jinghe River: the Liupan Mountains Nature Reserve (upstream), agricultural area (midstream), and urbanized area (downstream). Through combined analysis of river physical-chemical parameters with high-throughput sequencing of bacterial and fungal communities, the results indicated significant increases in river water electrical conductivity (19.60%) and ammonium nitrogen content (170.74%) from the protected area to the urban section. Conversely, significant decreases were observed in total carbon (31.74%), total organic carbon (32.60%), dissolved organic carbon (27.91%), and nitrate nitrogen concentrations (53.44%). The abundance-based coverage estimation (ACE) index of bacteria was significantly higher in urban areas than in the protected area. The ACE index of fungi and the relative abundance of Proteomycota and Ascomycota were significantly higher in the protected area than in the urban area. Co-occurrence network analysis revealed higher complexity in the microbial network within the protected area and greater modularity in agricultural area, whereas the network structure in the urban area was simplified, indicating weakened network stability due to human disturbance. Furthermore, the abundance and distribution of Acidobacteriota were significantly and negatively correlated with river carbon components ($P < 0.01$), highlighting its crucial potential role in carbon cycling. This research aims to elucidate changes in water physical-chemical characteristics, microbial community structure, diversity, and interaction networks under human influence, providing a scientific basis for water resource management and ecological conservation in arid areas.

Keywords: physical-chemical properties; microbial community; co-occurrence network; Acidobacteriota; human disturbance

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1 Introduction

In arid ecosystems, rivers play key roles in maintaining ecological balance (Preheim et al., 2016), sustaining biodiversity through habitat provision, and facilitating biogeochemical cycling through material transport (Karr and Chu, 2000). In recent years, agricultural activities and urbanization process have impacted the physical-chemical properties of river systems (Hao et al., 2014). For example, highly urbanized processes and agricultural production typically increase the content of dissolved organic matter in water bodies (Wang et al., 2020). Agricultural activities, particularly fertilizer application and irrigation practices, contribute large quantities of nutrients (including carbon, nitrogen, and phosphorus) to river ecosystems, thereby accelerating eutrophication processes (Vinagre et al., 2016; Cesoniene et al., 2019). Furthermore, agricultural runoff has been demonstrated to increase the electrical conductivity (EC) of water bodies owing to the dissolution of ionic compounds from fertilizers (Nehme et al., 2014). Numerous studies have shown that the conversion of land uses in river basins is the main factor affecting the physical-chemical properties of rivers. Wilson and Xenopoulos (2009) found that the dissolved organic carbon (DOC) content in river water flowing through farmlands was lower than that in water flowing through forest lands. As the natural vegetation coverage of the river water decreased and human interference increased, the carbon components (total carbon (TC) and DOC) in the water showed a decreasing trend. As an important ecosystem connecting the land and ocean (Albert et al., 2021), changes in the physical-chemical properties of rivers may further affect the microbial community structure and ecological functions. Consequently, investigating the influence of agricultural activities and urbanization process on the physical-chemical characteristics of rivers and their associated microbial communities is important for water resource management and ecological conservation in arid areas.

Microbial communities in rivers are highly diverse and perform a multitude of ecological functions, such as accelerating the degradation of complex organic pollutants in water and promoting the release and cycling of elements such as carbon, nitrogen, and phosphorus (Prosser, 2012; Yin et al., 2022; Fang et al., 2023). Ammonia-oxidizing and denitrifying bacteria in river water are pivotal for nitrogen transformation processes, and also play an indispensable role in regulating the carbon-nitrogen cycle process (Xie et al., 2016; Sun et al., 2026). The composition and metabolic activities of these microbial assemblages are gov-

erned by a suite of environmental factors (Hu et al., 2017), among which changes in pH, dissolved oxygen, nitrogen, phosphorus, salinity, and heavy metals can lead to differences in the structure and function of river microbial communities (Jin et al., 2014; Yan et al., 2015; Zhang et al., 2019b). Notably, human activity has emerged as a significant determinant of water microorganisms: the urbanization process in flowing river areas and the interference of agricultural activities have caused changes in the physical-chemical properties of river water, further altering the composition and diversity of river microbial communities (Hu et al., 2023). For example, human interference often results in a substantial increase in pollutant concentrations in water bodies, and high concentrations of $\text{NH}_4^+\text{-N}$ can inhibit the activity of autotrophic nitrifying microorganisms, thereby reducing the richness and diversity of nitrifying microorganisms (Zhang et al., 2021a). Changes in bacterial community structure are closely related to environmental factors, and their diversity can serve as important biological indicators for evaluating the degree of river pollution (Findlay, 2010). In urban rivers, the high abundance of microorganisms associated with carbon and nitrogen metabolism suggests that human activities may be the primary driving force behind the transformation of bacterial communities (Zhang et al., 2020).

Previous studies have established a significant correlation between the microbial diversity in river ecosystems and the complexity of their interaction networks (Escalas et al., 2019). The network structure of microbial communities plays an important role in enhancing ecosystem resistance, coping with external disturbances and maintaining ecosystem stability (Worden et al., 2015). In recent years, the extensive utilization of high-throughput sequencing technology has enabled researchers to analyze the network structure of microorganisms with greater precision

and to identify the influence of environmental alterations on them (Anderson and Harvey, 2022). Through network relationship analysis, the structure and mutual functions of microbial communities in a system can be understood, thereby allowing the analysis of key microorganisms in the microbial community (Zheng et al., 2021). Numerous studies have shown that the dominant microbial phyla in river ecosystems include Proteobacteria, Firmicutes, Bacteroidota, and Ascomycota (Bagagnan et al., 2024). Moreover, network interaction relationships among microorganisms vary according to changes in environmental conditions. Li et al. (2023) suggested that the combined effect of land-use patterns and river pollution in river systems could reduce microbial diversity and lead to a decrease in network modularity, thereby weakening ecosystem stability.

The Jinghe River is located inland at the eastern foot of the Ningxia Hui Autonomous Region, China (Chen et al., 2007), and is influenced by the continental monsoon climate. Its runoff is unevenly distributed throughout the year, with the flood season concentrated from July to October, accounting for over 60.00% of annual runoff (Wei and Lin, 1996). Jingyuan County, where the southern headwaters of the Jinghe River are located, is characterized by a less developed economy and a fragile ecological environment owing to both natural and histor-

ical constraints. In recent years, with the escalation of agricultural activities and the acceleration of urbanization, the water bodies of the Jinghe River have faced pressure from the input of nutrients, such as nitrogen and phosphorus, as well as organic pollutants, which in turn affect the material cycle and microbial community stability of the river ecosystem. Considering the high sensitivity of water-body microorganisms to environmental alterations (Zhang et al., 2016), it is important to explore the influence of agricultural activities and urbanization on the microbial community structure of water bodies. Previous studies have focused more on the ecological effects of large-scale rivers, such as the Yellow and Yangtze rivers, focusing on macro-ecological characteristics at the basin scale, such as the spatial distribution of bacterioplankton communities across the whole basin (Fan et al., 2016), while insufficient research has been conducted on the microbial response mechanisms of small- and medium-sized rivers, especially those directly affected by human activities. This study focuses on the regional span of small- and medium-sized rivers (Jinghe River), where environmental heterogeneity is strong and the temporal and spatial differences in microbial communities are more refined. The Yellow River Basin faces challenges such as water scarcity, severe fluctuations in runoff, and ecological fragility, making its tributaries (e.g., the Jinghe River) ecologically unique in terms of processes and microbial responses to environmental functions. These characteristics render such areas distinctive and irreplaceable as research subjects in other basins (Wang, 2022). Consequently, this study selected the upstream section of the Jinghe River (from the Liupan Mountains Nature Reserve to the urban area) as the study area. By measuring the physicochemical properties of the river and conducting microbial high-throughput sequencing, this research endeavors to address the following scientific issues: (1) the impact of agricultural activities and urbanization on the physicochemical properties and material accumulation of water bodies; and (2) how these physicochemical changes further affect the composition, diversity, and network relationships of microbial communities in water bodies, thus revealing the mechanism by which agricultural activities and urbanization influence river microbial ecology.

2 Materials and methods

2.1 Study area and sample collection

The Jinghe River originates from the eastern foothills of the Liupan Mountains in Jingyuan County, Guyuan City, Ningxia Hui Autonomous Region, flows through Yuanzhou District of Guyuan City and Jingyuan, Pengyang, and Yanchi counties (Zhang et al., 2024b), and then enters Gansu Province. The main stream in the Ningxia Hui Autonomous Region section is approximately 40 km. The sampling area of this study is located at the Liupan Mountains in Jingyuan County.

(35°38' 60" 'N, 106°19' 48" ' E), with the river source at an elevation of approximately 2850 m. The study area has a typical temperate semi-humid to semi-arid transition climate, with an annual average temperature of 4.1°C–9.5°C

and an average annual precipitation of 350.0–650.0 mm (Tang et al., 2019). The average runoff depth in the Jinghe River Basin is 65.9 mm, and the average water surface evaporation is 800.0 mm (Zhang et al., 2024b). Its hydrological seasonal characteristics are significant, with the dry season usually from November in each year to March–April of the following year, and the wet season concentrated from July to September, during which precipitation can account for more than 60.00% of the annual total precipitation (Tian et al., 2025).

Three sampling sections were set up along the riverbank from west to east: upstream, midstream, and downstream (Fig. 1). The upstream section is located at the river source within the Liupan Mountains Nature Reserve, the midstream section is distributed in the agricultural area on the eastern foothills of Liupan Mountains, and the downstream section is near Jingheyuan Town. Before sampling, it is necessary to remove debris, such as floating or submerged branches and dead leaves, from the water surface to water body. During water sample collection, the polyethylene plastic sampling bottles were rinsed three times with the water to be collected. The sampling points were set at 0.5 m below the water surface of the study section. If the water depth was less than 0.5 m, sampling was conducted at half the water depth. The flow of Jinghe River is relatively stable from May to June and from September to October. Therefore, the sample collection of this experiment was carried out in October 2023. Eight sampling points were randomly selected from each section within the same water surface range (3 m×river width), that is, eight duplicate samples, the collected samples were stored at 4°C and sent to the laboratory for subsequent determination of physical-chemical parameters and microorganisms.

Fig. 1 Schematic diagram of the upper reaches of the Jinghe River (a) and pictures of sampling sections (b–d)

2.2 Determination of water physical-chemical properties

We determined the physical-chemical indicators of the collected water samples according to the

Water and Wastewater Monitoring and Analysis Methods (State Environmental Protection Administration, 2002). The EC and pH were measured *in situ* using a Hach HQ40D handheld meter (Hach Company, Loveland, USA). TN was determined using the Kjeldahl method, the concentrations of $\text{NH}_4^+ - \text{N}$ and $\text{NO}_3^- - \text{N}$ were measured using potassium chloride extraction-spectrophotometry (Liu et al., 2010), and dissolved organic nitrogen (DON) was obtained by calculating the difference between TN and inorganic nitrogen. TC, total inorganic carbon (TIC), and DOC were quantitatively analyzed using a total organic carbon analyzer (Vario TOC cube, Elementar, Frankfurt, Germany). All physical-chemical data of water are expressed as mean±standard error. One-way analysis of variance (ANOVA) was performed using SPSS v.25.0 software, and Duncan's multiple range test was used to determine significant differences between means at a significance level ($P < 0.05$).

2.3 Deoxyribonucleic acid (DNA) extraction, polymerase chain reaction (PCR) amplification, and high-throughput sequencing of the Jinghe River water

Total bacteria and fungi in water were extracted using a modified cetyltrimethylammonium bromide (CTAB) method. The V3-V4 region of the bacterial 16S ribosome ribonucleic acid (rRNA) gene was amplified using primers 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'), and fungi were amplified using primers ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') and ITS2 (5'-GCTGCGTTCTTCATCGATGC-3') (Claesson et al., 2009). The PCR reaction system comprised 10×buffer 2.0 μ L, deoxyribonucleotide triphosphate (dNTPs; 2.5 mmol/L) 2.0 μ L, forward and reverse primers (10 μ mol/L) each 0.8 μ L, Phusion DNA polymerase 0.2 μ L, DNA template 1.0 μ L, and ddH₂O to make up 20.0 μ L. The PCR reaction conditions were as follows: 98°C for 1 min; 98°C for 10 s, 50°C for 30 s, 72°C for 30 s, 30 cycles; final extension at 72°C for 5 min (Wujisiguleng et al., 2022). DNA extraction (the optical density (OD)₂₆₀/OD₂₈₀ ratio falls within the range of 1.8–2.0), PCR amplification and sequencing were performed by Beijing Novogene Bioinformatics Technology Co., Ltd, China. The 16S rRNA and internal transcribed spacer (ITS) gene sequences obtained in this study were submitted to National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) database with serial numbers PRINA1357031 and PRINA1357039, respectively.

2.4 Data analysis

The sequencing data were processed using quantitative insights into microbial ecology (QIIME) v1.9.1 software. The species diversity and richness of microbial communities were systematically evaluated by calculating the operational taxonomic unit (OTU), Shannon, Simpson, Chao1, and abundance-based coverage estimation (ACE) indices of the water microbial communities. R v.4.1.0 software was used to analyze α -diversity. Based on the Bray-Curtis distance matrix, we performed non-metric multidimensional scaling (NMDS) using the “vegan” package to visualize differences in microbial community structure between different river sections, and analysis of similarities (ANOSIM) was used to test differences in community structure between groups (Pan et al., 2021). Visualization of community structure and ecological processes was achieved using the “ggplot2” package (Kang et al., 2022).

To explore the interactions between microorganisms, we constructed co-occurrence networks of bacteria and fungi based on Spearman's correlation ($|r| \geq 0.6$; $P < 0.050$), and used the “graph” package to calculate the key topological parameters of each network, including nodes, edges, average density, transitivity, diameter, and average path length, and to comprehensively analyze the complexity of community structure and the strength of interactions (Ma et al., 2016). Principal component analysis (PCA) of the subnetwork data revealed the complexity of the microbial network (Pan et al., 2024). The

correlation between microbial diversity indices and water physical-chemical parameters was analyzed using the Mantel test (Sunagawa et al., 2015), and a Random Forest regression model was used to identify the main environmental drivers affecting key microbial taxa (such as Acidobacteriota, Desulfobacterota, and Ascomycota). Finally, phylogenetic analysis was

performed on key taxa (such as Acidobacteriota), and the relationship between their members and environmental factors was discussed in combination with their relative abundance heat map.

3 Results

3.1 Physical-chemical properties of water bodies

As shown in Figure 2a, there was no significant difference in river pH among different river sections. In contrast, EC significantly increased as the river flowed downstream (Fig. 2b). TC, TIC, and DOC contents significantly decreased along the river flow direction, with downstream levels declining by 31.74%, 32.60%, and 27.91%, respectively, compared with upstream levels ($P < 0.050$; Fig. 2c-e). Further analysis revealed differences in the contents of various nitrogen indicators among different sections of the Jinghe River. TN contents in the upstream and midstream water bodies were similar and significantly higher than that in the downstream (Fig. 2f). NO_3^- -N content (Fig. 2g) decreased significantly in the downstream compared with the upper (53.44%) and middle reaches (56.59%). NH_4^+ -N content showed an opposite trend, being significantly higher in the downstream than in the upstream and midstream (Fig. 2h). The differences in DON content among different river sections were relatively small (Fig. 2i). According to the calculation of carbon and nitrogen contents in the river water, there was no significant difference in DOC:DON ratio among different river sections ($P > 0.050$; Fig. 2j).

Fig. 2 Differences in physical-chemical indicators of water bodies in different sections of the Jinghe River. (a), pH; (b), EC (electrical conductivity); (c), TC (total carbon); (d), TIC (total inorganic carbon); (e), DOC (dissolved organic carbon); (f), TN (total nitrogen); (g), NO_3^- -N (nitrate nitrogen); (h), NH_4^+ -N (ammonium nitrogen); (i), DON (dissolved organic nitrogen), (j), DOC:DON ratio. The abbreviations are the same in the following figures. Different lowercase letters indicate significant differences among different river sections at $P < 0.050$ level. Bars are standard errors.

3.2 Microbial diversity and its correlation with environmental factors

In each river section, there was no significant difference in the Shannon index of river bacteria; however, there was an increasing trend in the ACE index of bacteria from upstream to downstream, with the downstream being significantly higher than the upstream (Fig. 3a and b). The Shannon index of fungi in different river sections showed no significant differences, whereas the fungal

ACE index was significantly higher in the upstream than in the middle and downstream ($P < 0.050$; Fig. 3c and d). The Mantel test and Spearman's correlation analysis showed a significantly positive correlation among pH, EC, and TC (Fig. 3e). In addition, TC, TIC, and DOC were positively correlated with NO_3^- -N and negatively correlated with NH_4^+ -N ($P < 0.050$). Fungal diversity indices (Shannon and ACE) were significantly and negatively correlated with pH and EC, and positively correlated with TC, TIC, DOC, and TN ($P < 0.050$).

Fig. 3 Shannon and abundance-based coverage estimation (ACE) indices of bacterial (a and b) and fungal (c and d) community diversity, as well as the correlations between microbial diversity and physicochemical indicators (e) in different sections of the Jinghe River. In Fig. 3a–d, different lowercase letters indicate significant differences among different river sections at the $P < 0.050$ level. Bars are standard errors. , $P < 0.050$; , $P < 0.010$; , $P < 0.001$.

3.3 Microbial community structure characteristics and key environmental factors

NMDS analysis (Fig. 4a and b) showed differences in the bacterial community structure among the upstream, midstream, and downstream sections (stress = 0.0792; $R^2 = 0.9232$; $P = 0.001$). Similarly, the fungal community structure exhibited spatial heterogeneity across reaches, with significant differences observed between the upstream and downstream sections (stress = 0.0665; $R^2 = 0.9854$; $P = 0.001$). In the river-water bacterial community, DOC, TC, and EC were important factors affecting bacterial NMDS1, whereas NO_3^- -N, TN, and TIC were important factors affecting NMDS2. For fungal communities, factors such as DOC, TC, and TIC affected NMDS1, with NO_3^- -N, DOC, and TIC playing significant roles in NMDS2 (Fig. 4c and d).

The bacterial community in the Jinghe River was mainly composed of Proteobacteria, Firmicutes, Bacteroidota, and Verrucomicrobota, with high relative abundances in each river section. Differences were observed in the relative abundances of Firmicutes and Bacteroidota, with Firmicutes being significantly more abundant in the midstream than in the upstream (Figs. 5a and S1). The fungal communities were primarily Ascomycota, Chytridiomycota, and Rozellomycota. The relative abundance of Ascomycota was low along the river, whereas that of Rozellomycota was significantly higher in the downstream than in the upstream (Figs. 5b and S2). Desulfobacteria (59.22%), Acidobacteriota (46.36%), and Ascomycota (61.17%) were identified as the key taxa with high explanatory power in the model (Fig. 5c). Random Forest analysis showed that pH, TC, TIC, DOC, and TN were negatively correlated with Desulfobacteria and Acidobacteriota, whereas EC and NH_4^+ -N were positively correlated with both. Ascomycota showed the opposite trend (Fig. 5d).

3.4 Topological characteristics of microbial co-occurrence networks

Co-occurrence networks can reflect the stability of community structure and the efficiency of material and energy transfer. The constructed co-occurrence networks indicated that the bacterial-fungal co-occurrence network in the upstream section was more complex than those in the midstream and downstream sections. The numbers of nodes and edges in the upstream section were 1437 and 5823, respectively. In the midstream, these values were 1014 and 2986, respectively, while in the downstream, they were 919 and 2583, respectively (Table 1). In contrast, as the flow direction of the river decreased, the number of nodes and edges in the microbial co-occurrence network

Fig. 4 NMDS (non-metric multidimensional scaling) analysis of microbial community structure of bacteria (a) and fungi (b) in different sections of the Jinghe River, and the impact of environmental factors on NMDS of bacterial (c) and fungal (d) communities. MSE, mean squared error. *, $P < 0.050$ level; **, $P < 0.010$ level.

decreased, and the interactions between fungi and bacteria were more prominent in the upstream. The abundance of total nodes and edges indicated that the richness of components was high, and the network density was high, that is, the overall connection of the community was closer. The modularity coefficient reflected the degree of community clustering. The modularity coefficient of the midstream (0.7813) was higher than those of the upstream (0.7212) and downstream (0.7184), indicating stronger functional clustering of microbial communities in the midstream (Fig. 6). High modularity coefficient can improve the anti-risk ability of the community, and large average path length and diameter reflect the transmission efficiency and strong community connectivity.

3.5 Differentiation and driving factors of microbial community structure

PCA result revealed that PC1 (principal component) and PC2 accounted for 42.72% and 34.36% of the community differences, respectively. Microorganisms in each river section showed a certain degree of aggregation, indicating that environmental differences contributed to the formation of distinct microbial community structures (Fig. 7a). This study further found that Acidobacteriota played a dominant role in the core ecological process of the study area, and its ecological effect was more significant, therefore analysis of the relative abundance of Acidobacteriota and the niche competition index (NCI) showed a significantly positive

Fig. 5 Relative abundance of the top 10 phyla of bacteria (a) and fungi (b) in different river sections. (c), explanatory variation of environmental factors for dominant microbial phyla; (d), correlation and importance of environmental factors associated with dominant microbial phyla. In Figure 5d, circle size

represents the importance of each variable and background color indicates the strength and direction of the correlation.

Table 1 Network topology characteristics of water bodies in different river sections

Topological feature	Upstream	Midstream	Downstream
Total nodes	1437	1014	919
Total edges	5823	2986	2583
Positive edges	4303	1782	1539
Negative edges	1520	1204	1044
Modularity	0.7212	0.7813	0.7184
Average path length	5.5749	6.0973	6.1227
Graph diameter	14.6461	13.8583	18.4355
Graph density	0.0056	0.0058	0.0061
Clustering coefficient	0.4012	0.4163	0.3790
Betweenness centralization	0.0136	0.0281	0.0368
Degree centralization	0.0180	0.0120	0.0255

correlation ($P = 0.014$, $R^2 = 0.2092$; Fig. 7b). The heatmap of the phylogenetic relationship between the phylum Acidobacteriota and its physical-chemical properties showed that the members of the phylum mainly consisted of *Luteritalea*, Subgroup 10, *Blastocatella*, *Stenotrophobacter*, and *Bryobacter*. Most members showed a significantly positive correlation with the carbon components (TC, TIC, and DOC) and a negative correlation with EC and NH_4^+ -N (Fig. 7c).

4 Discussion

4.1 Factors affecting physical -chemical properties of the Jinghe River water

As a typical river basin with severe soil erosion in the arid area of northwestern China, the water

Fig. 6 Co-occurrence networks of bacteria and fungi in the upstream (a), midstream (b), and downstream (c) of the Jinghe River. Circles represent bacterial nodes, triangles represent fungal nodes, and different colors represent different phyla.

Fig. 7 Analysis of Acidobacteriota community structure, significance, and taxonomic composition in different sections of the Jinghe River. (a), PCA (principal component analysis) result; (b), correlation analysis between relative abundance of Acidobacteriota and NCI (niche competition index); (c), phylogenetic tree of Acidobacteriota taxonomic units and correlations with environmental factors. PC, principal component.

carbon content of the Jinghe River is influenced by multiple factors, including climate, land use type, agricultural activities, and urbanization process (Wang et al., 2020). In this study, under the influence of various factors, the carbon content of different forms of the Jinghe River showed significant differences in different river sections. The TC, TIC, and DOC contents in the upstream were significantly higher than those in the midstream and downstream. This result is consistent

with that of Zhu et al. (2020) at different locations of rivers in similar climate zones on the Loess Plateau. The Jinghe River is located in the arid northwestern area of China, where soil erosion is severe. The soil surface in the basin is rich in organic carbon, which flows into the Jinghe River through soil erosion, resulting in abundant organic carbon in the river water. Inorganic carbon in the river water is mainly derived from soil respiration, chemical weathering, and physical erosion, and is carried into rivers through surface runoff (Paula et al., 2017). Human activities also play a crucial role in the regulation of inorganic carbon levels. With the development of society and the increase in human activities, such as water conservancy measures, the carbon content of river water has changed. Related studies have shown that during the process of water flowing through rivers, as organic matter in the water decomposes and releases CO_2 , the organic carbon content in the water decreases (Ran et al., 2015), resulting in a significant decrease in the carbon content in the downstream compared with that in the upstream. The upstream of the Jinghe River is located in Liupan Mountains Nature Reserve, which is a forest ecosystem with high organic carbon content (Wang et al., 2020). There is a strong correlation between DOC in the river and organic carbon in the watershed (Li et al., 2017), resulting in a high DOC content in the upstream. However, the downstream is located at the gathering place of villages, and there is a large amount of farmland in the river basin, which damages the soil structure, resulting in a decrease in organic carbon content (Naipal et al., 2018). Consequently, the water-soluble organic carbon content in the downstream decreased.

Substances present in rivers are rich and diverse, and their forms and concentrations are strongly influenced by water conditions and the surrounding environment (Mayer et al., 2007). This influence is reflected in the observed variations in the distribution of different nitrogen compounds in the Jinghe River. The results indicated that the concentrations of TN, NO_3^- -N, and NH_4^+ -N in the Jinghe River varied significantly along the upstream to downstream gradient; however, the differences in DON were small, which was closely related to the environmental characteristics and intensity of agricultural activities and urbanization process in the watershed. The upstream of the Jinghe River is located in a forest community environment and is less affected by human activities, resulting in lower inflow of sewage and pollutants. Relatively intact riverine ecosystems exhibit greater stability and self-purification capacity, enabling the effective breakdown of incoming organic pollutants (Zhou et al., 2024). Therefore, there were generally fewer nitrogen compounds in the upstream, the concentrations of TN and NO_3^- -N were lower than those in the midstream. However, there

are villages clustered in the midstream and downstream areas surrounded by a large amount of farmland. The nitrogen fertilizer used in agriculture is not fully absorbed and flows into rivers through surface runoff, thereby increasing the TN content of river. As a result, the TN content in the midstream was higher than that in the upstream (Ji et al., 2007; Zhang et al., 2019a). The decomposition of animal and plant residues in agricultural activities and the discharge of human domestic wastewater have led to an increase in the TN and NO_3^- -N concentrations in water bodies (Elassassi et al., 2022), and may cause eutrophication in the downstream (Malik and Nadeem, 2011). Denitrification caused by environmental changes such as slow river flow, organic matter accumulation, water eutrophication in the downstream, and organic nitrogen mineralization led to the decrease of TN and NO_3^- -N concentrations and the increase of NH_4^+ -N concentration (Zhang et al., 2021b; Chen et al., 2024). The sediment adsorbed NO_3^- -N, which reduced the content of NO_3^- -N in water. This trend is aggravated by the desorption of NH_4^+ -N and the diffusion of NH_4^+ -N produced by mineralization into the water (Zhang et al., 2024a). In this study, the changes of physical-chemical properties (pH, EC, carbon, and nitrogen) of river water changed the living environment of microbial community, and then regulated the microbial community structure and metabolic activities.

4.2 Response of microbial community structure to environmental changes

The diversity of microbial communities in different river basins of the Jinghe River showed significant differences caused by the combined effects of various natural and human factors from

upstream to downstream (Liu et al., 2024). Previous studies have shown that agricultural activities and urbanization process can interfere with river flow and affect natural environmental conditions, leading to a decrease in the biodiversity of microbial communities (Mishra et al., 2021). The results of this study indicate that the bacterial ACE index decreased gradually from upstream to downstream, whereas the fungal ACE index exhibited an opposite trend, increasing along the same gradient. This result may be due to the migration and diffusion of microorganisms caused by the flow of river water, resulting in differences in the distribution of bacterial and fungal communities in the river, which also reflects their differential responses to environmental factors (Read et al., 2015). In addition, Proteobacteria, Firmicutes, and Bacteroidota were the dominant bacterial phyla in the Jinghe River water, whereas Ascomycota, Chytridiomycota, and Rozellomycota were the main fungal phyla, which is consistent with the conclusions of Bagagnan et al. (2024). Due to the accumulation of organic matter in the downstream, some members of Proteobacteria and Bacteroidota in bacteria can make full use of organic matter, thus improving the ACE index of bacterial community (Huang et al., 2022). Fungi such as Ascomycota are more suitable for decomposing and utilizing natural and complex organic carbon sources in plant litter, so they show a high ACE index in natural forest of the upstream

(Gutiérrez et al., 2011; Gruppuso et al., 2023).

The diversity of microbial communities in the Jinghe River is closely related to the physical-chemical properties of the water. Environmental factors such as organic matter, carbon, and nitrogen in the river water are important factors that affect structural changes in river microbial communities (Qin et al., 2017). Existing research has shown that physical chemical factors such as pH and organic matter content in the environment can drive changes in microbial community structure, and microbial metabolic activities can participate in the decomposition and transformation of carbon and nitrogen organic matter, thereby changing the soil environment and forming a feedback loop between physical-chemical factors and microbial communities (Hermans et al., 2020). This study found negative correlations of the fungal diversity index (Shannon and ACE) with pH and EC, as well as positive correlations of the fungal diversity index with TC, TIC, DOC, and TN, supporting the regulatory effect of river water physical-chemical characteristics on microbial diversity. Simultaneously dissolved oxygen (DO) and total phosphorus (TP) also have a regulatory effect on river microbial communities, and the gradient change of DO has a guiding role in the distribution of nitrogen cycling functional microorganisms. The enrichment of TP provides a nutritional basis for phosphorus metabolism related microorganisms, leading to an increase in the relative abundance of such microorganisms in the river water (Jiang et al., 2021; Liu et al., 2021). Notably, the intensity of the anthropogenic disturbance showed an increasing trend from upstream to downstream. Downstream pollution is more significantly affected by urbanization, and nitrogen enrichment in water bodies caused by human pollutant inputs is particularly prominent (Xu et al., 2023). Microorganisms are important for carbon and nitrogen cycling in rivers (Xu et al., 2023), which stimulates the functional response of microorganisms involved in nitrogen metabolism, altering their relative abundance and community composition (Lin et al., 2019; Chen et al., 2025). Previous studies have confirmed that excessive nitrogen input can reduce the diversity and abundance of bacterial communities (Zhu et al., 2023), which is consistent with the results of the present study.

Microbial community composition in the upstream, midstream, and downstream of the Jinghe River showed significant differences, which are closely related to the physical-chemical properties of the water and human activities. Studies have shown that the distribution of microbial communities is strongly influenced by environmental factors, such as carbon and nitrogen components, EC, pH, and land use patterns (Liu et al., 2018). In the upstream of the Jinghe River, natural environment of the forest ecosystem provides a stable ecological niche for microorganisms, where Proteobacteria, Firmicutes, and Bacteroidota are the dominant bacterial phyla, and Ascomycota and Chytridiomycota are the dominant fungal phyla. This community composition is closely related to the higher organic carbon (DOC and TC) content and lower EC in the upstream (Logue et al., 2016). In contrast, downstream areas are susceptible to nitrogen enrichment due to agricultural and urbanization activities, leading to a sim-

plification of the microbial community structure, manifested by a decrease in bacterial diversity (ACE index) and an increase in fungal diversity. These differences reflect the response mechanisms of microorganisms to different environmental pressures. Bacteria are more sensitive to nitrogen, whereas fungi may achieve adaptive growth by utilizing organic matter in eutrophic environments (Dai et al., 2018). Further analysis of the microbial symbiotic networks revealed differences in the stability of community composition.

4.3 Effects of agricultural activities and the urbanization process on the complexity and stability of microbial networks

River ecosystems are closely associated with land-use patterns (Liu et al., 2018). Many human activities (such as domestic sewage discharge and agricultural pollution inputs) can alter the physicochemical characteristics of water bodies (Peng et al., 2020). This in turn affects the structure and distribution of bacterial communities (Zhao et al., 2021) and reduces the biodiversity of microbial communities (Mishra et al., 2021). Microbial symbiotic networks are also disrupted (Savio et al., 2015), and both abiotic factors (such as DOC, temperature, TP, and sulfate) and biotic factors are significantly correlated with network indices (Liao et al., 2025). A previous study found that as the diversity index of bacterial communities increased, their network connectivity also increased (Wu et al., 2021). This study revealed significant differences in the network structures of microbial communities in the upstream, midstream, and downstream reaches of the Jinghe River, which were closely related to environmental differences and niche differentiation (Shi et al., 2025).

Research has shown that from upstream to downstream, the complexity of the microbial network gradually decreased, primarily due to differences in the land characteristics along the river. The upstream reach has high vegetation coverage within the reserve and is less affected by human interference, providing rich ecological niches for microorganisms and promoting the formation of highly correlated networks among microorganisms (Hui et al., 2023). In contrast, the downstream reach experiences intensive human activities, leading to the accumulation of pollutants and resulting in a decrease in the stability of microbial community networks and microbial diversity. These disruptions reflect a weakening of microbial interactions under environmental stress (Hernandez et al., 2021), indicating that agricultural activities and the urbanization process weaken the functional connections of microorganisms (Wagg et al., 2019). Previous studies have shown that network complexity can reduce the impact of environmental disturbances on community composition (Hui et al., 2023), further confirming this conclusion. In addition, the weakening of microbial network interactions can reduce the efficiency of ecological processes such as organic matter degradation and nitrogen cycling (Wagg et al., 2019). This functional degradation and pollutant accumulation may form a feedback loop, exacerbating the decline in the water's self-purification capacity.

4.4 Distribution pattern and environmental driving mechanism of the Acidobacteriota phylum

As an important component of the microbial community in the Jinghe River, Acidobacteriota exhibit significant differences in distribution and abundance across different river sections, mainly influenced by factors such as carbon composition, EC, and nitrogen (Jones et al., 2009). In this study, a significant positive correlation was observed between the relative abundance of Acidobacteriota and the NCI and was closely correlated with carbon components (TC, TIC, and DOC), indicating that Acidobacteriota may have strong ecological-niche adaptability in the Jinghe River. Thus, it can be used as a proxy microbial index to explore the impact of agricultural activities and urbanization on microbial distribution patterns. In addition, the main members of Acidobacteriota, such as *Lutritalea*, *Subgroup 10*, and *Blastocatella*, were positively correlated with carbon components (TC, TIC, and DOC) and negatively correlated with EC and $\text{NH}_4^+\text{-N}$. This result is consistent with those of previous studies, indicating that Acidobacteriota is highly dependent on carbon sources and may be sensitive to high EC and high $\text{NH}_4^+\text{-N}$ environments (Kielak et al., 2016; Yao et al., 2022).

The relatively high levels of TC, TIC, and DOC, coupled with the low EC and $\text{NH}_4^+ - \text{N}$ in the upstream, may provide a more suitable growth environment for Acidobacteriota. In contrast, the significant decrease in carbon components and increase in EC and $\text{NH}_4^+ - \text{N}$ in the downstream inhibited its growth. This phenomenon is consistent with the results of the random forest analysis, that is, Acidobacteriota is positively correlated with factors such as DOC and TC and negatively correlated with EC and $\text{NH}_4^+ - \text{N}$. Similar studies have also shown that Acidobacteriota is more active in oligotrophic or low-EC environments (Navarrete et al., 2015; Zhang et al., 2019b). Members of the Acidobacteriota phylum in the Jinghe River exhibited significant functional differentiation. For example, genera such as *Bryobacter* and *Stenotrophobacter* have a high abundance of functional genes related to carbon metabolism, which may be involved in the degradation of organic carbon in the Jinghe River (Eichorst et al., 2018). These findings further support the important role of Acidobacteriota in the carbon cycle. In summary, the differences in the distribution of Acidobacteriota in the Jinghe River were mainly driven by carbon composition, EC, and nitrogen form. This study revealed the distribution characteristics of Acidobacteriota and its association with environmental factors based on amplification and sequencing. However, due to the lack of quantitative data on metagenomes or functional genes, subsequent studies should be combined with isotope or macro-transcriptomics to further explore its carbon cycle function.

5 Conclusions

This study comprehensively analyzed the physical-chemical properties and microbial communities of different sections of the Jinghe River, and the result

revealed that with the development of agricultural activities and urbanization from upstream to downstream, the accumulation of organic matter and microbial community in the river water had significant changes. EC in the downstream increased significantly, whereas the contents of TC, TIC, DOC, and $\text{NO}_3^- - \text{N}$ decreased significantly, and $\text{NH}_4^+ - \text{N}$ showed an opposite trend, and accumulated in the downstream. The bacterial ACE index was significantly higher in the downstream, whereas the fungal ACE index was higher in the upstream. The composition of microbial community was obviously driven by DOC, TC, TIC, and $\text{NO}_3^- - \text{N}$ and other physical-chemical factors, among which Proteobacteria and Ascomycota were the dominant phyla of bacteria and fungi, respectively. Co-occurrence network analysis revealed more complex microbial interactions in the upstream, and more pronounced functional clustering in the midstream. In addition, the relative abundance of Acidobacteriota positively correlated with network complexity, and most of its members were related to carbon components, highlighting the critical role of carbon cycling in microbial ecology. These results provide an important theoretical basis for the interaction mechanism between microbial communities and environmental factors in river ecosystems and for the river ecological protection and functional assessment. It is suggested to control the input of nitrogen fertilizer in the process of agricultural activities to inhibit the accumulation of ammonium nitrogen and strengthen the sewage treatment of organic carbon and nitrogen components in urban river sections to maintain the stability of microbial network and river ecological function.

Conflict of 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.

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Author contributions

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Appendix

Fig. S1 Comparison of relative abundance of the top 10 bacterial phyla in different sections of the Jinghe River. (a), Proteobacteria; (b), Firmicutes; (c), Bacteroidota; (d), Verrucomicrobiota; (e), Actinobacteriota; (f), Acidobacteriota; (g), Desulfobacterota; (h), Cyanobacteria; (i), Myxococcota; (j), Chloroflexi. Different lowercase letters indicate significant differences among different river sections at $P < 0.050$ level. Bars are standard errors.

Fig. S2 Comparison of relative abundance of the top 7 fungal phyla in different sections of the Jinghe River. (a), Ascomycota; (b), Chytridiomycota; (c), Rozellomycota; (d), Basidiomycota; (e), Mortierellomycota; (f), Blastocladiomycota; (g), Mucoromycota. Different lowercase letters indicate significant differences among different river sections at $P < 0.050$ level. Bars are standard errors.

Note: Figure translations are in progress. See original paper for figures.

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