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Decoupling the heterogeneity of sediment microbial communities along the urbanization gradients: A Bayesian-based approach

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A B S T R A C T

Comprehending the response of microbial communities in rivers along urbanization gradients to hydrologic characteristics and pollution sources is critical for effective watershed management. However, the effects of complex factors on riverine microbial communities remain poorly understood. Thus, we established a bacteria-based index of biotic integrity (Ba-IBI) to evaluate the microbial community heterogeneity of rivers along an urbanization gradient. To examine the response of Ba-IBI to multiple stressors, we employed a Bayesian network based on structural equation modeling (SEM-BN) and revealed the key control factors influencing Ba-IBI at different levels of urbanization. Our findings highlight that waterborne nutrients have the most significant direct impact on Ba-IBI ($r = -0.563$), with a particular emphasis on ammonia nitrogen, which emerged as the primary driver of microbial community heterogeneity in the Liuyang River basin. In addition, our study confirmed the substantial adverse effects of urbanization on river ecology, as urban land use had the greatest indirect effect on Ba-IBI ($r = -0.460$). Specifically, the discharge load from wastewater treatment plants (WWTP) was found to significantly negatively affect the Ba-IBI of the entire watershed. In the low urbanized watersheds, rice cultivation (RC) and concentrated animal feeding operations (CAFO) are key control factors, and an increase in their emissions can lead to a sharp decrease in Ba-IBI. In moderately urbanized watersheds, the Ba-IBI tended to decrease as the level of RC emissions increased, while in those with moderate RC emissions, an increase in point source emissions mitigated the negative impact of RC on Ba-IBI. In highly urbanized watersheds, Ba-IBI was not sensitive to changes in stressors. Overall, our study presents a novel approach by integrating Ba-IBI with multi-scenario analysis tools to assess the effects of multiple stressors on microbial communities in river sediments, providing valuable insights for more refined environmental decision-making.

1. Introduction

River ecosystems face a multitude of stressors, both natural and anthropogenic in origin (Uddin et al., 2022). These stressors encompass changes in flow patterns, increased impervious surfaces, and industrial discharges (Javed et al., 2017). These stressors can significantly impact the ecological health and resilience of river ecosystems, leading to environmental problems, such as eutrophication and loss of biodiversity (Gammal et al., 2022; Zhu et al., 2022). The limited effectiveness of several river management measures can be attributed to a poor understanding of the combined effects of these stressors (de Vries et al., 2021). Therefore, comprehensive understanding of the factors influencing riverine ecosystems is critical for promoting their sustainable use and protection.

A variety of methods, including physical (e.g., ecological flows), chemical (e.g., water quality index), and biological (e.g., index of biotic integrity) have been used in river ecological health assessment (Karr, 1981; Uddin et al., 2022; Zheng et al., 2023). With the maturity of online monitoring technology, conventional physicochemical indicators are widely adopted in river ecological evaluation. Physicochemical methods offer a cost-effective and convenient approach for determining the pollution level of water bodies but lack specific response to stressors (Li et al., 2018). Biological indicators directly reflect the physicochemical properties of the river and are highly sensitive to changes in environmental factors (Sagova-Mareckova et al., 2021). Moreover, biological indicators contain valuable insights into the dynamics of river

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biogeochemical cycles, rendering them a superior choice for assessing and decoupling river ecology (Maavara et al., 2020). Hence, the use of biological indicators in ecological assessment has garnered increasing attention.

Current biomonitoring mostly focuses on macro-organisms, such as plankton and macroinvertebrates (Bolotov et al., 2012). Based on this, previous studies have considered the response of different biomes to different ecological conditions and developed multi-species-based indexes, such as the fish-based index of biological integrity (F-IBI) and Ephemeroptera, Plecoptera and Trichoptera (EPT) index (Bacigalupi et al., 2021; Li et al., 2018). Compared to macro-organisms, bacteria in sediments were more abundant and exhibited higher specificity between different habitats (Zhang et al., 2022). In addition, sediment bacterial communities are direct participants in river material cycling and energy flow and are highly responsive to changes in river physicochemical characteristics as well as to specific stressors (Caruso et al., 2016; Wang et al., 2018). Thus, sediment bacterial communities represent valuable information for decoupling and predicting the response of riverine ecosystems to stressors. The effects of stressors on riverine ecosystems are multifaceted, as they can impact all biotic levels and lead to changes in community structure and spatial distribution. Considering the uncertainties of complex response processes, cross-boundary indexes involving multiple taxonomic levels may be more reliable and reproducible than single-species indexes (Sagova-Mareckova et al., 2021). The bacteria-based index of biotic integrity (Ba-IBI) is a comprehensive index that considers multiple taxonomic and functional aspects of sediment bacteria. Many studies have developed the Ba-IBI and demonstrated its effectiveness in assessing the heterogeneity of rivers caused by fluvial inputs (Zhang et al., 2020) and dam construction (Yang et al., 2019).

Urbanization has profoundly altered both the hydrological patterns and pollutant discharges within watersheds. Alterations in land use patterns caused by urbanization can significantly impact the hydrologic cycle of rivers, leading to altered dispersion patterns of nonpoint source pollutants (Zhang et al., 2021). Urbanization plays a direct role in increasing point source discharges, adding to the complexity of water pollution. The combined effects of multiple stressors are typically either antagonistic, where the joint effects are less than the sum of individual effects, or synergistic, where the joint effects are greater than the sum of individual effects (Birk et al., 2020). The majority of previously developed Ba-IBI have been utilized to investigate the response of microbial communities to individual sources of pollution or the physicochemical properties of rivers (Wang et al., 2021; Zhang et al., 2022). Nevertheless, few studies have employed Ba-IBI for decoupling analysis in the presence of combined impacts from multiple pressure sources, which constrains the full potential of Ba-IBI for analyzing complex scenarios involving pressure source interactions. Assuming that the common effects of stressors are simply additive may result in an overestimation or underestimation of their actual effects. This gap in the literature highlights the need for further research to better understand the intricate interactions among different stressors in river environments.

Model simulations present an opportunity to elucidate the intricate relationships between stressors and microbial communities and facilitate the prediction of potential changes. Quantitative analytical tools like Random Forests, Generalized Linear Models (GLMs), and Bayesian Networks (BNs) have gained increasing popularity in environmental predictions (Aguilera et al., 2011; Li et al., 2017; Wang et al., 2022). Compared to linear-based or black box models, Bayesian Networks (BNs) offer an interpretable model structure through directed acyclic graphs (DAG) and consider the uncertainty of predictions and relationships among variables (Aguilera et al., 2011). Moreover, BNs can integrate expert knowledge and observational data, which holds great promise for microbial community decoupling and prediction (de Vries et al., 2021). The core of BN modeling is establishing the network structure, which can be achieved using statistical tools such as Structural Equation Modeling (SEM). SEM shares a similar structure with BNs and can test the significance of the model structure (Marcot and Penman, 2019). Combining SEM with BNs can provide a robust structure for BN modeling and also leverage the attribution analysis advantages of SEM (Kim et al., 2022; Li et al., 2018).

Building upon the strengths of sediment bacterial communities in evaluating river ecological quality and the outstanding capabilities of SEM-BN in decoupling, the objectives of this study are threefold: (1) to establish Ba-IBI as a tool for assessing river ecological quality in complex watersheds; (2) to employ Ba-IBI to investigate the impacts of multiple stressors on microbial community heterogeneity; and (3) to quantify the responses of microbial communities to stressors along an urbanization gradient. In this study, we harness the full potential of Ba-IBI for environmental decoupling, enabling an exploration of complex scenarios involving multiple stressors. It offers a fresh perspective on ecological conservation and restoration in intricate watersheds.

2. Materials and methods

2.1. Study area

The Liuyang River is a secondary tributary of the Yangtze River, located in Changsha, China, with the total length of 234.8 km and the watershed area of 4665 km^2. The Liuyang River Basin (LYRB) experiences an average annual temperature of 17.4 °C and an average annual precipitation of 1601.1 mm, primarily concentrated from April to September. Originating from the northern foothills of Dawei Mountain, the Liuyang River flows westward, eventually joining the Xiangjiang River. Along its course, it traverses diverse landscapes. The LYRB's upper reaches are predominantly forested (68%), while the middle reaches traverse agricultural lands (19%), and the lower reaches run through highly populated urban areas (9%). Due to the rapid urbanization and population growth in recent decades, the aquatic ecosystem of the Liuyang River has been seriously damaged (Jia et al., 2018).

The proportion of urban land use is a widely used indicator for measuring the degree of urbanization (de Jesús-Crespo and Ramírez, 2011; Simonin et al., 2019). In the LYRB, woodland cover is negatively correlated with the degree of urbanization, while cropland proportion is positively correlated in low and moderately urbanized watersheds, with the highest proportion found in the moderately urbanized areas (Fig. S1).

2.2. Data sources

Fifty-one sampling sites were set up along the LYRB and sampling was carried out in January and July 2021 respectively. In this study, the sampling sites were positioned at the river's center to ensure both representativeness and sample stability. At each sampling site, the dissolved oxygen (DO), pH, and water temperature were measured in situ using HACH HQ30d portable meters (HACH Company, Loveland, Colorado, USA). Water samples were collected from subsurface water using sterilized water collectors and transported on ice to the laboratory for determining of total nitrogen (TN), ammonia nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N), total phosphorus (TP), chloride (Cl^-) and sulfate (SO_4^{2-}). Sediment samples were collected using the portable sludge samplers and stored at −20 °C prior to DNA extraction and sediment physicochemical analysis. Some parts of the sediment samples were sent to Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China) for 16s rRNA sequencing, and the rest were used for measuring the same chemical parameters as the water sample.

In order to evaluate the impact of stressors on river ecology, we conducted an analysis of environmental factors which have been established or postulated to possess the potential for ecological influence. The environmental factors including water quality, land use, the emission loads from point and non-point sources, and flow in each subbasin are summarized in Table S1. The input data for the Soil and Water

Assessment Tool (SWAT) model used for hydrological simulation included Digital Elevation Model (DEM), land use, soil, and weather data are listed in Table S2.

2.3. Establishment of Ba-IBI

2.3.1. Selection of reference sites

Ba-IBI was developed following standard protocols (Huang et al., 2015), and reference sites selection was deemed critical since all metrics are compared to these sites. The reference sites are considered to be in an environment with minimal disturbance (Karr, 1981). To assess the level of contamination at each sampling site, we utilized the Comprehensive Water Quality Identification (CWQI) index from a previous study (Wu et al., 2019). The CWQI is based on the five levels classified by the Chinese surface water environmental quality standards, with TN, NH_4^+-N, TP and DO as the main variable. The calculation of CWQI is detailed in the supplementary material (Text S1). Sampling sites were divided into three groups based on the 25th and 75th percentiles of the CWQI range. The sampling sites with the lowest CWQI were set as the reference sites, and those with the highest CWQI was set as the impaired sites.

2.3.2. Metrics selection and index development

The candidate metrics dataset can be divided into 4 categories based on 4 aspects of microbial communities: diversity, composition, tolerance, and function (Zhang et al., 2020). The composition metrics consist of the dominant taxa's relative abundance in various taxonomic levels. The tolerance metrics consist of the proportion of genus-level taxa that are tolerant or sensitive to a single stressor based on redundancy analysis (Li et al., 2017). Meanwhile, functional metrics investigate changes in bacterial characteristics, metabolic processes, and functional pathways at varying levels of contamination. To reduce the effect of extreme values and ensure that the data can be analyzed on the same scale, all proportional metrics (between 0 and 1) were transformed by the arcsine-square root, while other metrics were log-transformed (Jia et al., 2013). All metrics are then screened in three steps. Firstly, range tests were conducted to exclude metrics with zero values in the samples. Next, the Mann-Whitney *U* test was used to remove metrics that showed no significant differences between the reference and impaired sites. Finally, redundancy tests were performed to exclude metrics that showed significant correlation. Further details on the development of our index can be found in the supplementary material (Text S2).

2.4. BN model establishment and scenario analysis

Referring to previous modeling experience, our BN modeling process consists of 3 steps (Aguilera et al., 2011; de Vries et al., 2021; Marcot and Penman, 2019). 1) Model structure and parameterization: Based on the results of the SEM, an influence diagram was established, revealing the relationship between environmental variables and Ba-IBI. The nodes were then classified into 3 or 4 classes using the Self-organizing Maps (SOM) Toolbox in MATLAB (Li et al., 2018); 2) Model training and validation: The BN model is trained and validated using Netica 6.0.9 software, which provides a visual graphical interface and can input continuous data. We employed 10-fold cross-validation, and use the accuracy and the area under the receiver operating characteristic curve (AUC) as the evaluation metrics of the model; and 3) Sensitivity and scenario analysis: Sensitivity analysis complements the attribution analysis performed in SEM by identifying the primary drivers of Ba-IBI across areas with varying degrees of urbanization. Scenario analysis, on the other hand, allows predicting changes in the probability distribution of a target node by introducing or removing specific stressors. In addition, for the given inputs, the scenario simulation can predict the change of the target node by the expected value of the Conditional Probability Table (CPT) (de Vries et al., 2021). In this study, we simulated the variations of Ba-IBI under different levels of urbanization with imposing or reducing stressor scenarios based on the observed data.

2.5. Data analysis method

Based on the Operational Taxonomic Units (OTUs), the diversity indexes were calculated using the vegan package in R (version 4.1.2). Functional metrics were selected using METAGENassist (Arndt et al., 2012). To evaluate the performance of Ba-IBI in quantitatively evaluating the ecological quality of rivers, we fitted a least squares regression between Ba-IBI and CWQI using IBM SPSS 24.

In this study, the Spearman correlation between Ba-IBI and stressors was calculated using the Correlation Plots in Origin (2022b) (version 9.75). To further investigate the effect of environmental variables on Ba-IBI, we introduced SEM path analysis to quantify their direct and indirect effects on Ba-IBI. We considered the multilevel relationships between urbanization processes, human activities, environmental changes, and ecological status and provided a high-confidence structure for BN modeling. The SEM was built using AMOS 24.0 (Amos Development Corp., Chicago, IL, USA), with evaluation of Chi-square/Degree of Freedom (CMN/DF) < 4, Root Mean Square Error of Approximation (RMSEA) < 0.08, Comparative Fit Index (CFI) > 0.95, and Goodness of Fit Index (GFI) > 0.90. Principal component analysis (PCA) was used to reduce the dimensionality of the water contaminants and sediment contaminants variables before establishing the SEM (Table S4).

3. Results and discussion

3.1. Ba-IBI for assessing river ecological heterogeneity

In this study, 13 reference sites and 13 impaired sites were defined separately (Fig. 1). A candidate metric library of river ecological heterogeneity consisting of 131 metrics was developed (Table S3). To ensure that candidate metrics were well-distributed across all samples, exhibited substantial variability between impaired and reference sites, and were free from redundant biological information, all metrics were then screened by range, heterogeneity, and redundancy tests. The comprehensive screening process is outlined in the supplementary material (Text S2). Only five core metrics were retained for the development of Ba-IBI: M18 (*Gammaproteobacteria*), M59 (*Xanthobacteraceae*), M71 (*Bradyrhizobiaceae*), M88 (*Pedomicrobium*), and M112 (Ammonia oxidizer). Among them, M18 and M112 increased with the decline of ecological quality, while M59, M71, and M88 decreased with the decline of ecological quality. Overall, the significant difference in river ecological quality is well reflected by the core metrics (Fig. S2).

Ecological heterogeneity of rivers was quantitatively assessed by Ba-IBI, which ranged from 1.05 to 4.66 in all samples with a mean of 3.17. To discern variations in river ecological quality, we categorized it into three levels based on the 25th and 75th percentiles of Ba-IBI: "low" (1.05–2.71), "medium" (2.71–3.84), and "high" (3.84–4.66), representing severely, moderately, and mildly impaired river ecology, respectively. According to the box plots, significant differences were exhibited between reference and impaired sites, with most reference sites at "high" levels and most impaired sites at "low" levels (Fig. 2a). Besides, a strong negative correlation was shown between Ba-IBI and CWQI (Spearman's $r = -0.80$), indicating that Ba-IBI decreased with the deterioration of water quality (Fig. 2b). Both of these findings underscore the effectiveness of Ba-IBI in assessing variations in river ecology.

The Ba-IBI at the sampling sites exhibited significant differences along the urbanization gradient. Notably, most impaired sites were situated in towns and cultivated areas characterized by high to moderate levels of urbanization, while all reference sites were located in upstream regions covered by forests. This pattern suggests that human activities associated with urbanization are likely the primary drivers of river ecology (Fig. 1). Furthermore, all five core indicators are considered to be closely related to human activities or water quality (Fig. S3).

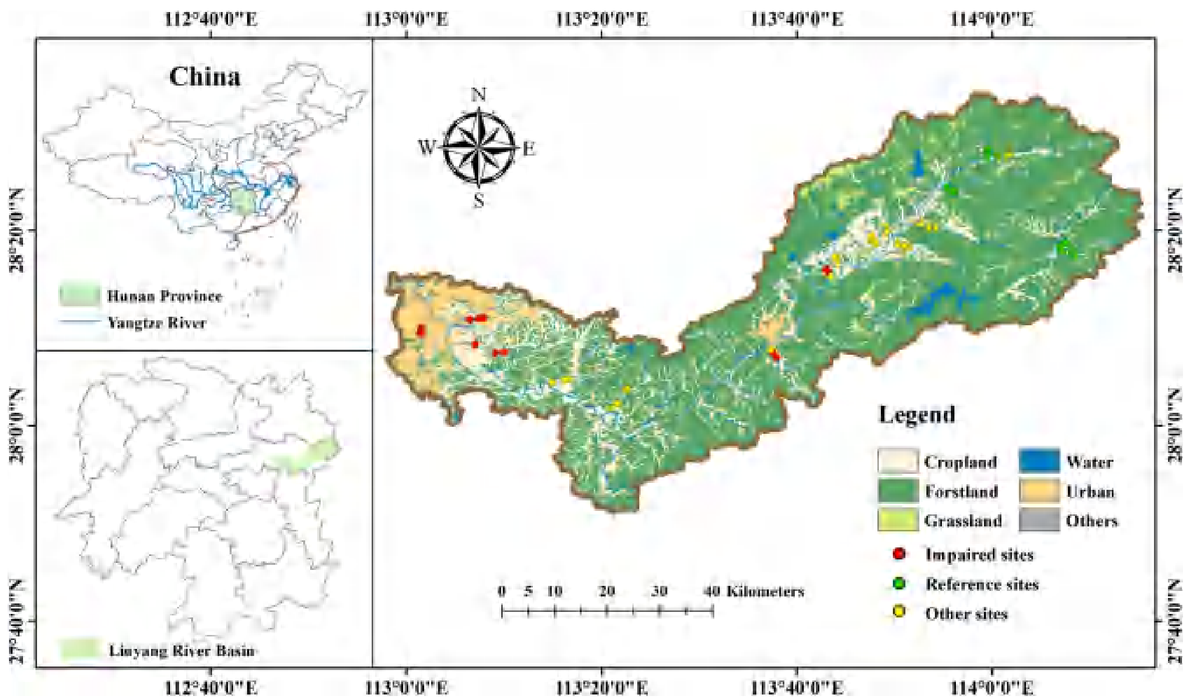

Fig. 1. Study area and distribution of sampling sites (Red for impaired sites, green for reference sites, and yellow for other sites).

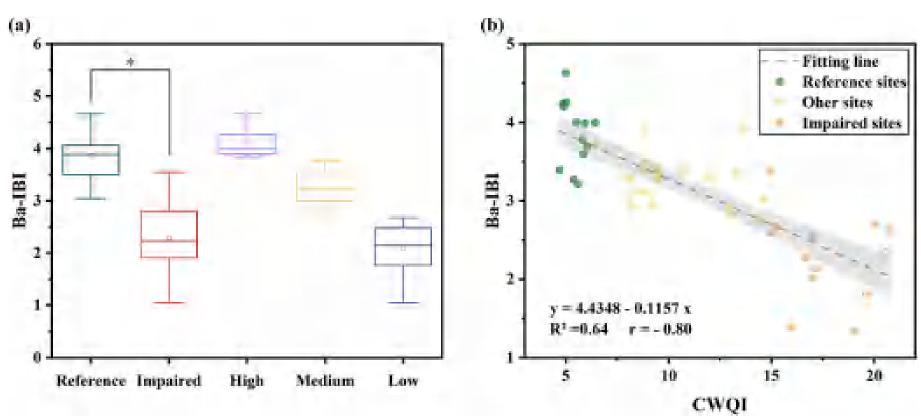

Fig. 2. Sensitivity test for Ba-IBI. (a) Sensitivity of Ba-IBI in distinguishing river ecological impairment. (b) Ordinary least squares regression of the Ba-IBI and comprehensive water quality identification (CWQI) index.

Gammaproteobacteria exhibit a significant positive correlation with the daily average discharge volume (m^3/day) from wastewater treatment plants (WWTP) within the sub-basin. This could potentially be attributed to the fact that *Gammaproteobacteria* are known to contain various pathogenic bacterial species, such as *E. coli* and *Vibrio cholerae*, which are found in higher concentrations in treated medical wastewater (Kiersztyn et al., 2019; Li et al., 2012). *Bradyrhizobiaceae*, a nitrogen-fixing bacteria, showed a significant negative correlation with the emission from RC and WWTP, while a positive correlation was observed for ammonia-oxidizing bacteria (AOB). The discharge of nitrogen-rich effluents from WWTP and RC may lead to an increase in the abundance of nitrogen-fixing and ammonia-oxidizing bacteria in the river (Lin et al., 2020; Zhu et al., 2023). The relative abundance of *Xanthobacteraceae* decreases with increasing industrial emissions and WWTP. While *Xanthobacteraceae* are known for their significant role in the degradation of toxic organic compounds, such as polycyclic aromatic hydrocarbons, in polluted environments, recent studies suggest that their relative abundance decreases in urbanized areas (Simonin et al., 2019). *Pedomicrobium*, a bacterium sensitive to heavy metals, exhibited a significant negative correlation with industrial discharges (Chen et al., 2022; Yang et al., 2022). The aforementioned findings demonstrate that the core indicators of Ba-IBI reveal a strong response to stressors, particularly anthropogenic.

3.2. Relative importance of environmental factors

To account for the intricate interplay between sediment microbes and stressors, we constructed a conceptual framework that connects various environmental factors, such as urban land use, pollution sources, flow dynamics, and water and sediment properties, to Ba-IBI (Fig. 3 and S5). The model exhibits high fitting performance (CMIN/DF = 1.37, GFI = 0.94, CFI = 0.97, RSMEA = 0.06), which demonstrates the plausibility of the conceptual model.

As illustrated in Fig. 3, Urban had a significant negative indirect effect on Ba-IBI ($r = -0.460$), which was primarily mediated by changes in water nutrient concentrations ($r = 0.745$). This result underscores the adverse impact of urbanization on riverine ecology and supports the discussion on the distribution of Ba-IBI in Section 3.1. On the one hand,

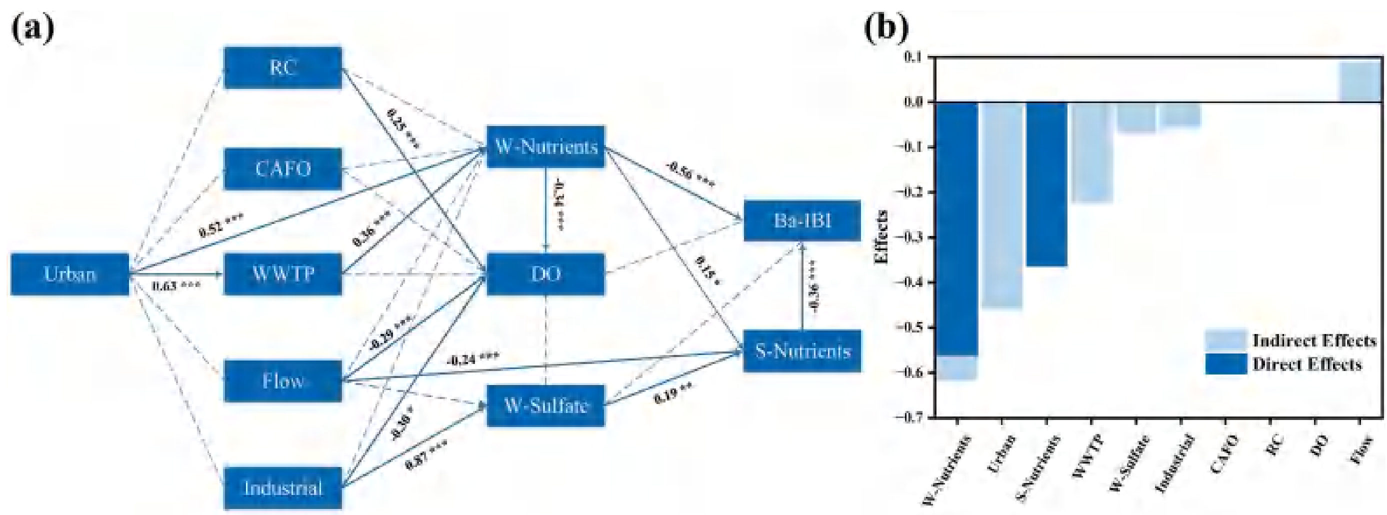

Fig. 3. (a) The SEM path framework and model results of the impact of environmental factors on Ba-IBI. The solid line represents a significant effect ($p < 0.1$), while the dashed line represents an insignificant effect ($p > 0.1$), *** represents $p < 0.01$, ** represents $p < 0.05$, and * represents $p < 0.1$. (b) Direct and indirect effects of each explanatory variable on Ba-IBI. RC stands for the emission of rice cultivation, CAFO stands for the emission of concentrated animal feeding operation, and WWTP stands for the emission of waste water treatment plant. "W-" and "S-" represent environmental factors in water and sediment, respectively.

high urban land cover leads to the accumulation of pollutants on impervious surfaces, which can weaken the self-purification capacity of natural water bodies and soils, ultimately exacerbating river pollution (Ding et al., 2016). This is supported by the significant direct impact of Urban on W-Nutrients ($r = 0.516$). On the other hand, highly urbanized areas often feature higher concentrations of anthropogenic point source emissions, including those from sewage treatment plants and industrial facilities. In our model, Urban has a significant indirect effect on W-Nutrients through WWTP ($r = 0.229$). In addition, emissions from industrial sources exhibited a weak negative indirect effect on Ba-IBI ($r = -0.059$). Industrial wastewater and sewage plant effluent contain high concentrations of nutrients and chemicals that have the potential to modify the structure and metabolism of microbial communities. Additionally, even low levels of antibiotics present in wastewater can promote the development of antibiotic resistance in pathogenic bacteria and accumulate in sediments, thereby posing a potential threat to human health (Mello et al., 2018). Overall, CAFO and RC had no significant effect on Ba-IBI (Table S6), which may be attributed to the non-linear relationship among the variables (Kim et al., 2022). However, sensitivity analysis of the BN model indicated that CAFO and RC were more important stressors than others in low and mid-urban areas (Fig. S7). This may be due to the fact that most of the cultivated land and breeding farms are located in the middle and upper reaches of the watershed, where urban distribution is sparse.

W-Nutrients had a significant effect on Ba-IBI ($r = -0.617$). Based on the Principal Component Analysis (PCA) results (Table S4), TN and NH_4^+-N in water were identified as the primary contributors to the first principal component of water nutrients, accounting for more than 80% of the variance. This finding indicates that nitrogen loading, particularly the presence of ammonia nitrogen, is likely the primary factor contributing to the observed heterogeneity in sediment microbial communities in the LYRB. Rivers in urban areas typically carry higher nitrogen loads, and ammonia nitrogen, predominantly of anthropogenic origin, is a leading cause of river impairment in China (Huang et al., 2021; Xuan et al., 2022). Furthermore, W-Sulfate had significant negative effects on Ba-IBI ($r = -0.068$) through S-Nutrients. It has been proven that the S^{2-} after SO_4^{2-} reduction could stimulate the release of PO_4^{3-} in sediments, and the high concentration of W-Sulfate may exacerbate river eutrophication (Chen et al., 2016). The significant direct effect of Industrial on W-Sulfates ($r = 0.869$) is consistent with previous studies demonstrating that industrial emissions and fuel combustion increase riverine sulfate (Torres-Martínez et al., 2020). It is worth to mention that the relative importance of W-Sulfate in highly urbanized watersheds were higher than other watersheds (Fig. S7). This observation is likely a consequence of sulfate pollution in rivers stemming from industrial emissions and combustion processes.

Flow has weak positive indirect effects on Ba-IBI ($r = 0.088$). The impact of flow on river ecology is multifaceted. On the one hand, The increase in flow may promote the resuspension of NH_4^+-N in the sediment and inhibit the sedimentation of NO_3^- in the water, which may increase the rate of nitrification and decrease the rate of mineralization, resulting in the transfer of nitrogen from the sediment to the water (Karthäuser et al., 2021; Lü et al., 2022). On the other hand, high-flow conditions have the potential to dilute pollutants and can exert a positive influence on river water quality (Tian et al., 2019).

3.3. Effects of multiple stressors on Ba-IBI under different urbanization scenarios

BN was developed based on the structure validated by SEM (Fig. 4). The SOM clustering algorithm was used to discretize them into 3 or 4 states (Table S6). The K-fold cross-validation results illustrated that the average accuracy of the model was 0.70 and the average AUC was 0.78, indicating the strong performance of the model in predicting Ba-IBI (Fig. S6).

The results of the scenario analysis reveal a significant difference in Ba-IBI along the urbanization gradient. As the level of urbanization shifts from "Low" to "High", the proportion of Ba-IBI at "Low" increases by 18.2% (Fig. 5). Taking into account the relationship between land use patterns and urbanization (Fig. S1), the impact of urbanization on Ba-IBI can be summarized as follows: Ba-IBI is highest in forested watersheds, followed by agricultural and urban watersheds. Furthermore, Ba-IBI in watersheds with different degrees of urbanization exhibit different responses to stressors (Fig. 6). For the entire basin, Ba-IBI is linearly related to RC, WWTP, Industrial, and Flow, whereby the proportion of Ba-IBI at "Low" increases as these stressors change from "Low" to "High" (Fig. 6a, i, m, q). In contrast, the proportion of Ba-IBI at "High" decreased as CAFO changed from "Low" to "High" (Fig. 6e), suggesting that watersheds with better ecological quality may be more vulnerable to CAFO than impaired areas. WWTP has negative impacts on all types of watersheds, highlighting its significance as a key control factor for the ecology of the entire watershed (Fig. 6i~l).

Specifically, the increase of all stressors significantly increases the proportion of Ba-IBI at "Low" in low urbanized watersheds (Fig. 6). In

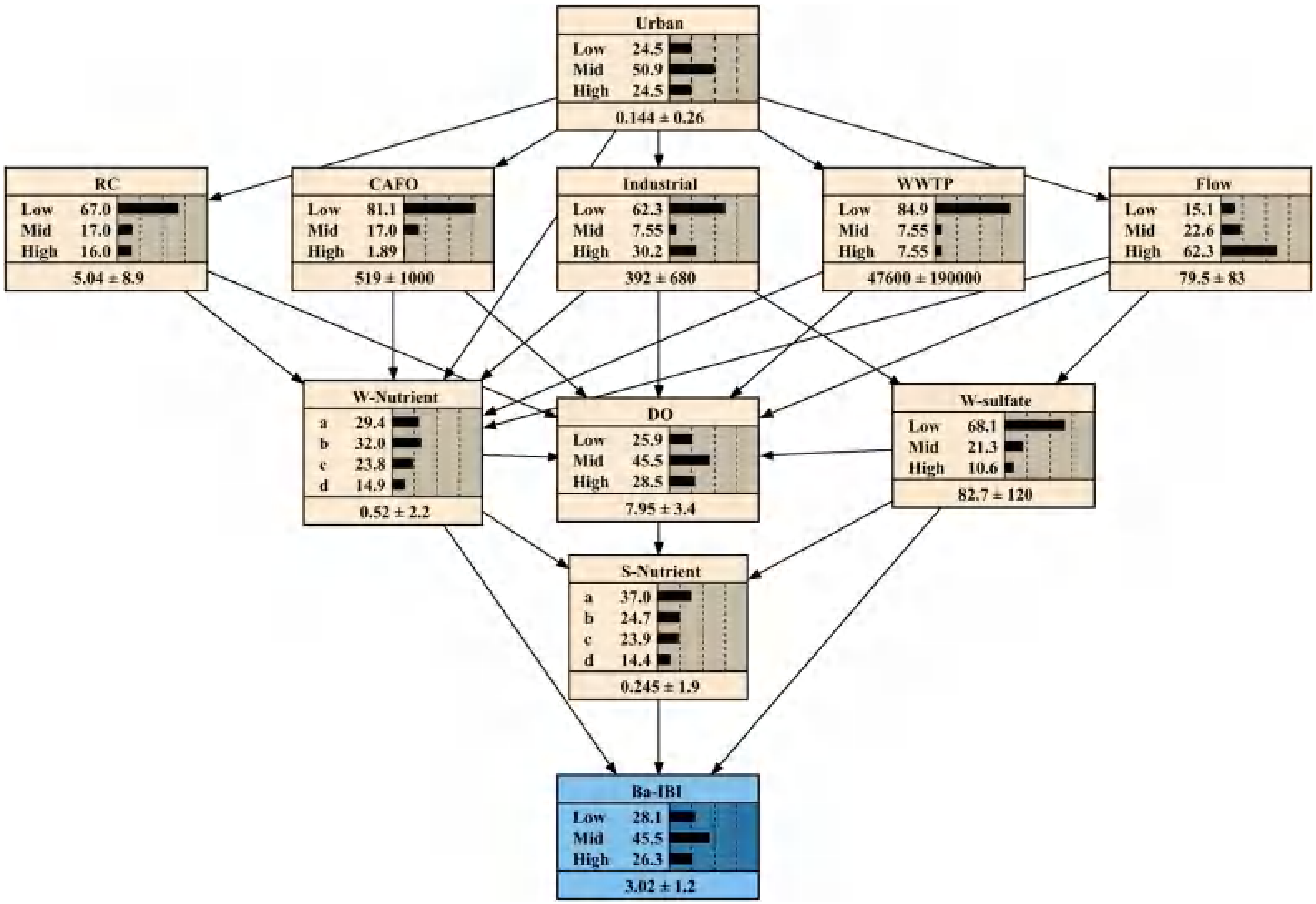

Fig. 4. BN model linking Ba-IBI to environmental variables after training with observational data. Blue represents target nodes and yellow represents input nodes. Node states are listed in Table S6.

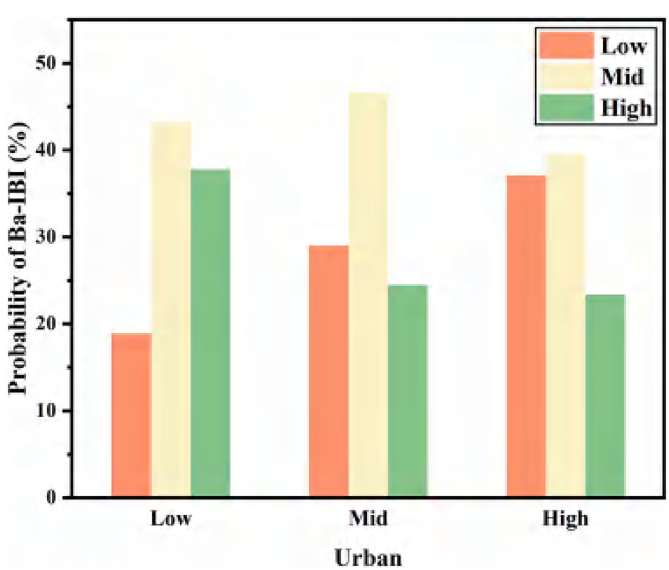

Fig. 5. Scenario analysis results for the state probability distribution of Ba-IBI (Red for "Low", yellow for "Mid" and green for "High") along the urbanization gradient.

particular, the proportion of Ba-IBI at "Low" will increase by 16.5% as the elevated TN in the watershed due to RC emissions increases from 2.0 mg/L to 4.4 mg/L (Fig. 6b). Similarly, with the increase of CAFO emission loadings from 205 m^3/d to 816 m^3/d, the percentage of Ba-IBI at the "Low" level will increase by 17.5% (Fig. 6f). Massive fertilization during rice cultivation resulted in a dramatic increase in the diffuse nitrogen load on the ground surface (Liang et al., 2023). CAFO wastewater, characterized by high pollutant and pathogen contents, has the potential to disrupt the ecological balance of water bodies (Gržinić et al., 2023; Zhu et al., 2023). The watersheds with "High" flow resulted in a significantly higher proportion of Ba-IBI at the "Low" level, as compared to those with "Mid" and "Low" flow (Fig. 6r). As the base concentration of pollutants is low, high-flow watersheds are often accompanied by more precipitation, which may cause the silt in the forest to be washed into the river channel, adding pollutants to the water and making the river turbid (Bu et al., 2014).

In moderately urbanized watersheds, there was a negative correlation between Ba-IBI and increasing levels of RC emissions. In detail, as RC emissions escalated from "Low" to "High", the proportion of Ba-IBI at the "Low" level increased by 5.4% (Fig. 6c). However, when examining the scenario of moderate RC emissions, the proportion of Ba-IBI in the "low" category exhibited a significantly lower trend compared to other scenarios. Conversely, WWTP, Industrial, and CAFO showed an opposite trend (Fig. 6g, k, o). It reveals that there is an antagonistic relationship between point source pollution and RC in the watershed. Specifically, increased point source emissions were found to offset the negative effects of RC on Ba-IBI. One potential explanation for this phenomenon is the ongoing urbanization process, which has led to the transformation of large areas of previously cultivated land in many agricultural land-dominated watersheds into non-agricultural zones, including industrial and residential areas (Han et al., 2022). The positive effect of flow

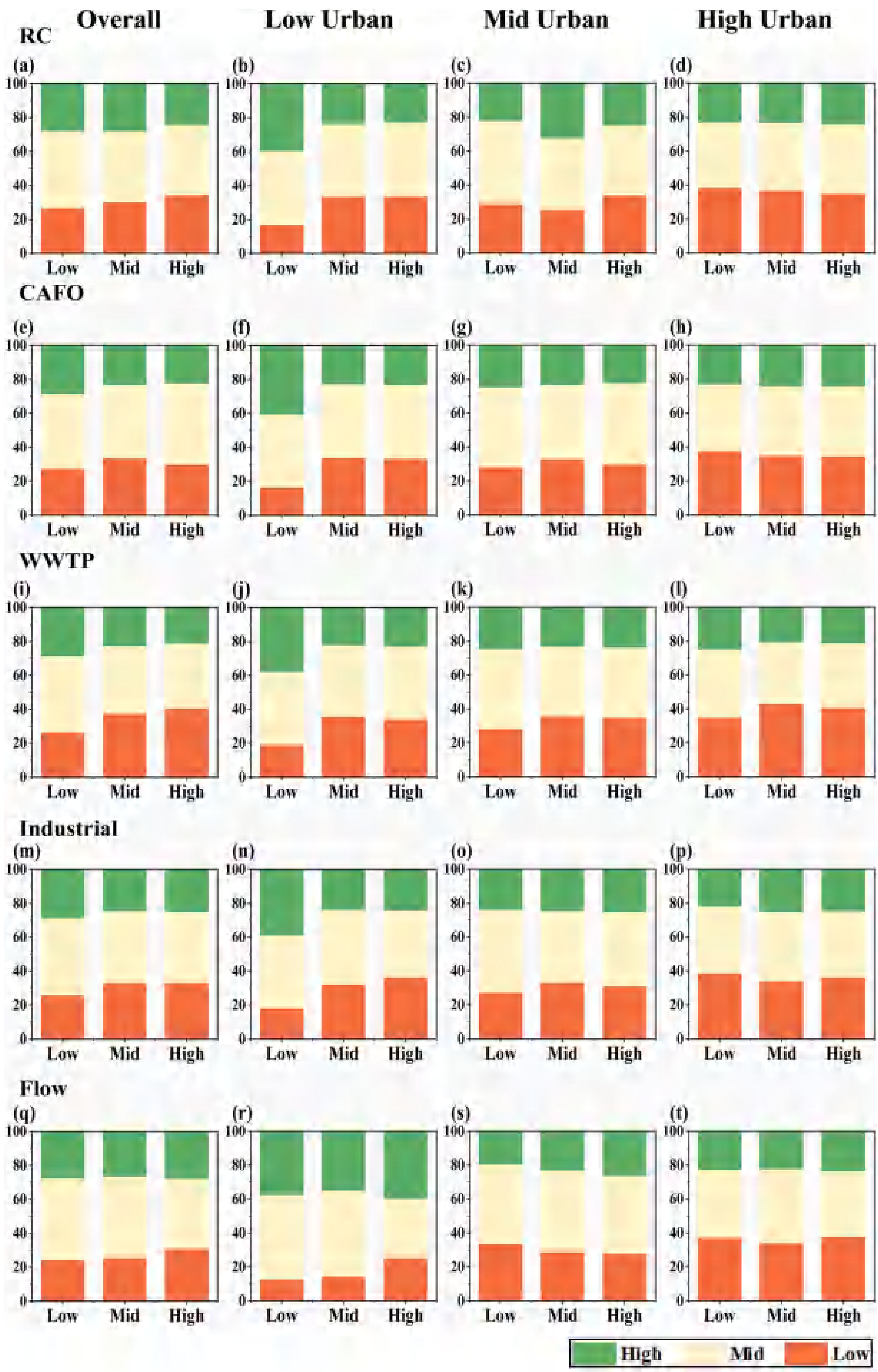

Fig. 6. Scenario analysis result of the state probability (%) distribution of Ba-IBI (Red for "Low", yellow for "Mid" and green for "High") changes with different states of the stressors (a–d: RC, e–h: CAFO, i–l: WWTP, m–p: Industrial, q–t: Flow) under different levels of urbanization: Overall (first row), Low-Urban: (second row), Mid-Urban (third row) and High-Urban (last row).

on Ba-IBI suggests that the dilution effect of flow on pollutants plays a more important role in this watershed (Fig. 6s). The increase in flow resulted in the accelerated transfer of pollutants, leading to greater dilution and transfer effects on pollutants compared to mixing and diffusion effects in this watershed (Müller et al., 2020).

It's noteworthy that Ba-IBI in highly urbanized watersheds showed insensitivity to changes in stressors (Fig. 6d, h, l, p, t). Previous studies have explored alternative stable theories in riverine microbial communities, suggesting that high environmental pressures can lead to a catastrophic shift in microbial communities within riverine sediment to a new, low-biodiversity steady state (Shang et al., 2021). In this stable state, controlling the input of pollution sources alone proves to be limited in restoring the functions of the river benthic ecosystem. Additional restoration measures, such as bioremediation, become necessary (Gonze et al., 2017; Scheffer et al., 2001).

3.4. Implications in riverine environmental management

Current river management faces the challenge of identifying effective measures for improving river ecology (Sendzimir and Schmutz, 2018). Sediment microbial communities respond rapidly to environmental changes and can provide valuable insights into the effects of pollution sources on river ecosystems. Ba-IBI is a versatile index that comprehensively assesses microbial community characteristics, including diversity, structure, and sensitivity to environmental factors. Attribution analysis tools help identify key factors influencing river ecology. Our study showcases the efficacy of Ba-IBI in decoupling and predicting the impacts of diverse stressors on distinct microbial communities in watershed sediments, even in complex scenarios involving multiple stressor interactions. These findings can inform the prioritization of targeted environmental stress mitigation measures for effective management practices.

The differences in land use patterns and sources of pollutants contribute to variations in the influencing factors of benthic microbial communities in watersheds with different degrees of urbanization (Feng et al., 2022; Qu et al., 2017). The discharge load from WWTP has a significant negative impact on Ba-IBI throughout the basin. In the case of the irreversible urbanization process, enhancing the effluent quality from WWTPs can potentially benefit the ecology of the LYRB. Our study reveals that the controlling factors for benthic microbial communities in low-urbanization watersheds are CAFO and RC. In moderately urbanized watersheds, we observed antagonistic effects between RC and point source pollution emissions. These findings offer valuable insights into strategies for enhancing water quality in the basin. For instance, in low-urbanization areas, it is crucial to regulate the scale of farms and the expansion of cultivated land. In moderately urbanized areas, efforts should focus on controlling agricultural non-point source emissions, as well as preventing complex sources of watershed pollution resulting from urban expansion. Due to the intense environmental pressure in highly urbanized watersheds, the response of Ba-IBI to changes in stressors is limited. Therefore, additional research is necessary to investigate the benthic habitat of this watershed and identify potential solutions for river restoration (Fu et al., 2020).

4. Conclusion

Our study introduces Ba-IBI as a tool for decoupling the impacts of multiple stressors on river sediment microbial communities. We employed SEM-BN to quantitatively analyze the primary drivers of microbial communities across an urbanization gradient. The results illustrated that nutrients in water exerted the greatest direct effect on Ba-IBI ($r = -0.563$). Additionally, PCA results highlight that nitrogen loading in the water, particularly ammonia nitrogen, emerges as the predominant driver of sediment microbial community heterogeneity within the LYRB. Urban was the most important indirect factor for Ba-IBI heterogeneity ($r = -0.460$). The discharge load of WWTP has a significant negative impact on Ba-IBI throughout the basin. In low urbanized watersheds, RC and CAFO are the key controlling factors. The proportion of Ba-IBI classified as "Low" is projected to increase by 16.5% as the elevated TN in the watershed, caused by RC emissions, increases from 2.0 mg/L to 4.4 mg/L. Similarly, the percentage of Ba-IBI classified as "Low" is expected to increase by 17.5% when the wastewater emission loadings from CAFO increases from 205 m^3/d to 816 m^3/d. In moderately urbanized watersheds, a shift from "Low" to "High" RC emissions leads to a 5.4% increase in the proportion of Ba-IBI at "Low." Moreover, our findings indicate that, under a moderate scenario of rice cultivation (RC) emissions, an increase in point source emissions offset the negative impact of RC on Ba-IBI. In contrast, Ba-IBI in highly urbanized watersheds were not sensitive to the changes in stressors, suggesting a new steady state of low biodiversity. Our study emphasizes the significance of controlling key factors in watersheds with low to moderate levels of urbanization and implementing restoration measures promptly in highly urbanized watersheds.

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Human subjects research statements

Our study does not involve human subjects.

CRediT authorship contribution statement

Jie Liang: Writing – review & editing, Conceptualization. **Junjie Ding:** Writing – original draft, Methodology, Software. **Ziqian Zhu:** Data curation, Writing – review & editing. **Xiang Gao:** Data curation, Writing – review & editing. **Shuai Li:** Writing – review & editing. **Xin Li:** Writing – review & editing. **Min Yan:** Writing – review & editing. **Qinxue Zhou:** Writing – review & editing. **Ning Tang:** Data curation. **Lan Lu:** Writing – review & editing. **Xiaodong Li:** Writing – review & editing.

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

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2023.117255.

References

Aguilera, P.A., Fernández, A., Fernández, R., Rumí, R., Salmerón, A., 2011. Bayesian networks in environmental modelling. Environ. Model. Software 26, 1376–1388. https://doi.org/10.1016/j.envsoft.2011.06.004.

Arndt, D., Xia, J., Liu, Y., Zhou, Y., Guo, A.C., Cruz, J.A., Sinelnikov, I., Budwill, K., Nesbo, C.L., Wishart, D.S., 2012. METAGENassist: a comprehensive web server for comparative metagenomics. Nucleic Acids Res. 40, W88–W95. https://doi.org/10.1093/nar/gks497.

Bacigalupi, J., Staples, D.F., Treml, M.T., Bahr, D.L., 2021. Development of fish-based indices of biological integrity for Minnesota lakes. Ecol. Indicat. 125, 107512 https://doi.org/10.1016/j.ecolind.2021.107512.

Birk, S., Chapman, D., Carvalho, L., Spears, B.M., Andersen, H.E., Argillier, C., Auer, S., Baattrup-Pedersen, A., Banin, L., Beklioğlu, M., Bondar-Kunze, E., Borja, A., Branco, P., Bucak, T., Buijse, A.D., Cardoso, A.C., Couture, R.-M., Cremona, F., de Zwart, D., Feld, C.K., Ferreira, M.T., Feuchtmayr, H., Gessner, M.O., Gieswein, A., Globevnik, L., Graeber, D., Graf, W., Gutiérrez-Cánovas, C., Hanganu, J., Işkın, U., Järvinen, M., Jeppesen, E., Kotamäki, N., Kuijper, M., Lemm, J.U., Lu, S., Solheim, A. L., Mischke, U., Moe, S.J., Nõges, P., Nõges, T., Ormerod, S.J., Panagopoulos, Y., Phillips, G., Posthuma, L., Pouso, S., Prudhomme, C., Rankinen, K., Rasmussen, J.J., Richardson, J., Sagouis, A., Santos, J.M., Schäfer, R.B., Schinegger, R., Schmutz, S., Schneider, S.C., Schülting, L., Segurado, P., Stefanidis, K., Sures, B., Thackeray, S.J., Turunen, J., Uyarra, M.C., Venohr, M., von der Ohe, P.C., Willby, N., Hering, D., 2020. Impacts of multiple stressors on freshwater biota across spatial scales and ecosystems. Nat. Ecol. Evol. 4, 1060–1068. https://doi.org/10.1038/s41559-020-1216-4.

Bolotov, S.E., Tsvetkov, A.I., Krylov, A.V., 2012. Zooplankton in the zones of confluence of unregulated rivers. Inland Water Biol. 5, 184–191. https://doi.org/10.1134/S1995082912020034.

Bu, H., Meng, W., Zhang, Y., Wan, J., 2014. Relationships between land use patterns and water quality in the Taizi River basin, China. Ecol. Indicat. 41, 187–197. https://doi.org/10.1016/j.ecolind.2014.02.003.

Caruso, G., La Ferla, R., Azzaro, M., Zoppini, A., Marino, G., Petochi, T., Corinaldesi, C., Leonardi, M., Zaccone, R., Fonda Umani, S., Caroppo, C., Monticelli, L., Azzaro, F., Decembrini, F., Maimone, G., Cavallo, R.A., Stabili, L., Hristova Todorova, N., Karamfilov, V.K., Rastelli, E., Cappello, S., Acquaviva, M.I., Narracci, M., De Angelis, R., Del Negro, P., Latini, M., Danovaro, R., 2016. Microbial assemblages for environmental quality assessment: knowledge, gaps and usefulness in the European Marine Strategy Framework Directive. Crit. Rev. Microbiol. 42, 883–904. https://doi.org/10.3109/1040841X.2015.1087380.

Chen, J., Li, W., Tan, Q., Sheng, D., Li, Y., Chen, S., Zhou, W., 2022. Effect of disinfectant exposure and starvation treatment on the detachment of simulated drinking water biofilms. Sci. Total Environ. 807, 150896 https://doi.org/10.1016/j.scitotenv.2021.150896.

Chen, M., Li, X.-H., He, Y.-H., Song, N., Cai, H.-Y., Wang, C., Li, Y.-T., Chu, H.-Y., Krumholz, L.R., Jiang, H.-L., 2016. Increasing sulfate concentrations result in higher sulfide production and phosphorous mobilization in a shallow eutrophic freshwater lake. Water Res. 96, 94–104. https://doi.org/10.1016/j.watres.2016.03.030.

de Jesús-Crespo, Ramírez, A., 2011. Effects of urbanization on stream physicochemistry and macroinvertebrate assemblages in a tropical urban watershed in Puerto Rico. J. North Am. Benthol. Soc. 30, 739–750. https://doi.org/10.1899/10-081.1.

de Vries, J., Kraak, M.H.S., Skeffington, R.A., Wade, A.J., Verdonschot, P.F.M., 2021. A Bayesian network to simulate macroinvertebrate responses to multiple stressors in lowland streams. Water Res. 194, 116952 https://doi.org/10.1016/j.watres.2021.116952.

Ding, J., Jiang, Y., Liu, Q., Hou, Z., Liao, J., Fu, L., Peng, Q., 2016. Influences of the land use pattern on water quality in low-order streams of the Dongjiang River basin, China: a multi-scale analysis. Sci. Total Environ. 551–552, 205–216. https://doi.org/10.1016/j.scitotenv.2016.01.162.

Feng, W., Gao, J., Wei, Y., Liu, D., Yang, F., Zhang, Q., Bai, Y., 2022. Pattern changes of microbial communities in urban river affected by anthropogenic activities and their environmental driving mechanisms. Environ. Sci. Eur. 34, 93. https://doi.org/10.1186/s12302-022-00669-1.

Fu, D., Yan, Y., Yang, X., Rene, E.R., Singh, R.P., 2020. Bioremediation of contaminated river sediment and overlying water using biologically activated beads: a case study from Shedu river, China. Biocatal. Agric. Biotechnol. 23, 101492 https://doi.org/10.1016/j.bcab.2019.101492.

Gammal, J., Hewitt, J., Gladstone-Gallagher, R., Thrush, S., Douglas, E., Lohrer, A., Pilditch, C., 2022. Stressors Increase the Impacts of Coastal Macrofauna Biodiversity Loss on Ecosystem Multifunctionality. Ecosystems. https://doi.org/10.1007/s10021-022-00775-4.

Gonze, D., Lahti, L., Raes, J., Faust, K., 2017. Multi-stability and the origin of microbial community types. ISME J. 11, 2159–2166. https://doi.org/10.1038/ismej.2017.60.

Gržinić, G., Piotrowicz-Cieślak, A., Klimkowicz-Pawlas, A., Górny, R.L., Ławniczek-Wałczyk, A., Piechowicz, L., Olkowska, E., Potrykus, M., Tankiewicz, M., Krupka, M., Siebielec, G., Wolska, L., 2023. Intensive poultry farming: a review of the impact on the environment and human health. Sci. Total Environ. 858, 160014 https://doi.org/10.1016/j.scitotenv.2022.160014.

Han, H., Peng, H., Li, S., Yang, J., Yan, Z., 2022. The non-agriculturalization of cultivated land in karst mountainous areas in China. Land 11, 1727. https://doi.org/10.3390/land11101727.

Huang, Q., Gao, J., Cai, Y., Yin, H., Gao, Y., Zhao, J., Liu, L., Huang, J., 2015. Development and application of benthic macroinvertebrate-based multimetric indices for the assessment of streams and rivers in the Taihu Basin, China. Ecol. Indic. 48, 649–659. https://doi.org/10.1016/j.ecolind.2014.09.014.

Huang, J., Zhang, Y., Bing, H., Peng, J., Dong, F., Gao, J., Arhonditsis, G.B., 2021. Characterizing the river water quality in China: recent progress and on-going challenges. Water Res. 201, 117309 https://doi.org/10.1016/j.watres.2021.117309.

Javed, S., Ali, A., Ullah, S., 2017. Spatial assessment of water quality parameters in Jhelum city (Pakistan). Environ. Monit. Assess. 189, 119. https://doi.org/10.1007/s10661-017-5822-9.

Jia, Y., Sui, X., Chen, Y., 2013. Development of a fish-based index of biotic integrity for wadeable streams in Southern China. Environ. Manage. 52, 995–1008. https://doi.org/10.1007/s00267-013-0129-2.

Jia, Y., Wang, L., Qu, Z., Yang, Z., 2018. Distribution, contamination and accumulation of heavy metals in water, sediments, and freshwater shellfish from Liuyang River, Southern China. Environ. Sci. Pollut. Res. 25, 7012–7020. https://doi.org/10.1007/s11356-017-1068-x.

Karr, J.R., 1981. Assessment of biotic integrity using fish communities. Fisheries 6, 21–27. https://doi.org/10.1577/1548-8446(1981)006<0021:AOBIUF>2.0.CO;2.

Karthäuser, C., Ahmerkamp, S., Marchant, H.K., Bristow, L.A., Hauss, H., Iversen, M.H., Kiko, R., Maerz, J., Lavik, G., Kuypers, M.M.M., 2021. Small sinking particles control anammox rates in the Peruvian oxygen minimum zone. Nat. Commun. 12, 3235. https://doi.org/10.1038/s41467-021-23340-4.

Kiersztyn, B., Chróst, R., Kaliński, T., Siuda, W., Bukowska, A., Kowalczyk, G., Grabowska, K., 2019. Structural and functional microbial diversity along a eutrophication gradient of interconnected lakes undergoing anthropopressure. Sci. Rep. 9, 11144 https://doi.org/10.1038/s41598-019-47577-8.

Kim, T., Lee, D., Shin, J., Kim, Y., Cha, Y., 2022. Learning hierarchical Bayesian networks to assess the interaction effects of controlling factors on spatiotemporal patterns of fecal pollution in streams. Sci. Total Environ. 812, 152520 https://doi.org/10.1016/j.scitotenv.2021.152520.

Li, D., Sharp, J.O., Saikaly, P.E., Ali, S., Alidina, M., Alarawi, M.S., Keller, S., Hoppe-Jones, C., Drewes, J.E., 2012. Dissolved organic Carbon influences microbial community composition and diversity in managed aquifer recharge Systems. Appl. Environ. Microbiol. 78, 6819–6828. https://doi.org/10.1128/AEM.01223-12.

Li, Jin, Alvarez, B., Siwabessy, J., Tran, M., Huang, Z., Przeslawski, R., Radke, L., Howard, F., Nichol, S., 2017. Application of random forest, generalised linear model and their hybrid methods with geostatistical techniques to count data: predicting sponge species richness. Environ. Model. Software 97, 112–129. https://doi.org/10.1016/j.envsoft.2017.07.016.

Li, Jie, Li, Y., Qian, B., Niu, L., Zhang, W., Cai, W., Wu, H., Wang, P., Wang, C., 2017. Development and validation of a bacteria-based index of biotic integrity for assessing the ecological status of urban rivers: a case study of Qinhuai River basin in Nanjing, China. J. Environ. Manag. 196, 161–167. https://doi.org/10.1016/j.jenvman.2017.03.003.

Li, J., Luo, G., He, L., Xu, J., Lyu, J., 2018. Analytical approaches for determining chemical oxygen demand in water bodies: a review. Crit. Rev. Anal. Chem. 48, 47–65. https://doi.org/10.1080/10408347.2017.1370670.

Li, X., Zhang, Y., Guo, F., Gao, X., Wang, Y., 2018. Predicting the effect of land use and climate change on stream macroinvertebrates based on the linkage between structural equation modeling and bayesian network. Ecol. Indicat. 85, 820–831. https://doi.org/10.1016/j.ecolind.2017.11.044.

Liang, J., Tang, W., Zhu, Z., Li, S., Wang, K., Gao, X., Li, Xin, Tang, N., Lu, L., Li, Xiaodong, 2023. Spatiotemporal variability and controlling factors of indirect N2O emission in a typical complex watershed. Water Res. 229, 119515 https://doi.org/10.1016/j.watres.2022.119515.

Lin, J., Chen, N., Yuan, X., Tian, Q., Hu, A., Zheng, Y., 2020. Impacts of human disturbance on the biogeochemical nitrogen cycle in a subtropical river system revealed by nitrifier and denitrifier genes. Sci. Total Environ. 746, 141139 https://doi.org/10.1016/j.scitotenv.2020.141139.

Lü, J., Wang, S., Liu, B., Zheng, W., Tan, K., Song, X., 2022. Slight flow volume rises increase nitrogen loading to nitrogen-rich river, while dramatic flow volume rises promote nitrogen consumption. Sci. Total Environ. 844, 157013 https://doi.org/10.1016/j.scitotenv.2022.157013.

Maavara, T., Chen, Q., Van Meter, K., Brown, L.E., Zhang, J., Ni, J., Zarfl, C., 2020. River dam impacts on biogeochemical cycling. Nat. Rev. Earth Environ. 1, 103–116. https://doi.org/10.1038/s43017-019-0019-0.

Marcot, B.G., Penman, T.D., 2019. Advances in Bayesian network modelling: integration of modelling technologies. Environ. Model. Software 111, 386–393. https://doi.org/10.1016/j.envsoft.2018.09.016.

Mello, K. de, Valente, R.A., Randhir, T.O., dos Santos, A.C.A., Vettorazzi, C.A., 2018. Effects of land use and land cover on water quality of low-order streams in Southeastern Brazil: watershed versus riparian zone. Catena 167, 130–138. https://doi.org/10.1016/j.catena.2018.04.027.

Müller, A., Österlund, H., Marsalek, J., Viklander, M., 2020. The pollution conveyed by urban runoff: a review of sources. Sci. Total Environ. 709, 136125 https://doi.org/10.1016/j.scitotenv.2019.136125.

Qu, X., Ren, Z., Zhang, H., Zhang, M., Zhang, Y., Liu, X., Peng, W., 2017. Influences of anthropogenic land use on microbial community structure and functional potentials of stream benthic biofilms. Sci. Rep. 7, 15117 https://doi.org/10.1038/s41598-017-15624-x.

Sagova-Mareckova, M., Boenigk, J., Bouchez, A., Cermakova, K., Chonova, T., Cordier, T., Eisendle, U., Elersek, T., Fazi, S., Fleituch, T., Frühe, L., Gajdosova, M., Graupner, N., Haegerbaeumer, A., Kelly, A.-M., Kopecky, J., Leese, F., Nõges, P., Orlic, S., Panksep, K., Pawlowski, J., Petrusek, A., Piggott, J.J., Rusch, J.C., Salis, R., Schenk, J., Simek, K., Stovicek, A., Strand, D.A., Vasquez, M.I., Vrålstad, T., Zlatkovic, S., Zupancic, M., Stoeck, T., 2021. Expanding ecological assessment by integrating microorganisms into routine freshwater biomonitoring. Water Res. 191, 116767 https://doi.org/10.1016/j.watres.2020.116767.

Scheffer, M., Carpenter, S., Foley, J.A., Folke, C., Walker, B., 2001. Catastrophic shifts in ecosystems. Nature 413, 591–596. https://doi.org/10.1038/35098000.

Sendzimir, J., Schmutz, S., 2018. Challenges in riverine ecosystem management. In: Schmutz, S., Sendzimir, J. (Eds.), Riverine Ecosystem Management: Science for Governing towards a Sustainable Future, Aquatic Ecology Series. Springer International Publishing, Cham, pp. 1–16. https://doi.org/10.1007/978-3-319-73250-3_1.

Shang, J., Zhang, W., Chen, X., Li, Y., Niu, L., Wang, L., Zhang, H., 2021. How environmental stress leads to alternative microbiota states in a river ecosystem: a new insight into river restoration. Water Res. 203, 117538 https://doi.org/10.1016/j.watres.2021.117538.

Simonin, M., Voss, K.A., Hassett, B.A., Rocca, J.D., Wang, S., Bier, R.L., Violin, C.R., Wright, J.P., Bernhardt, E.S., 2019. In search of microbial indicator taxa: shifts in stream bacterial communities along an urbanization gradient. Environ. Microbiol. 21, 3653–3668. https://doi.org/10.1111/1462-2920.14694.

Tian, Y., Jiang, Y., Liu, Q., Dong, M., Xu, D., Liu, Y., Xu, X., 2019. Using a water quality index to assess the water quality of the upper and middle streams of the Luanhe River, northern China. Sci. Total Environ. 667, 142–151. https://doi.org/10.1016/j.scitotenv.2019.02.356.

Torres-Martínez, J.A., Mora, A., Knappett, P.S.K., Ornelas-Soto, N., Mahlknecht, J., 2020. Tracking nitrate and sulfate sources in groundwater of an urbanized valley using a multi-tracer approach combined with a Bayesian isotope mixing model. Water Res. 182, 115962 https://doi.org/10.1016/j.watres.2020.115962.

Uddin, M.G., Nash, S., Rahman, A., Olbert, A.I., 2022. A comprehensive method for improvement of water quality index (WQI) models for coastal water quality assessment. Water Res. 219, 118532 https://doi.org/10.1016/j.watres.2022.118532.

Wang, J., Fan, H., He, X., Zhang, F., Xiao, J., Yan, Z., Feng, J., Li, R., 2021. Response of bacterial communities to variation in water quality and physicochemical conditions in a river-reservoir system. Glob. Ecol. Conserv. 27, e01541 https://doi.org/10.1016/j.gecco.2021.e01541.

Wang, K., Li, S., Zhu, Z., Gao, X., Li, X., Tang, W., Liang, J., 2022. Identification of priority conservation areas based on ecosystem services and systematic conservation planning analysis. Environ. Sci. Pollut. Res. https://doi.org/10.1007/s11356-022-24883-9.

Wang, L., Zhang, J., Li, H., Yang, H., Peng, C., Peng, Z., Lu, L., 2018. Shift in the microbial community composition of surface water and sediment along an urban river. Sci. Total Environ. 627, 600–612. https://doi.org/10.1016/j.scitotenv.2018.01.203.

Wu, H., Li, Y., Zhang, W., Wang, C., Wang, P., Niu, L., Du, J., Gao, Y., 2019. Bacterial community composition and function shift with the aggravation of water quality in a heavily polluted river. J. Environ. Manag. 237, 433–441. https://doi.org/10.1016/j.jenvman.2019.02.101.

Xuan, Y., Mai, Y., Xu, Y., Zheng, J., He, Z., Shu, L., Cao, Y., 2022. Enhanced microbial nitrification-denitrification processes in a subtropical metropolitan river network. Water Res. 222, 118857 https://doi.org/10.1016/j.watres.2022.118857.

Yang, C., Zeng, Z., Wang, Y., He, G., Hu, Y., Gao, D., Dai, Y., Li, Q., Zhang, H., 2022. Ecological risk assessment and identification of the distinct microbial groups in heavy metal-polluted river sediments. Environ. Geochem. Health. https://doi.org/10.1007/s10653-022-01343-4.

Yang, N., Li, Y., Zhang, W., Wang, L., Gao, Y., 2019. Reduction of bacterial integrity associated with dam construction: a quantitative assessment using an index of biotic integrity improved by stability analysis. J. Environ. Manag. 230, 75–83. https://doi.org/10.1016/j.jenvman.2018.09.071.

Zhang, W., Yang, G., Wang, H., Li, Y., Niu, L., Zhang, H., Wang, L., 2022. Predicting bend-induced heterogeneity in sediment microbial communities by integrating bacteria-based index of biotic integrity and supervised learning algorithms. J. Environ. Manag. 304, 114267 https://doi.org/10.1016/j.jenvman.2021.114267.

Zhang, W., Zhu, M., Li, Y., Wang, C., Qian, B., Niu, L., Wang, P., Gu, J., Yang, N., 2020. How fluvial inputs directly and indirectly affect the ecological status of different lake regions: a bio-assessment framework. J. Hydrol. 582, 124502 https://doi.org/10.1016/j.jhydrol.2019.124502.

Zhang, Z., Zhang, F., Du, J., Chen, D., Zhang, W., 2021. Impacts of land use at multiple buffer scales on seasonal water quality in a reticular river network area. PLoS One 16, e0244606. https://doi.org/10.1371/journal.pone.0244606.

Zheng, Y., Yang, T., Wang, N., Wan, X., Hu, C., Sun, L., Yan, X., 2023. Quantifying hydrological-ecological response relationships based on zooplankton index of biotic integrity and comprehensive habitat quality index - a case study of typical rivers in Xi"an, China. Sci. Total Environ. 858, 159925 https://doi.org/10.1016/j.scitotenv.2022.159925.

Zhu, Z., Li, Xin, Bu, Q., Yan, Q., Wen, L., Chen, X., Li, Xiaodong, Yan, M., Jiang, L., Chen, G., Li, S., Gao, X., Zeng, G., Liang, J., 2023. Land–water transport and sources of nitrogen pollution affecting the structure and function of riverine microbial communities. Environ. Sci. Technol. 57, 2726–2738. https://doi.org/10.1021/acs.est.2c04705.

Zhu, Z., Wang, K., Lei, M., Li, Xin, Li, Xiaodong, Jiang, L., Gao, X., Li, S., Liang, J., 2022. Identification of priority areas for water ecosystem services by a techno-economic, social and climate change modeling framework. Water Res. 221, 118766 https://doi.org/10.1016/j.watres.2022.118766.