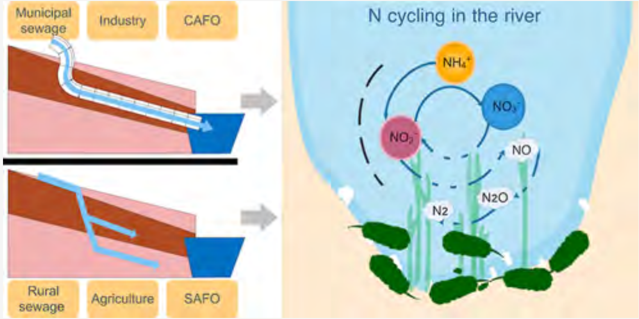
Environmental Science & Technology

pubs.acs.org/est Article

Land–Water Transport and Sources of Nitrogen Pollution Affecting the Structure and Function of Riverine Microbial Communities

Ziqian Zhu, Xin Li, Qiurong Bu, Qingcheng Yan, Liqun Wen, Xiaolei Chen, Xiaodong Li, Ming Yan, Longbo Jiang, Gaojie Chen, Shuai Li, Xiang Gao, Guangming Zeng, and Jie Liang*

Cite This: Environ. Sci. Technol. 2023, 57, 2726–2738 Read Online



ABSTRACT: The characterization of variations in riverine microbiota that stem from contaminant sources and transport modes is important for understanding biogeochemical processes. However, the association between complex anthropogenic nitrogen pollution and bacteria has not been extensively investigated owing to the difficulties faced while determining the distribution of nitrogen contaminants in watersheds. Here, we employed the Soil and Water Assessment Tool alongside microbiological analysis to explore microbial characteristics and their responses to complex nitrogen pollution patterns. Significant variations in microbial communities were observed in sub-basins with distinct land–water pollution transport modes. Point source-dominated areas (PSDAs) exhibited reduced microbial diversity, high number of denitrification groups, and increased nitrogen cycling compared with others. The negative relative deviations (−3.38) between the measured and simulated nitrate concentrations in PSDAs indicated that nitrate removal was more effective in PSDAs. Pollution sources were also closely associated with microbiota. Effluents from concentrated animal feeding operations were the primary factors relating to the microbiota compositions in PSDAs and balanced areas. In nonpoint source-dominated areas, contaminants from septic tanks become the most relevant sources to microbial community structures. Overall, this study expands our knowledge regarding microbial biogeochemistry in catchments and beyond by linking specific nitrogen pollution scenarios to microorganisms.

KEYWORDS: *complex nitrogen sources, SWAT, microbial community, point sources, nonpoint sources*

1. INTRODUCTION

Surplus nitrogen is one of the greatest threats to water quality and has been implicated in several ecological disasters.[1] Nitrogen biogeochemical processes, mostly controlled by microorganisms, are essential for the self-purification of water. These microbes undergo multiple environmental pressures, including inflow contamination from various nitrogen pollution sources, which alter the nitrogen cycling pathway or rate in rivers.[2−4] Generally, the microbial nitrogen cycling network involves nitrogen fixation, nitrification, denitrification, anaerobic ammonium oxidation (ANAMMOX), and dissimilatory nitrate reduction to ammonium.[5] The effluents primarily affect microbial states by importing nutrients and wastewater-originated microbes and altering habitat features, thereby affecting water biogeochemistry.[6,7] Effluent-influenced reaches are beneficial for ammonia-oxidizing bacteria (AOB), which are considered the primary microorganisms responsible for nitrification.[8,9] Two nitrite reductase genes (*nirS* and *nirK*) exhibit opposite correlations with the environmental nitrogen concentration, suggesting that organisms possessing different nitrite reductase genes might occupy distinct niches.[10,11] The *nirK*/*nirS* ratio is lower in areas affected by effluents than in unaffected areas, and *nirS*-type denitrifiers constitute the primary contributors to denitrification.[12] Moreover, incomplete heterotrophic denitrification is frequently observed in rivers strongly affected by effluents, as reflected by a low *nosZ*/*nir* ratio and an increased N_2O emission potential.[12−14] To the best of knowledge, so far, most studies have only explored the effect of a single pollution source on microorganisms, whereas few studies have examined complex nitrogen contamination scenarios. Hence, the microbial characteristics and their responses to various nitrogen pollution sources warrant clarification.

Nitrogen pollution can be classified into nonpoint and point sources based on their transportation modes.[15] Correspondingly, nitrogen runoff via soil erosion and leaching comprise the main transport pathways of contaminants from nonpoint

Received: July 14, 2022
Revised: November 23, 2022
Accepted: January 26, 2023
Published: February 6, 2023

https://doi.org/10.1021/acs.est.2c04705

sources into rivers; contaminants from point sources tend to flow directly into rivers via pipe networks.[16] Owing to the differences in hydrological, physicochemical, and biotic conditions between the two distinct transport modes, the microbial composition and functionality in rivers in nonpoint source-dominated areas (NPSDAs) might differ from those in point source-dominated areas (PSDAs).[1] Therefore, elucidating the mechanism of water self-purification requires further clarification regarding microbial characteristics under various contaminant transport modes.

Identifying pollution sources can be difficult because of the complex sources and patterns of transport of nitrogen contaminants.[17,18] Several ecohydrological models have been developed to simulate the transport and conversion of contaminants from diverse regions of basins to receiving water.[19,20] Herein, the Soil and Water Assessment Tool (SWAT) was used as it is an open-source software with a wide range of applications. Furthermore, it can simulate the transport process from multiple points and nonpoint sources of contaminants in basins.[4,21,22]

Characterizing the biogeochemical feature is crucial for environmental protection when the nitrogen pollution sources are complex. Accordingly, we focused on the mutual feedback mechanism between microbial activities and the sources and land−water transport modes of river nitrogen contaminants to address the following objectives: (1) to determine the characteristics of prokaryotic microorganisms and their responses to different transport modes of nitrogen pollution; (2) to identify the primary nitrogen pollution sources correlating with microbial communities; and (3) to evaluate whether and to what extent changes in microbial communities affect nitrogen transformation. We used SWAT in conjunction with 16S rRNA gene sequencing and the quantitative polymerase chain reaction (qPCR) to analyze the characteristics of the microbiota in regions with varied nitrogen pollution transport modes and sources.

2. MATERIALS AND METHODS

2.1. Study Site and Sample Collection. This study was conducted in the Liuyang River Basin, which is located downstream of the Xiangjiang River, a major tributary of the Yangtze River (see Supporting Information, Text S1, for details). According to the existing status of water pollution in the main channel and tributaries of the Liuyang River, 17 reaches with various nitrogen contamination scenarios were sampled. Each reach had three sample locations near the sub-basin outlet (Figure S1). The water and sediment samples were collected in January 2021. At each site, water samples (10−30 cm in depth) were collected in triplicate using precleaned polyethylene bottles. Sediment samples were also gathered in triplicate from the top layer of riverbeds (0−5 cm in depth) with a grab sampler (XDB0201, Pusen, China). The water and sediment samples were then homogenized, sealed in presterilized bags, and stored at −80 °C for further analysis. Following the collection of samples, bacterial DNA was extracted from the sediments, and physicochemical analysis of the water and sediments was conducted.

2.2. Measurement of Physicochemical Parameters. A total of 15 environmental parameters were monitored in this study, including 9 water variables and 6 sediment variables (Figure S2). The dissolved oxygen (DO), water temperature (T), and pH were measured in situ with a portable multi-meter (HQ2200, HACH, USA). The concentrations of nitrate (NO_3^-−N), ammonium nitrogen (NH_4^+−N), total phosphorus (TP), total nitrogen (TN), sulfate (SO_4^{2-}), and chloride (Cl^-) of water and sediments were measured using the corresponding methods described in Supporting Information, Text S2.

2.3. DNA Extraction and Sequencing Analysis. DNA was isolated from homogenized sediment samples using a soil genomic DNA extraction kit (Solarbio, Beijing, China). The quality of isolated DNA was evaluated using 1% agarose gel electrophoresis, and it was subsequently frozen at −20 °C. PCR amplification and high-throughput sequencing were performed at Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China). PCR amplification was conducted using the 806R (5′-GGACTACHVGGGTWTCTAAT-3′) and 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) primers, which targeted the V3−V4 regions of 16S rRNA (Supporting Information, Text S3). The sample sequence data have been deposited in Sequence Read Archive (http://www.ncbi.nlm.nih.gov/sra/) for public access (bioproject number: PRJNA845821).

2.4. Quantitative PCR. Six target genes were amplified using PCR with different primers (Table S1). The reactions were performed in a 20 μL reaction mixture comprising 10 μL of qPCR mix, 0.4 μL of reference dye, 0.8 μL of each primer (5 μM), 6 μL of ddH_2O, and 2 μL of template DNA. The ABI 7300 system (Applied Biosystems, USA) and SYBR green were used to perform PCR reactions (Supporting Information, Text S4).

2.5. SWAT Model Establishment. SWAT utilizes land use data, soil properties, digital elevation model data, meteorological data, daily streamflow data, and monthly observations of TN, NH_4^+−N, and NO_3^-−N (Figure S3), as detailed in Supporting Information, Text S5. Both point and nonpoint contaminant sources contribute to water contamination. In our analysis, point sources comprised industrial zones, industries located outside of the zone, municipal wastewater treatment facilities, rural sewage treatment plants, concentrated animal feeding operations (CAFOs), and refuse landfills (Supporting Information, Text S6 and Table S2). Nonpoint sources of contaminants included crop cultivation (Table S3 provides details on crop management practices for double-crop rice rotation), scattered small-scale animal feeding operations (SAFOs), and rural sewage without centralized treatment (Supporting Information, Text S6).

SWAT calibration and uncertainty procedure (SWAT-CUP) software was used for calibration and validation (Supporting Information, Text S7).[23] Herein, we calibrated the selected parameters with several iterations of 500 simulations calibration (Table S4). The model accurately stimulated monthly discharges and nitrogen species loadings for the calibration and validation periods (R^2 of all >0.66 and Nash−Sutcliffe model efficiency (NSE) of all >0.58) (Table S5 and Figure S4).

The approach for nitrogen source attribution is provided in Supporting Information, Text S8.

2.6. Statistical Analyses. Specific information regarding statistical analyses is included in Supporting Information, Text S9. Briefly, Mantel tests, canonical correlation analysis (CCA), principal coordinate analysis (PCoA), and redundancy analysis (RDA) were conducted using the vegan package in R 4.0.2. Wilcoxon rank-sum tests, Kruskal−Wallis test, one-way analysis of variance (ANOVA), and Spearman correlation analysis were performed in the SPSS 22.0 platform. Random

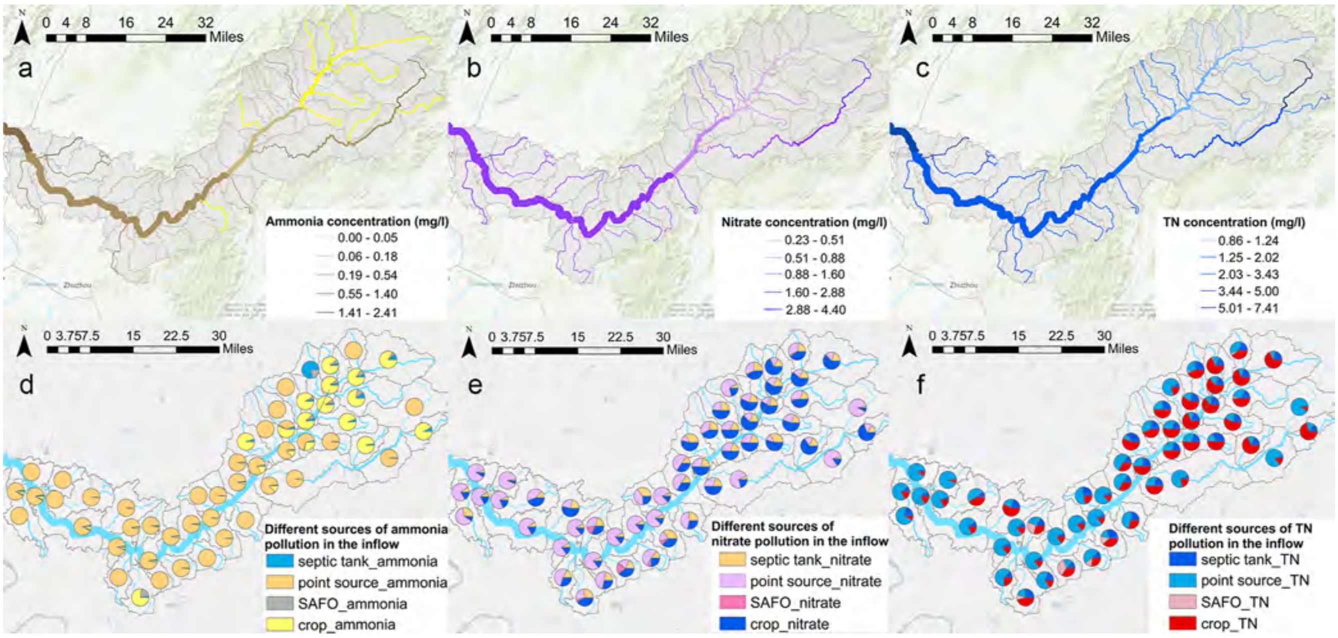


Figure 1. Simulated average nitrogen loadings from various sources and the concentration of nitrogen species in the river. The simulated average riverine concentration of (a) ammonia, (b) nitrate, and (c) TN are shown in the color maps. The simulated proportions of the average nitrogen loading of (d) ammonia, (e) nitrate, and (f) TN in the river from different sources are shown in the pie charts.

forests analysis was conducted using the randomForest package in R 4.0.2. The phenotype and metabolic function of microbiota were predicted using the BugBase database and functional annotation of prokaryotic taxa, respectively. Linear discriminant analysis and effect size (LEfSe) analysis were performed using LEfSe software. Bipartite association network analysis was conducted using the NetworkX package in the Python 3.8.2 platform. Structural equation modeling (SEM) was conducted using the "piecewiseSEM" R package.

3. RESULTS AND DISCUSSION

3.1. Key Environmental Variables Relating to Microbial Community Composition. All sequences were classified into 59 phyla (Figure S5). The Mantel test indicated that the TN concentration in water ($R = 0.40$, $p = 0.001$) was the most significant factor correlated with microbiota composition (Table S6). CCA results also showed that the TN concentration in water (with the longest arrow and highest value of R^2) was the most relevant factor ($R^2 = 0.67$, $p = 0.001$) for microbiota compositions (Figure S6 and Table S7). In addition, nitrate and ammonia were associated substantially with microbial populations (Tables S6 and S7). These results verified the hypothesis that the nitrogen concentration in the Liuyang River was one of the primary factors associated with the organization of microbial communities. Therefore, further investigation into the particular states of microorganisms under diverse nitrogen pollution patterns is warranted.[24,25]

3.2. Nitrogen Concentration Simulation and Source Attribution. The concentrations of TN, NH_4^+−N, and NO_3^-−N increased from upstream to downstream locations, ranging from 0.86 to 7.41, 0.05 to 2.41, and 0.23 to 4.40 mg/L, respectively, as determined by the SWAT simulation (Figure 1a−c).

The source of TN and NO_3^-−N pollution was comparable at each outlet of the reach (Figure 1e,f). In the upstream area, agriculture produced >50% of the total TN and NO_3^-−N load, while SAFOs contributed <1%. In certain midstream subbasins, SAFOs accounted for >33% of the total load of these two nitrogen species as there were many small hog farms scattered along the middle stream. The contribution percentages of agriculture to TN and NO_3^-−N decreased from the upstream to downstream regions. The proportion of point sources increased gradually downstream, as urban domestic sewage treated by municipal plants and industrial wastewater generated by commercial output increased downstream.[26,27]

NH_4^+−N was derived from distinct sources than TN and NO_3^-−N (Figure 1d). Except a few agricultural regions in the upper reaches where cultivation constituted the primary pollution source, point sources comprised the primary source of riverine NH_4^+−N in the middle and lower reaches (i.e., point sources comprised >50% of the pollution sources). This was possible because soil colloids are negatively charged while NH_4^+−N is positively charged; hence, NH_4^+−N tends to bind to soil particles. Additionally, when contaminants are transferred from land to water bodies via nonpoint source transport, it might take a considerable amount of time for them to transit through the surface, topsoil, vadose zone, and shallow aquifer, ultimately reaching the main channel. During these phases, native microorganisms could rapidly convert NH_4^+−N to various forms via nitrification and ANAMMOX processes. Conversely, point source contaminants can be discharged directly into rivers via pipe networks in a shorter time. The short hydraulic retention time makes ammonia less susceptible to transformation during land−water transport; thus, point source pollution accounts for a relatively large proportion of ammonia in rivers.[28,29]

3.3. Association between Contaminant Transport Mode and Microbial Characteristics. Per the land−water transport modes of nitrogen pollution in each reach simulated by the SWAT model (Figure 1d−f), the sampling locations were categorized into three types: PSDAs, NPSDAs, and balanced areas (BAs) exhibiting equal contributions from point and nonpoint sources. According to PCoA results (Figure 2a),

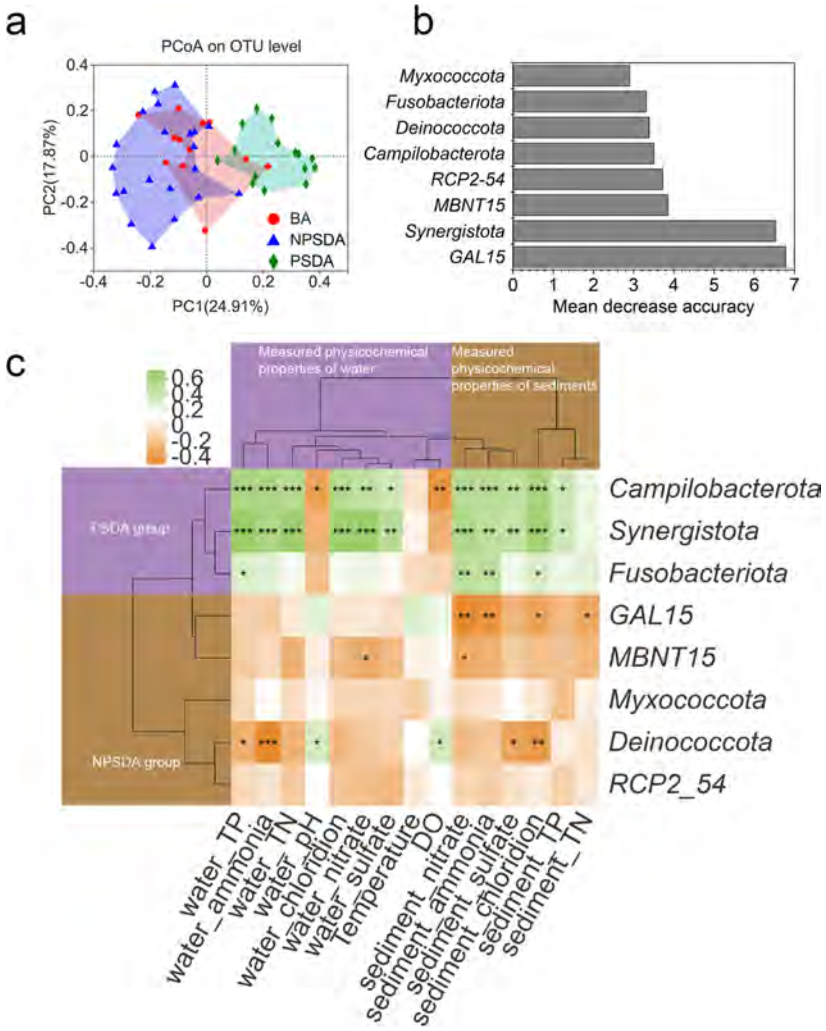


Figure 2. (a) PCoA diagram of the composition of microbial communities in the PSDA group, the NPSDA group, and the BA group. (b) The top eight most important features at the phylum level were used as the biomarkers to distinguish between the PSDA and NPSDA groups. (c) Spearman correlations between the relative abundance of biomarkers and the measured environmental factors.

the samples from PSDAs and NPSDAs were clustered into two distinct groups, thereby confirming the hypothesis that subbasins with different contaminant transport modes support distinct microbial communities.

The microbiota of NPSDAs exhibited significantly higher species richness ($p = 0.041$; Wilcoxon rank-sum tests) (Figure S7a) and community diversity ($p = 0.020$; Wilcoxon rank-sum tests) (Figure S7b) than those of PSDAs and BAs, indicating increased within-habitat diversity. NPSDAs demonstrated a higher number of microbial species than PSDAs (Figure S8). This may be due to the following: (1) the presence of hazardous substances in the effluents of point sources, such as halogenated organics and antibiotics, possibly prevent the propagation of certain microbes in the receiving rivers[30] and (2) in point source-influenced regions, excessive nitrogen levels (Figure S2) may help homogenize the microbial communities, engendering the loss of majority of the species and substitution with less expanding species, thereby reducing biodiversity.[31] Our findings indicated that the microbial community in PSDAs exhibit reduced microbial population size and diversity, thereby potentially favoring certain functional groups and causing biotic homogenization.[32,33]

The eight most important features were used as biomarkers to differentiate between PSDAs and NPSDAs in random forests analysis (Figure 2b), and the abundances of the biomarkers are detailed in Table S8. Three biomarkers were more prevalent in PSDAs than in NPSDAs, while five biomarkers were more prevalent in NPSDAs than in PSDAs (Figure S9). Analysis of the correlation between the relative abundance of the selected biomarkers and the measured physiochemical properties of rivers revealed two distinct clusters (Figure 2c). The three enriched biomarkers in the PSDA group were positively correlated with the majority of environmental factors, except for the pH and DO in water. However, six bacterial families from the NPSDA group were negatively correlated with the majority of environmental factors. Therefore, areas with diverse land−water transport modes of nitrogen contaminants exhibited heterogeneity in the microenvironment of sediments and dissimilarity among microbial communities.[34,35]

A significantly lower ratio of ammonia-oxidizing archaea (AOA)/AOB *amoA* genes [mean value, 1.13; standard error of the mean (SE), 0.04; standard deviation (SD), 0.15; $p < 0.05$; one-way ANOVA] was observed in PSDAs compared with NPSDAs (mean value, 1.28; SE, 0.04; SD, 0.19; $p < 0.05$; one-

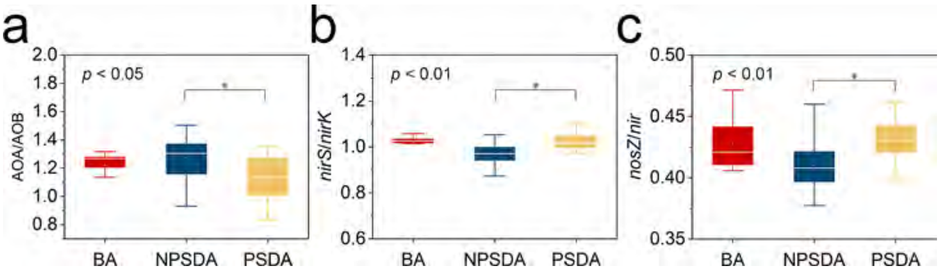


Figure 3. Boxplots of the relative abundance of (a) AOA/AOB, (b) *nirS*/*nirK*, and (c) *nosZ*/*nir* among the PSDA, NPSDA, and BA groups.

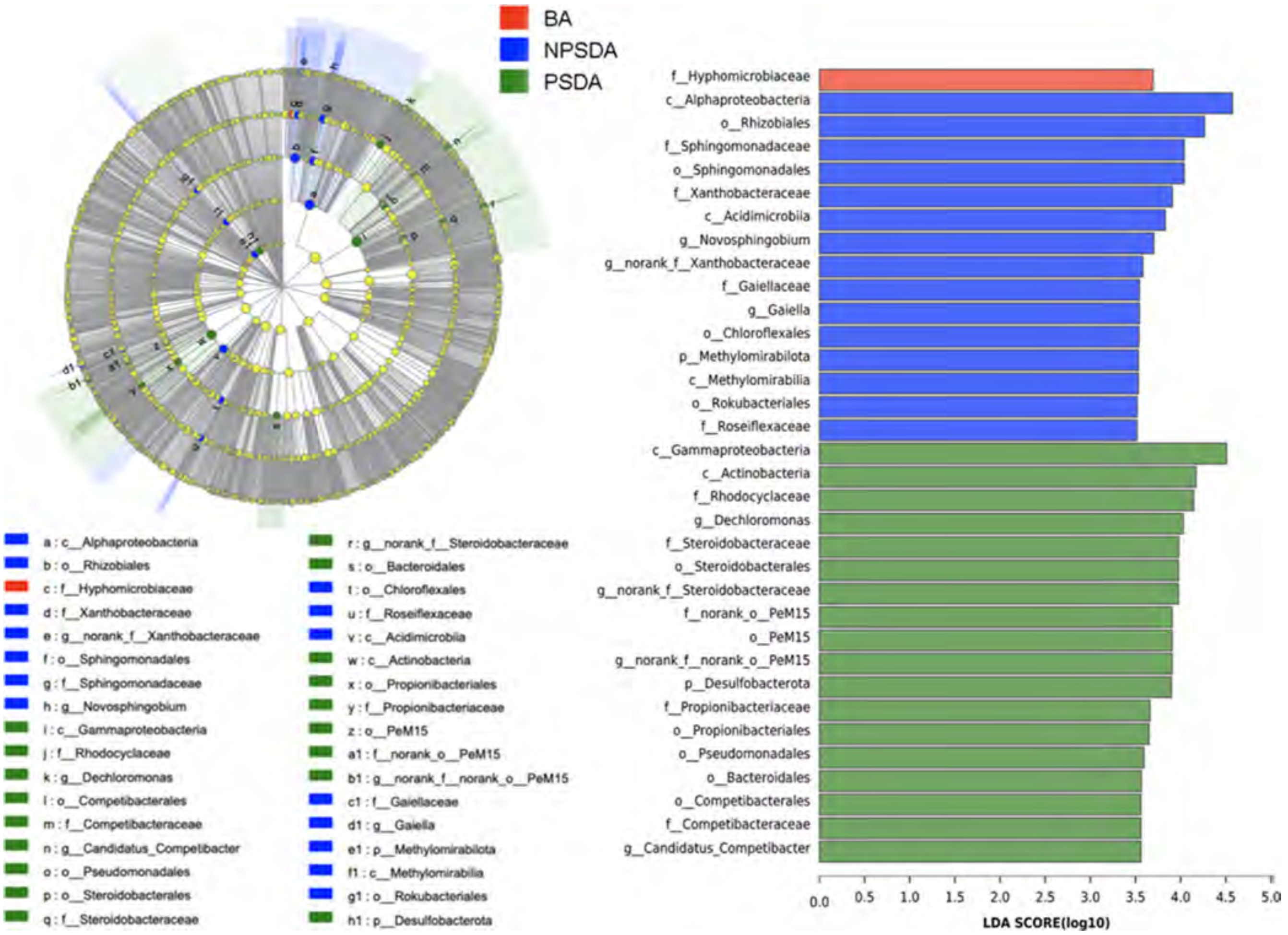


Figure 4. Taxonomic differences between sediments in the PSDA, the NPSDA group, and BA groups. Cladogram indicating the phylogenetic distribution of microbial lineages in the sediment of the PSDA group (green), the NPSDA group (blue), and the BA group (red).

way ANOVA) (Figure 3a). As AOB are considered the major contributors to ammonia oxidation, nitrification might be stimulated in PSDAs.[8,9,36] Studies have reported that increase in the available nitrogen concentration, ammonia in particular, is beneficial for the growth of AOB over AOA, as AOB occupy a broader ecological niche than AOA.[37] The higher sediment ammonia concentration (Figure S2) explains why the AOA/AOB ratio is lower in PSDAs than in other places. Additionally, manure is a major nitrogen source in NPSDAs and has been proven to provide energy to ammonia oxidizers and significantly increase the number of AOA.[38] The ratio of *nirS*/*nirK* was significantly increased by 1.06 times in the PSDA group (mean value, 1.02; SE, 0.01; SD, 0.06; $p < 0.01$; Kruskal–Wallis test) than in the NPSDA group (mean value, 0.96; SE, 0.01; SD, 0.07; $p < 0.01$; Kruskal–Wallis test) (Figure 3b). Previous research has shown that high *nirS*/*nirK* ratios might increase denitrification capacity.[39] Furthermore, the presence of one or more metals (Zn and Cu) in sediments was reported to beneficially impact the *nirS*/*nirK* ratio.[40] The cumulative heavy metals (Zn and Cu) originating from the point sources may cause a higher ratio of *nirS*/*nirK* in the PSDA group than in the other groups.[41] The *nosZ*/*nir* ratio was significantly higher (mean value, 0.43; SE, 0.00; SD, 0.02; $p < 0.01$; one-way ANOVA) in the PSDA group than in the NPSDA group (mean value, 0.41; SE, 0.00; SD, 0.02; $p < 0.01$; one-way ANOVA), suggesting that denitrification is more complete and the N_2O/N_2 emission ratio is lower in PSDAs than in NPSDAs (Figure 3c).[13,42] Besides, the *amoA* of AOA

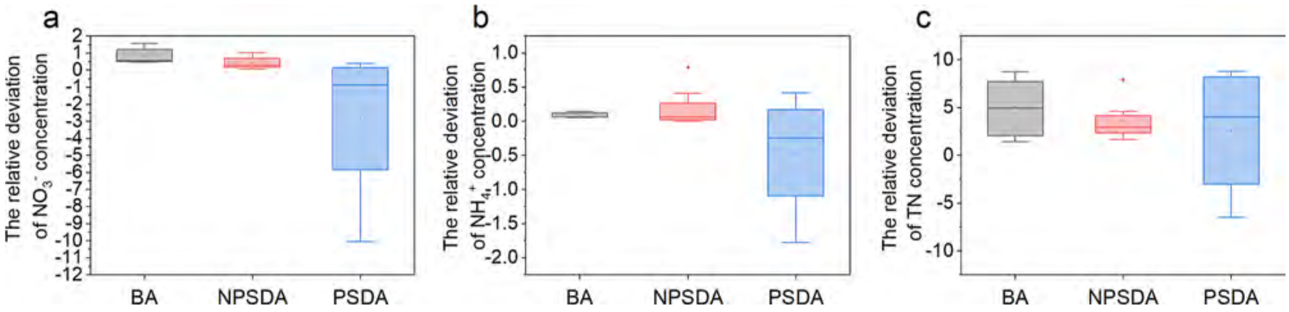


Figure 5. Relative deviations (i.e., the deviation between the measured concentrations and the simulated concentrations divided by the measured concentrations) of (a) NO_3^-, (b) NH_4^+, and (c) TN in the PSDA, NPSDA, and BA groups.

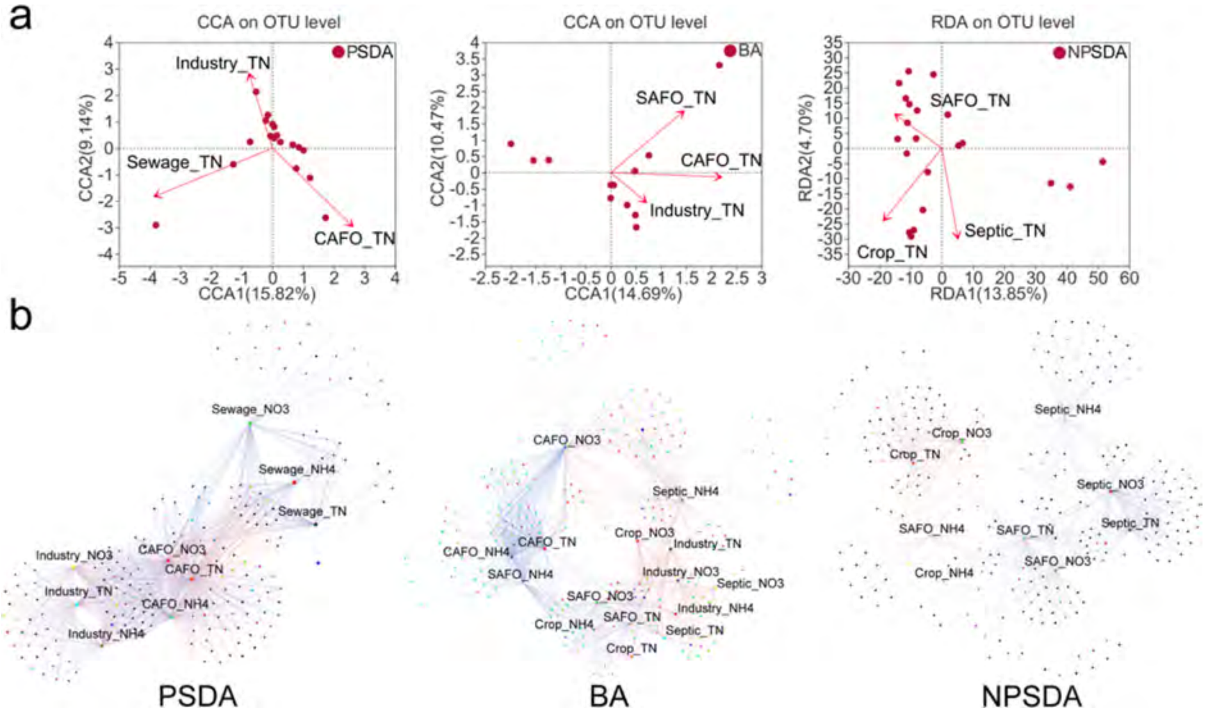


Figure 6. (a) Relationship between the composition of microbial communities and nitrogen sources (point sources include CAFO, municipal and rural sewage treatment plants, and industrial facilities; nonpoint sources include crop cultivation, SAFO, and rural sewage from septic tanks without centralized processing) as indicated by the CCA and RDA diagram and (b) bipartite association network in the PSDA, BA, and NPSDA groups.

was more abundant in NPSDAs (mean value, 5.88; SE, 0.19; SD, 0.94; $p < 0.05$; one-way ANOVA) than in PSDAs (mean value, 5.27; SE, 0.17; SD, 0.72; $p < 0.05$; one-way ANOVA), indicating that the ammonia-oxidizing archaea were enriched in the NPSDAs (Figure S10). These results confirmed the variations in nitrogen-related metabolic pathways under distinct pollutant transport modes.

Significantly higher levels of predicted functions involved in nitrate reduction ($p < 0.05$; Kruskal–Wallis test), nitrate respiration ($p < 0.1$; Kruskal–Wallis test), and nitrogen respiration ($p < 0.1$; Kruskal–Wallis test) were observed in PSDAs than in NPSDAs (Figure S11a). These results were consistent with the findings of the aforementioned analysis showing that point sources can accelerate the transformation of nitrogen in rivers. The following factors may account for the increased rate of nitrogen transformation in point than in nonpoint sources: (1) the majority of sewage from point sources is treated by various wastewater treatment technologies before discharge into the river, which creates unique microenvironments and promotes the aggregation of nitrogen-related taxa[43] and (2) the colonization and proliferation of nitrogen-related genera are promoted in point sources via the high concentration of nitrogen in the riverine sediments of PSDAs.[44] Additionally, anaerobic microbes were more abundant in PSDAs than in NPSDAs (Figure S11b), suggesting that PSDAs are ideal sites for anaerobic denitrification. However, field data indicated no significant difference between PSDAs and NPSDAs in terms of DO levels (Figure S2). A probable explanation for this is that the pipe networks of point sources may have a relatively anaerobic environment and that the effluents from the pipeline thus carry a large concentration of anaerobic microbes, thereby generating this behavior. Additionally, metabolic inferences from functional annotation of prokaryotic taxa and BugBase predictions warrant additional validation.

Sphingomonadaceae, *Rhizobiales*, *Rokubacteriales*, *Acidimicrobiia*, *Roseiflexaceae*, and *Gaiella* were shown to be enriched in NPSDAs. *Rhizobiales* and *Sphingomonadaceae* are known to fix nitrogen and are symbionts with plant roots.[45] In PSDAs, the most prevalent taxa included *Competibacteraceae*, *Pseudomona-*

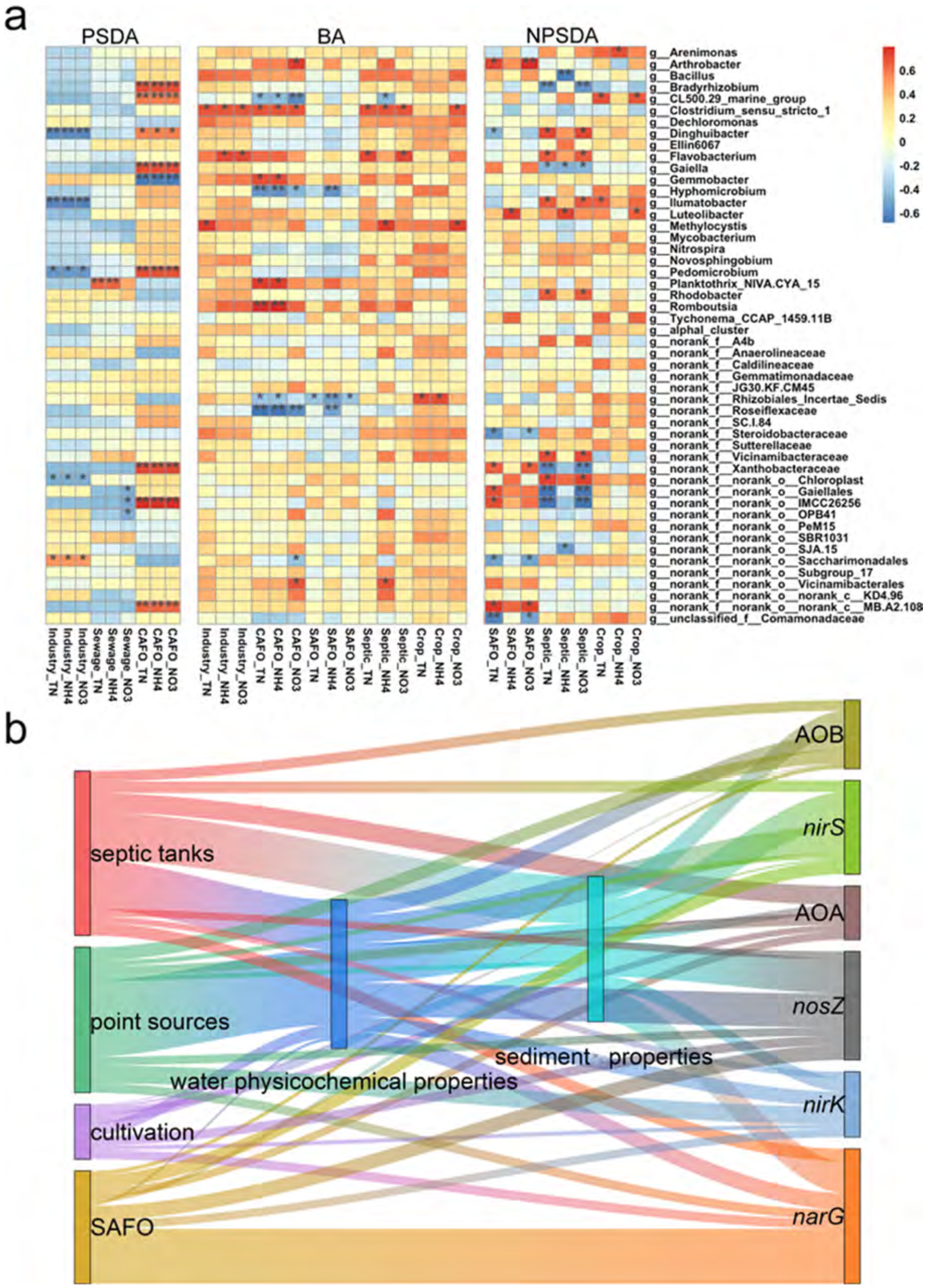


Figure 7. (a) Relationship between the composition of microbial communities and nitrogen sources indicated by a heatmap of the results of Spearman correlation analyses. (b) SEM image of the relationships among the pollution sources of nitrogen species, physicochemical properties of water, and sediment characteristics.

dales, *Steroidobacteraceae*, *Desulfobacterota*, *Rhodocyclaceae*, *PeM15*, *Propionibacteriaceae*, and *Bacteroidales*. Several microorganisms present in PSDAs belonged to the orders that contributed to denitrification (e.g., *Rhodocyclaceae*, *Competibacteraceae*, *Steroidobacteraceae*, and *Bacteroidales*) (Figure 4).[46,47] Accordingly, the results of LEfSe analysis indicated that the presence of nitrogen cycling-related microbes makes PSDAs the active regions for denitrification.

The complete nutrient cycle in hydrological response units was modeled using SWAT by simulating nitrogen transport and microbial nitrogen transformation. Detailed information is provided in previous studies.[48] To characterize the microbial nitrogen transformation level in the river of the three groups, the riverine microbial-related parameters were made identical for each reach to ensure that the SWAT-simulated riverine microbial nitrogen transformation was identical in all rivers. The relative deviations (i.e., the deviation between the measured and simulated concentrations divided by the measured concentrations) of nitrogen species varied among the different regions (Figure 5a−c). The relative deviation of NO_3^- in the PSDA group was lower than that in other regions, and the majority of observed deviations were negative, indicating that active denitrifier-induced activities induced stronger NO_3^- removal in PSDAs (Figure 5a). The relative TN concentration deviations in the BAs and NPSDAs were positive, thereby indicating that the measured concentrations were consistently greater than the simulated findings (Figure 5c). This could be attributed to the weaker nitrogen microbial transformation via the nonpoint source pathway.[49] These findings allow us to expand our knowledge regarding microbial biogeochemistry of the catchment and beyond, which can also be used to optimize the performances of simulation models involving nitrogen migration and transformation.

3.4. Relationship between Nitrogen Sources and Microbiota Compositions. According to CCA and RDA of the operational taxonomic unit (OTU) (Figure 6a), nitrogenous contaminants from CAFOs and sewage treatment plants comprised the most significant variables in the PSDAs. In the BA group, the contaminants from two types of feedlots (CAFOs and SAFOs) constituted the most important factors associated with microbiota composition. In the NPSDA group, nitrogen contamination from rural septic tanks and crop cultivation was most strongly associated with microbiota composition.

The bipartite association network of the PSDA group (Figure 6b) revealed that 141 OTUs were significantly correlated with CAFO contamination (node degree, 141 for TN), which showed the highest number of close links between pollution sources and OTUs. In the BA group, the maximum number of OTUs was associated with CAFOs (node degree, 114 for nitrate). In the NPSDA group, OTUs were also strongly linked with septic tanks (node degree, 81 for TN), thus indicating that septic tank contamination exhibited a substantial relationship with sediment microbial communities.

The sensitivity of dominant species to various contamination sources was analyzed at the genus level (Figure 7a). *Xanthobacteraceae* and *Bradyrhizobium* (both nitrogen-fixing bacteria) were significantly positively correlated with CAFOs in PSDAs and negatively correlated with septic tanks in NPSDAs.[50−52] The relative abundance of *Clostridium* (nitrogen-fixing bacteria) increased with increase in the levels of industry effluents, CAFOs, and septic tanks in BAs.[53,54] *Arthrobacter*, a functional microorganism involved in uncommon heterotrophic nitrification processes, propagated in BAs and NPSDAs in the presence of the effluents of CAFOs and SAFOs, respectively.[55] The denitrifier *Gaiella* was positively correlated with CAFO contaminants in PSDAs and negatively correlated with septic tank contaminants in NPSDAs.[52] The denitrifier *Flavobacterium* was positively correlated with septic tank contaminants in both the BA and NPSDA groups.[56] *Comamonadaceae*, a family of common aquatic denitrifying bacteria, was negatively associated with the nitrogen species of SAFOs in the NPSDA group.[57] *Hyphomicrobium*, exhibiting both denitrification and nitrogen fixation genes, was negatively correlated with the nitrogen species of CAFOs and SAFOs in the BA group.[58] *Planktothrix*, a harmful cyanobacterium existing in eutrophic waters, was positively correlated with sewage plant contaminants in the PSDA group and CAFO contaminants in the BA group. According to Spearman correlation analysis, CAFOs were significantly correlated with the majority of the microorganisms involved in the nitrogen cycles in the PSDA and BA groups. The discharge standard for effluents from CAFOs is less stringent than that of wastewater from sewage plants and industrial facilities; thus, the higher nutrient levels of CAFO contaminants may explain their strong correlations with the majority of microorganisms.[59] Rural septic tank effluents were significantly correlated with numerous genera in the NPSDA group, followed by SAFOs and crop cultivation. Because various pollution sources also introduce large quantities of bacteria to the rivers, Figure 7a indicates the bacteria imported from various sources as well. For instance, the genus *Arenimonas*, which is reported to be abundantly enriched in cropland, was found to be significantly associated with NH_4^+−N from agriculturally polluted effluents.[60] In addition, the genus *Bacillus* indicates the movement of septic tank effluents, which was also positively relevant for NH_4^+−N from septic tanks.[61] Moreover, the predominance of *Romboutsia* in jejuna ruminants has been frequently reported previously; thus, a significant relationship between *Romboutsia* and nitrogen series from CAFOs was assumed.[62] The above findings indicate that the most significant factor associated with microbiota composition among the selected pollution sources was the nitrogen series from CAFOs in the PSDA and BA groups and the nitrogenous contaminants from rural septic tanks in the NPSDA group.

SEM results revealed that septic tank and point source contaminants exerted the strongest direct correlation with the physicochemical properties of water (Figure 7b). Septic tanks had a direct relationship with sediment properties as well, while other pollution sources demonstrated only indirect correlations with sediment properties by altering the physicochemical properties of water. The denitrification-related genes *narG*, *nirS*, and *nosZ* were directly associated with sediments, suggesting that sediments were hotspots for denitrification.[63,64] SAFOs showed direct relevance with *narG*, indicating their involvement in nitrate reduction.

3.5. Implications for Environmental Remediation and Supervision of River Courses. Determining how river pollution affects the structure and function of microorganisms is necessary for optimizing the trade-offs associated with nitrogen pollution reduction strategies. The findings of this study shed new light on how microorganisms react to various riverine pollution scenarios.

3.5.1. Relationships between Microbial Characteristics and Pollution Transport Modes. Compared with NPSDAs and BAs, PSDAs appear to be hotspots for microbial nitrogen removal. The transport mode of pollution does not act on the microbes directly and is a comprehensive definition and hence microorganisms are unable to detect the transport mode of their habitats. However, pollution transport modes have been significantly associated with several small-scale factors that affect microbiota directly (Figure 2c). Furthermore, as freshwater biodiversity is structured by a hierarchy of characteristics acting at different spatial scales, the transport modes of nitrogen pollution cannot account for all differences in the microbial populations. Nonetheless, as the transport mode is the integrative attribute of polluting scenarios on a catchment scale, it is relevant for the regional estimates of microbial states. The heterogeneity in the microbial composition and functionality at locations with various nitrogen transport modes may be explained as follows: (1) because of hydrological and biogeochemical nitrogen legacy effects, it may take years for contaminants from nonpoint sources to reach rivers. Contaminants from nonpoint sources may have therefore impacted water quality in the past and continue to degrade it presently, although the sources might not be active.[65] Conversely, pollution from point sources flows into rivers via pipe networks with a short hydraulic retention time; (2) when contaminants are transported from land to water bodies via nonpoint source transport modes, the contaminants need to permeate the surface, topsoil, vadose zone, and shallow aquifer to ultimately reach the main channel, thereby importing the native bacteria present in the different phases to the rivers.[66] Furthermore, end-of-pipe treatment, which is frequently used to treat domestic and industrial wastewaters, is also applied to point sources, which could cause effluents to include a significant number of functional microorganisms.[67] Additionally, the release of bacteria from the pipe biofilm affects bulk water;[68] (3) diverse levels of physicochemical variables, including temperature, pH, DO, and nutrition, exist in areas with separate transport modes (Figure 2c). Hence, differences in retaining nutrients, importing microorganisms, and modifying microenvironmental characteristics may cooperate to regulate bacterial states under different pollution transport modes. Alternatively, laboratory-based experiments that manipulate contaminant transport would allow us to account for the impacts of other processes, thereby isolating variance caused exclusively by pollution transport modes.

Nitrogen abatement policies have been focusing on improving the discharge standard for wastewater from point sources. However, the self-purification of point source discharge is inevitably disregarded. In addition, despite the poor capacity of NPSDAs for microbial nitrogen removal, nonpoint source nitrogen emissions are not regulated to some extent. Therefore, stronger regulation is necessary to control pollution originating from NPSDAs. Furthermore, natural-based environmental remediation strategies should be developed for NPSDAs' discharge that can increase effluent self-purification capacity, such as by adding inoculants containing functional microorganisms.[69] Furthermore, the insufficient nitrogen transformation capacity of NPSDAs renders them a hotspot for greenhouse gas emissions.[70−72]

3.5.2. Relationships between Microbial Characteristics and Pollution Sources. Numerous studies have demonstrated that river influents from various sources impact the micro-environments of rivers.[40,73,74] The microbiota of the river sediments might be altered due to the changes in the relative proportions of contaminants from various sources. CAFO contaminants exhibited the strongest correlation with the microbiota composition in the PSDA and BA groups, while septic tanks constituted the most relevant factor in the NPSDA group. These findings enhance knowledge regarding the self-purification process of rivers and provide insights of optimizing trade-offs associated with water quality protection strategies. For example, the amounts of contaminants emitted by point sources and septic tanks should be carefully considered while developing pollution abatement plans as they may significantly alter the microbial community and nitrogen transformation capacities in rivers.

3.5.3. Limitations and Future Directions. Regarding the limitations and outlooks of this work, we observed the following: although nitrogen was the most significant factor associated with microbial compositions in this study and was confirmed to be one of the important variables affecting microbes, the nitrogen-centric representation of pollution inputs remains insufficient. In future work, the more comprehensive measurement of environmental physicochemical parameters could afford sufficient background data for statistical analyses, helping us to identify factors that correlated best with microorganisms. Moreover, the integrated models based on the large-scale data collection of other significant elements are necessary to provide sufficient coverage of contaminants, particularly those reported to regulate microbial status. Thus, we may examine the connections between pollution scenarios and microbial states more comprehensively.

Physical transport as well as microbial transformation processes are commonly believed to regulate nitrogen concentration in rivers.[75−79] However, the effect of abiotic nitrogen transformation requires further investigation. Therefore, the potential denitrification, dissimilatory nitrate reduction to ammonium, and ANAMMOX rates of sediments should be evaluated using the ^{15}N-labeled isotope experiment to determine the microbial nitrogen removal capacities.[80,81] To distinguish between abiotic and biotic nitrogen transformation pathways, isotopic tracing data might be compared with nitrogen simulation and measurement differences. Hence, these results can serve as the foundation for improving the modeling of nitrogen conversion and strengthening the conclusion.

The integrated analysis of other tools in conjunction with hydrological simulation and microbial community compositional and functional analyses may shed additional light on microbial characteristics under various pollution scenarios. In particular, the SourceTracker analysis, a method to understand the dispersal dynamics of sediment microorganisms, can be coupled with hydrological models to demonstrate how shifts in imported microbes constrain riverine microbiota.[82] Moreover, the multiple stable isotope technique, which identifies the source and destination of nitrogen, can be combined with hydrological simulations to further enhance the ability to distinguish pollution patterns.[83,84] Therefore, the combination of technologies provides a feasible method to distinguish specific riverine pollution patterns and examine differences in microbial composition and functionality between river catchments with various pollution scenarios.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.2c04705.

> Descriptions of study areas, measurement of physicochemical parameters, procedures for sequence analysis, establishment of the SWAT model, microbial composition of samples, correlations indicated by CCA, Chao and Simpson indexes, numbers of species, relative

proportions of biomarkers, abundances of functional genes, metabolic functions, and procedure of qPCR (PDF)

AUTHOR INFORMATION

Corresponding Author

Jie Liang – *College of Environmental Science and Engineering, Hunan University, Changsha 410082, P. R. China; Key Laboratory of Environmental Biology and Pollution Control (Hunan University), Ministry of Education, Changsha 410082, P. R. China;* orcid.org/0000-0002-1979-9356; Email: liangjie@hnu.edu.cn

Authors

Ziqian Zhu – *College of Environmental Science and Engineering, Hunan University, Changsha 410082, P. R. China; Key Laboratory of Environmental Biology and Pollution Control (Hunan University), Ministry of Education, Changsha 410082, P. R. China*

Xin Li – *College of Environmental Science and Engineering, Hunan University, Changsha 410082, P. R. China; Key Laboratory of Environmental Biology and Pollution Control (Hunan University), Ministry of Education, Changsha 410082, P. R. China*

Qiurong Bu – *National Engineering Research Centre of Advanced Technologies and Equipment for Water Environmental Pollution Monitoring, Changsha 410205, P. R. China*

Qingcheng Yan – *National Engineering Research Centre of Advanced Technologies and Equipment for Water Environmental Pollution Monitoring, Changsha 410205, P. R. China*

Liqun Wen – *National Engineering Research Centre of Advanced Technologies and Equipment for Water Environmental Pollution Monitoring, Changsha 410205, P. R. China*

Xiaolei Chen – *National Engineering Research Centre of Advanced Technologies and Equipment for Water Environmental Pollution Monitoring, Changsha 410205, P. R. China*

Xiaodong Li – *College of Environmental Science and Engineering, Hunan University, Changsha 410082, P. R. China; Key Laboratory of Environmental Biology and Pollution Control (Hunan University), Ministry of Education, Changsha 410082, P. R. China*

Ming Yan – *College of Environmental Science and Engineering, Hunan University, Changsha 410082, P. R. China; Key Laboratory of Environmental Biology and Pollution Control (Hunan University), Ministry of Education, Changsha 410082, P. R. China*

Longbo Jiang – *College of Environmental Science and Engineering, Hunan University, Changsha 410082, P. R. China; Key Laboratory of Environmental Biology and Pollution Control (Hunan University), Ministry of Education, Changsha 410082, P. R. China*

Gaojie Chen – *School of Mathematics, Hunan University, Changsha 410082, P. R. China*

Shuai Li – *College of Environmental Science and Engineering, Hunan University, Changsha 410082, P. R. China; Key Laboratory of Environmental Biology and Pollution Control (Hunan University), Ministry of Education, Changsha 410082, P. R. China*

Xiang Gao – *College of Environmental Science and Engineering, Hunan University, Changsha 410082, P. R. China; Key Laboratory of Environmental Biology and Pollution Control (Hunan University), Ministry of Education, Changsha 410082, P. R. China*

Guangming Zeng – *College of Environmental Science and Engineering, Hunan University, Changsha 410082, P. R. China; Key Laboratory of Environmental Biology and Pollution Control (Hunan University), Ministry of Education, Changsha 410082, P. R. China;* orcid.org/0000-0002-4230-7647

Complete contact information is available at:
https://pubs.acs.org/10.1021/acs.est.2c04705

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (51979101, 72088101, 51679082, and 51521006), the Natural Science Foundation of Hunan Province (2019JJ20002), the Science and Technology Program of the Water Resources Department of Hunan Province (XSKJ2021000-06), and Lihe Technology (Hunan) Co., Ltd.

REFERENCES

(1) Zhang, C.; Ju, X.; Powlson, D.; Oenema, O.; Smith, P. Nitrogen surplus benchmarks for controlling N pollution in the main cropping systems of China. *Environ. Sci. Technol.* **2019**, *53*, 6678–6687.

(2) Wang, Q.; Liang, J.; Zhao, C.; Bai, Y.; Liu, R.; Liu, H.; Qu, J. Wastewater treatment plant upgrade induces the receiving river retaining bioavailable nitrogen sources. *Environ. Pollut.* **2020**, *263*, 114478.

(3) Goldberg, S. J.; Nelson, C. E.; Viviani, D. A.; Shulse, C. N.; Church, M. J. Cascading influence of inorganic nitrogen sources on DOM production, composition, lability and microbial community structure in the open ocean. *Environ. Microbiol.* **2017**, *19*, 3450–3464.

(4) Guo, G.-X.; Deng, H.; Qiao, M.; Yao, H.-Y.; Zhu, Y.-G. Effect of long-term wastewater irrigation on potential denitrification and denitrifying communities in soils at the watershed scale. *Environ. Sci. Technol.* **2013**, *47*, 3105–3113.

(5) Kuypers, M. M. M.; Marchant, H. K.; Kartal, B. The microbial nitrogen-cycling network. *Nat. Rev. Microbiol.* **2018**, *16*, 263–276.

(6) Guo, X.; Yang, Y.; Niu, Z.; Lu, D.-P.; Zhu, C.; Feng, J.; Wu, J.; Chen, Y.; Tou, F.; Liu, M.; Hou, L. Characteristics of microbial community indicate anthropogenic impact on the sediments along the Yangtze Estuary and its coastal area. *Sci. Total Environ.* **2019**, *648*, 306–314.

(7) Lofton, D. D.; Hershey, A. E.; Whalen, S. C. Evaluation of denitrification in an urban stream receiving wastewater effluent. *Biogeochemistry* **2007**, *86*, 77–90.

(8) Laanbroek, H.; Keijzer, R.; Verhoeven, J.; Whigham, D. Changes in community composition of ammonia-oxidizing betaproteobacteria from stands of Black mangrove (*Avicennia germinans*) in response to ammonia enrichment and more oxic conditions. *Front. Microbiol.* **2013**, *4*, 343.

(9) Bollmann, A.; Bullerjahn, G. S.; McKay, R. M. Abundance and diversity of ammonia-oxidizing archaea and bacteria in sediments of trophic end members of the Laurentian Great Lakes, Erie and Superior. *PLoS One* **2014**, *9*, No. e97068.

(10) Ligi, T.; Truu, M.; Truu, J.; Nõlvak, H.; Kaasik, A.; Mitsch, W. J.; Mander, Ü. Effects of soil chemical characteristics and water regime on denitrification genes (*nirS*, *nirK*, and *nosZ*) abundances in a created riverine wetland complex. *Ecol. Eng.* **2014**, *72*, 47–55.

(11) Magalhães, C. M.; Machado, A.; Matos, P.; Bordalo, A. A. Impact of copper on the diversity, abundance and transcription of

nitrite and nitrous oxide reductase genes in an urban European estuary. *FEMS Microbiol. Ecol.* **2011**, *77*, 274−284.

(12) Knapp, C. W.; Dodds, W. K.; Wilson, K. C.; O'Brien, J. M.; Graham, D. W. Spatial heterogeneity of denitrification genes in a highly homogenous urban stream. *Environ. Sci. Technol.* **2009**, *43*, 4273−4279.

(13) Jéglot, A.; Audet, J.; Sørensen, S. R.; Schnorr, K.; Plauborg, F.; Elsgaard, L. Microbiome structure and function in woodchip bioreactors for nitrate removal in agricultural drainage water. *Front. Microbiol.* **2021**, *12*, 678448.

(14) Conthe, M.; Lycus, P.; Arntzen, M. Ø.; Ramos da Silva, A.; Frostegård, Å.; Bakken, L. R.; Kleerebezem, R.; van Loosdrecht, M. C. M. Denitrification as an N_2O sink. *Water Res.* **2019**, *151*, 381−387.

(15) Wu, Y.; Chen, J. Investigating the effects of point source and nonpoint source pollution on the water quality of the East River (Dongjiang) in South China. *Ecol. Indic.* **2013**, *32*, 294−304.

(16) Ye, H.; Tang, C.; Cao, Y. Sources and transformation mechanisms of inorganic nitrogen: Evidence from multi-isotopes in a rural-urban river area. *Sci. Total Environ.* **2021**, *794*, 148615.

(17) Yi, Q.; Chen, Q.; Hu, L.; Shi, W. Tracking nitrogen sources, transformation, and transport at a basin scale with complex plain river networks. *Environ. Sci. Technol.* **2017**, *51*, 5396−5403.

(18) Yang, X.; Liu, Q.; Fu, G.; He, Y.; Luo, X.; Zheng, Z. Spatiotemporal patterns and source attribution of nitrogen load in a river basin with complex pollution sources. *Water Res.* **2016**, *94*, 187−199.

(19) He, B.; Kanae, S.; Oki, T.; Hirabayashi, Y.; Yamashiki, Y.; Takara, K. Assessment of global nitrogen pollution in rivers using an integrated biogeochemical modeling framework. *Water Res.* **2011**, *45*, 2573−2586.

(20) Chen, X.; Strokal, M.; Kroeze, C.; Supit, I.; Wang, M.; Ma, L.; Chen, X.; Shi, X. Modeling the contribution of crops to nitrogen pollution in the Yangtze River. *Environ. Sci. Technol.* **2020**, *54*, 11929−11939.

(21) Lee, S.; McCarty, G. W.; Moglen, G. E.; Li, X.; Wallace, C. W. Assessing the effectiveness of riparian buffers for reducing organic nitrogen loads in the Coastal Plain of the Chesapeake Bay watershed using a watershed model. *J. Hydrol.* **2020**, *585*, 124779.

(22) Zhang, J. L.; Li, Y. P.; Huang, G. H.; Baetz, B. W.; Liu, J. Uncertainty analysis for effluent trading planning using a Bayesian estimation-based simulation-optimization modeling approach. *Water Res.* **2017**, *116*, 159−181.

(23) Abbaspour, K. C.; Rouholahnejad, E.; Vaghefi, S.; Srinivasan, R.; Yang, H.; Kløve, B. A continental-scale hydrology and water quality model for Europe: Calibration and uncertainty of a high-resolution large-scale SWAT model. *J. Hydrol.* **2015**, *524*, 733−752.

(24) Li, Y.; Hui, C.; Zhang, W.; Wang, C.; Niu, L.; Zhang, H.; Wang, L. Integrating microbial community assembly and fluid kinetics to decouple nitrogen dynamics in an urban channel confluence. *Environ. Sci. Technol.* **2020**, *54*, 11237−11248.

(25) Zhang, H.; Sun, L.; Li, Y.; Zhang, W.; Niu, L.; Wang, L. The bacterial community structure and N-cycling gene abundance in response to dam construction in a riparian zone. *Environ. Res.* **2021**, *194*, 110717.

(26) Hu, Y.; Long, C. M.; Wang, Y.-C.; Kerkez, B.; Scavia, D. Urban total phosphorus loads to the St. Clair-Detroit River System. *J. Great Lake. Res.* **2019**, *45*, 1142−1149.

(27) Bai, H.; Chen, Y.; Wang, Y.; Song, Z.; Tong, H.; Wei, Y.; Yu, Q.; Xu, Z.; Yang, S. Contribution rates analysis for sources apportionment to special river sections in Yangtze River Basin. *J. Hydrol.* **2021**, *600*, 126519.

(28) Xu, J.; Han, C.; Jiang, Y.; Zhong, W. Spatial distribution and co-occurrence of aerobic ammonia oxidation and anaerobic ammonium oxidation activities in the water-soil interface, bulk, and rhizosphere regions of paddy soil. *Plant Soil* **2021**, *466*, 557−568.

(29) Maguire, T. J.; Fulweiler, R. W. Fate and effect of dissolved silicon within wastewater treatment effluent. *Environ. Sci. Technol.* **2017**, *51*, 7403−7411.

(30) Zhang, L.; Zhong, M.; Li, X.; Lu, W.; Li, J. River bacterial community structure and co-occurrence patterns under the influence of different domestic sewage types. *J. Environ. Manage.* **2020**, *266*, 110590.

(31) Monchamp, M.-E.; Spaak, P.; Domaizon, I.; Dubois, N.; Bouffard, D.; Pomati, F. Homogenization of lake cyanobacterial communities over a century of climate change and eutrophication. *Nat. Ecol. Evol.* **2018**, *2*, 317−324.

(32) Drury, B.; Rosi-Marshall, E.; Kelly, J. J. Wastewater treatment effluent reduces the abundance and diversity of benthic bacterial communities in urban and suburban rivers. *Appl. Environ. Microbiol.* **2013**, *79*, 1897−1905.

(33) Lu, H.; Chandran, K.; Stensel, D. Microbial ecology of denitrification in biological wastewater treatment. *Water Res.* **2014**, *64*, 237−254.

(34) Zhang, S.-Y.; Tsementzi, D.; Hatt, J. K.; Bivins, A.; Khelurkar, N.; Brown, J.; Tripathi, S. N.; Konstantinidis, K. T. Intensive allochthonous inputs along the Ganges River and their effect on microbial community composition and dynamics. *Environ. Microbiol.* **2019**, *21*, 182−196.

(35) Devane, M. L.; Moriarty, E. M.; Robson, B.; Lin, S.; Wood, D.; Webster-Brown, J.; Gilpin, B. J. Relationships between chemical and microbial faecal source tracking markers in urban river water and sediments during and post-discharge of human sewage. *Sci. Total Environ.* **2019**, *651*, 1588−1604.

(36) Straka, L. L.; Meinhardt, K. A.; Bollmann, A.; Stahl, D. A.; Winkler, M.-K. H. Affinity informs environmental cooperation between ammonia-oxidizing archaea (AOA) and anaerobic ammonia-oxidizing (Anammox) bacteria. *ISME J.* **2019**, *13*, 1997−2004.

(37) You, L.; Ros, G. H.; Chen, Y.; Yang, X.; Cui, Z.; Liu, X.; Jiang, R.; Zhang, F.; de Vries, W. Global meta-analysis of terrestrial nitrous oxide emissions and associated functional genes under nitrogen addition. *Soil Biol. Biochem.* **2022**, *165*, 108523.

(38) Ali, Md. M.; Khanom, A.; Nahar, K.; Ali, M. Y.; Azad, Md. A. K.; Rahman, M. M. Effect of manure application on net nitrification rates, heavy metal concentrations and nitrifying archaea/bacteria in soils. *Bull. Environ. Contam. Toxicol.* **2021**, *106*, 707−713.

(39) Ai, C.; Liang, G.; Wang, X.; Sun, J.; He, P.; Zhou, W. A distinctive root-inhabiting denitrifying community with high $N_2O/(N_2O+N_2)$ product ratio. *Soil Biol. Biochem.* **2017**, *109*, 118−123.

(40) Martínez-Santos, M.; Lanzén, A.; Unda-Calvo, J.; Martín, I.; Garbisu, C.; Ruiz-Romera, E. Treated and untreated wastewater effluents alter river sediment bacterial communities involved in nitrogen and sulphur cycling. *Sci. Total Environ.* **2018**, *633*, 1051−1061.

(41) Liu, W. X.; Li, X. D.; Shen, Z. G.; Wang, D. C.; Wai, O. W. H.; Li, Y. S. Multivariate statistical study of heavy metal enrichment in sediments of the Pearl River Estuary. *Environ. Pollut.* **2003**, *121*, 377−388.

(42) Du, L.; Zhao, Y.; Wang, C.; Wu, Z.; Zhou, Q. Effects of plant on denitrification pathways in integrated vertical-flow constructed wetland treating swine wastewater. *Ecotoxicol. Environ. Saf.* **2020**, *201*, 110752.

(43) Potgieter, S. C.; Dai, Z.; Venter, S. N.; Sigudu, M.; Pinto, A. J. Microbial nitrogen metabolism in chloraminated drinking water reservoirs. *mSphere* **2020**, *5*, No. e00274.

(44) Li, D.; Chu, Z.; Zeng, Z.; Sima, M.; Huang, M.; Zheng, B. Effects of design parameters, microbial community and nitrogen removal on the field-scale multi-pond constructed wetlands. *Sci. Total Environ.* **2021**, *797*, 148989.

(45) Brown, P. J. B.; de Pedro, M. A.; Kysela, D. T.; Van der Henst, C.; Kim, J.; De Bolle, X.; Fuqua, C.; Brun, Y. V. Polar growth in the Alphaproteobacterial order Rhizobiales. *Proc. Natl. Acad. Sci. U.S.A.* **2012**, *109*, 1697−1701.

(46) Jia, L.; Sun, H.; Zhou, Q.; Zhao, L.; Wu, W. Pilot-scale two-stage constructed wetlands based on novel solid carbon for rural wastewater treatment in southern China: Enhanced nitrogen removal and mechanism. *J. Environ. Manage.* **2021**, *292*, 112750.

(47) Scholes, R. C.; Vega, M. A.; Sharp, J. O.; Sedlak, D. L. Nitrate removal from reverse osmosis concentrate in pilot-scale open-water unit process wetlands. *Environ. Sci.: Water Res. Technol.* **2021**, *7*, 650−661.

(48) Schilling, K. E.; Wolter, C. F. Modeling nitrate-nitrogen load reduction strategies for the Des Moines River, Iowa using SWAT. *Environ. Manage.* **2009**, *44*, 671−682.

(49) Van Meter, K.; Van Cappellen, P.; Basu, N. Legacy nitrogen may prevent achievement of water quality goals in the Gulf of Mexico. *Science* **2018**, *360*, 427−430.

(50) Fei, Y.; Huang, S.; Zhang, H.; Tong, Y.; Wen, D.; Xia, X.; Wang, H.; Luo, Y.; Barceló, D. Response of soil enzyme activities and bacterial communities to the accumulation of microplastics in an acid cropped soil. *Sci. Total Environ.* **2020**, *707*, 135634.

(51) Albuquerque, L.; França, L.; Rainey, F. A.; Schumann, P.; Nobre, M. F.; da Costa, M. S. *Gaiella occulta* gen. nov., sp. nov., a novel representative of a deep branching phylogenetic lineage within the class *Actinobacteria* and proposal of *Gaiellaceae* fam. nov. and *Gaiellales* ord. nov. *Syst. Appl. Microbiol.* **2011**, *34*, 595−599.

(52) Li, Y.; Yuan, L.; Xue, S.; Liu, B.; Jin, G. Artificial root exudates excite bacterial nitrogen fixation in the subsurface of mine soils. *Appl. Soil Ecol.* **2021**, *157*, 103774.

(53) Davis, T. W.; Bullerjahn, G. S.; Tuttle, T.; McKay, R. M.; Watson, S. B. Effects of increasing nitrogen and phosphorus concentrations on phytoplankton community growth and toxicity during *Planktothrix* blooms in Sandusky Bay, Lake Erie. *Environ. Sci. Technol.* **2015**, *49*, 7197−7207.

(54) Carnahan, J. E.; Mortenson, L. E.; Mower, H. F.; Castle, J. E. Nitrogen fixation in cell-free extracts of *Clostridium pasteurianum*. *Biochim. Biophys. Acta* **1960**, *44*, 520−535.

(55) Verstraete, W.; Alexander, M. Heterotrophic nitrification by *Arthrobacter* sp. *J. Bacteriol.* **1972**, *110*, 955−961.

(56) Abdelhamed, H.; Nho, S. W.; Karsi, A.; Lawrence, M. L. The role of denitrification genes in anaerobic growth and virulence of *Flavobacterium columnare*. *J. Appl. Microbiol.* **2021**, *130*, 1062−1074.

(57) Lv, J.; Yuan, R.; Wang, S. Water diversion induces more changes in bacterial and archaeal communities of river sediments than seasonality. *J. Environ. Manage.* **2021**, *293*, 112876.

(58) Ogden, K. L.; Gadgill, J.; Akin, T. Biological denitrification of hydrolysates from octahydro-1,3,5,7 tetranitro-1,3,5,7-tetrazocine. *Water Environ. Res.* **2002**, *74*, 338−345.

(59) Hu, Y.; Cheng, H.; Tao, S. Environmental and human health challenges of industrial livestock and poultry farming in China and their mitigation. *Environ. Int.* **2017**, *107*, 111−130.

(60) Zhang, L.; Zhao, F.; Li, X.; Lu, W. Contribution of influent rivers affected by different types of pollution to the changes of benthic microbial community structure in a large lake. *Ecotoxicol. Environ. Saf.* **2020**, *198*, 110657.

(61) Henry, H.; Thorn, R.; Brady, E. An assessment of the suitability of a range of chemical and biological tracers to monitor the movement of septic tank effluents to groundwater. *Ir. Geogr.* **1991**, *24*, 91−105.

(62) Guerra, V.; Tiago, I.; Aires, A.; Coelho, C.; Nunes, J.; Martins, L. O.; Veríssimo, A. The gastrointestinal microbiome of browsing goats (*Capra hircus*). *PLoS One* **2022**, *17*, No. e0276262.

(63) Wang, S.; Wang, W.; Liu, L.; Zhuang, L.; Zhao, S.; Su, Y.; Li, Y.; Wang, M.; Wang, C.; Xu, L.; Zhu, G. Microbial nitrogen cycle hotspots in the plant-bed/ditch system of a constructed wetland with N_2O mitigation. *Environ. Sci. Technol.* **2018**, *52*, 6226−6236.

(64) Liu, Y.; Liu, C.; Nelson, W. C.; Shi, L.; Xu, F.; Liu, Y.; Yan, A.; Zhong, L.; Thompson, C.; Fredrickson, J. K.; Zachara, J. M. Dissimilatory nitrate/nitrite reduction processes in river sediments a cross climatic gradient : influences of biogeochemical controls and climatic temperature regime. *Environ. Sci. Technol.* **2017**, *51*, 4877−4886.

(65) Basu, N. B.; Van Meter, K. J.; Byrnes, D. K.; Van Cappellen, P.; Brouwer, R.; Jacobsen, B. H.; Jarsjö, J.; Rudolph, D. L.; Cunha, M. C.; Nelson, N.; Bhattacharya, R.; Destouni, G.; Olsen, S. B. Managing nitrogen legacies to accelerate water quality improvement. *Nat. Geosci.* **2022**, *15*, 97−105.

(66) Marinos, R. E.; Campbell, J. L.; Driscoll, C. T.; Likens, G. E.; McDowell, W. H.; Rosi, E. J.; Rustad, L. E.; Bernhardt, E. S. Give and take: a watershed acid rain mitigation experiment increases baseflow nitrogen retention but increases stormflow nitrogen export. *Environ. Sci. Technol.* **2018**, *52*, 13155−13165.

(67) Macintosh, K. A.; Mayer, B. K.; McDowell, R. W.; Powers, S. M.; Baker, L. A.; Boyer, T. H.; Rittmann, B. E. Managing diffuse phosphorus at the source versus at the sink. *Environ. Sci. Technol.* **2018**, *52*, 11995−12009.

(68) Chan, S.; Pullerits, K.; Keucken, A.; Persson, K. M.; Paul, C. J.; Rådström, P. Bacterial release from pipe biofilm in a full-scale drinking water distribution system. *npj Biofilms Microbiomes* **2019**, *5*, 9.

(69) Li, W.; Zhang, S.; Zhang, L.; Li, X.; Wang, F.; Li, G.; Li, J.; Li, W. In-situ remediation of sediment by calcium nitrate combined with composite microorganisms under low-DO regulation. *Sci. Total Environ.* **2019**, *697*, 134109.

(70) Yu, Z.; Deng, H.; Wang, D.; Ye, M.; Tan, Y.; Li, Y.; Chen, Z.; Xu, S. Nitrous oxide emissions in the Shanghai river network: implications for the effects of urban sewage and IPCC methodology. *Global Change Biol.* **2013**, *19*, 2999−3010.

(71) Wang, R.; Zhang, H.; Zhang, W.; Zheng, X.; Butterbach-Bahl, K.; Li, S.; Han, S. An urban polluted river as a significant hotspot for water−atmosphere exchange of CH_4 and N_2O. *Environ. Pollut.* **2020**, *264*, 114770.

(72) Zhang, W.; Li, H.; Xiao, Q.; Li, X. Urban rivers are hotspots of riverine greenhouse gas (N_2O, CH_4, CO_2) emissions in the mixed-landscape Chaohu Lake Basin. *Water Res.* **2021**, *189*, 116624.

(73) Wang, J.; Li, Y.; Wang, P.; Niu, L.; Zhang, W.; Wang, C. Response of bacterial community compositions to different sources of pollutants in sediments of a tributary of Taihu Lake, China. *Environ. Sci. Pollut. Res.* **2016**, *23*, 13886−13894.

(74) Wang, L.; Zhang, J.; Li, H.; Yang, H.; Peng, C.; Peng, Z.; Lu, L. Shift in the microbial community composition of surface water and sediment along an urban river. *Sci. Total Environ.* **2018**, *627*, 600−612.

(75) Reisinger, A. J.; Groffman, P. M.; Rosi-Marshall, E. J. Nitrogen-cycling process rates across urban ecosystems. *FEMS Microbiol. Ecol.* **2016**, *92*, fiw198.

(76) Guan, Y.; Hou, T.; Li, X.; Feng, L.; Wang, Z. Metagenomic insights into comparative study of nitrogen metabolic potential and microbial community between primitive and urban river sediments. *Environ. Res.* **2022**, *212*, 113592.

(77) Su, X.; Cui, L.; Tang, Y.; Wen, T.; Yang, K.; Wang, Y.; Zhang, J.; Zhu, G.; Yang, X.; Hou, L.; Zhu, Y.-G. Denitrification and N_2O emission in estuarine sediments in response to ocean acidification: from process to mechanism. *Environ. Sci. Technol.* **2022**, *56*, 14828−14839.

(78) Guo, Z.; Su, R.; Zeng, J.; Wang, S.; Zhang, D.; Yu, Z.; Wu, Q. L.; Zhao, D. *NosZI* microbial community determined the potential of denitrification and nitrous oxide emission in river sediments of Qinghai-Tibetan Plateau. *Environ. Res.* **2022**, *214*, 114138.

(79) Lv, K.; Guo, X.; Wang, C.; Su, Q.; Liu, D.; Xiao, S.; Yang, Z. Sediment nitrogen contents controlled by microbial community in a eutrophic tributary in Three Gorges Reservoir, China. *Environ. Pollut.* **2022**, *314*, 120312.

(80) Wang, X.; Yang, R.; Guo, Y.; Zhang, Z.; Kao, C. M.; Chen, S. Investigation of COD and COD/N ratio for the dominance of anammox pathway for nitrogen removal via isotope labelling technique and the relevant bacteria. *J. Hazard. Mater.* **2019**, *366*, 606−614.

(81) Chen, Y.-J.; Leung, P. M.; Cook, P. L. M.; Wong, W. W.; Hutchinson, T.; Eate, V.; Kessler, A. J.; Greening, C. Hydrodynamic disturbance controls microbial community assembly and biogeochemical processes in coastal sediments. *ISME J.* **2022**, *16*, 750−763.

(82) Zhang, W.; Gu, J.; Li, Y.; Lin, L.; Wang, P.; Wang, C.; Qian, B.; Wang, H.; Niu, L.; Wang, L.; Zhang, H.; Gao, Y.; Zhu, M.; Fang, S. New insights into sediment transport in interconnected river−lake

systems through tracing microorganisms. *Environ. Sci. Technol.* **2019**, *53*, 4099−4108.

(83) Cejudo, E.; Hood, J. L.; Schiff, S. L.; Aravena, R. O. Using the δ^{15}N of submerged biomass for assessing changes in the nitrogen cycling in a river receiving wastewater treated effluent. *Ecol. Indic.* **2018**, *95*, 645−653.

(84) Hu, M.; Liu, Y.; Zhang, Y.; Dahlgren, R. A.; Chen, D. Coupling stable isotopes and water chemistry to assess the role of hydrological and biogeochemical processes on riverine nitrogen sources. *Water Res.* **2019**, *150*, 418−430.