Anthropogenic land uses shape denitrification-related microbial communities in freshwater river ecosystems

Xing Liu[1], Baozhu Pan[1], Zhenyu Huang[1], Xinyuan Liu[1], Haoran He[1], and Xinzheng Zhu[1]

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Abstract

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Abstract

Microbiota play essential roles in nitrogen (N) cycling in freshwater river ecosystems. However, microbial functional groups associated with N cycling (especially denitrification) in freshwater rivers under anthropogenic disturbance are still poorly understood. Here, we studied the impacts of different land-use types on denitrification-related microbial communities in Weihe River, Hanjiang River, and their tributaries, in the Qinling Mountains, China. The major land-use types in the three river areas were divided into natural (forest, shrub, grassland, and open water) and anthropogenic (agricultural and urbanized land) types. A

landscape survey of microbiota in the river water and sediment was carried out with extensive sample sources based on deep 16S rRNA gene sequencing, which yielded operational taxonomic units for predicting functional groups. With increases in proportions of agricultural and urbanized land areas, electrical conductivity, total N, ammonium-N, and nitrate-N all increased in water samples. Conversely, microbial diversity exhibited a decreasing trend in water samples, whereas the relative abundance of denitrification-related functional groups increased, with increases in the proportions of agricultural and urbanized land areas. The relative abundances of denitrification- and human-related microbial functional groups in sediment samples were distinctively higher in Weihe River (mainly under agriculture and urbanization), when compared with those of Hanjiang River and Qinling tributaries (dominated by forests). The results indicate that anthropogenic land-use types, such as agricultural and urbanized land, result in simple microbial community structure and stimulate microbe-mediated denitrification in freshwater rivers.

Keywords: Land-use type, Denitrification, Microbiota, Freshwater ecosystems, Agricultural land, Urbanized land

Background

Freshwater is a fundamental component of aquatic ecosystems and is essential for coupling of biogeochemical cycles between continents and oceans (Aufdenkampe et al., 2011). The emission of greenhouse gases (GHG) from freshwater ecosystems is source of concern globally (Searchinger et al., 2008, Robertson et al., 2000 and Liu et al., 2020). Freshwater systems emit 0.3 Tg N yr^{-1} to the atmosphere in the form of nitrous oxide (N_2O) under natural conditions (Tian et al., 2020), with implications for global warming. N_2O is one of the most long-lived GHGs (Prinn et al., 2018). The atmospheric N_2O burden increased from 1,462 Tg N in the 1980s to 1,555 Tg N in 2007–2016, with anthropogenic N_2O emissions at 7.3 (4.2–11.4) Tg N yr^{-1} and natural N_2O emissions at 9.7 (8.0–12.0) Tg N yr^{-1}, respectively (Tian et al., 2020). Consequently, it is essential to explore the mechanisms of nitrogen (N) cycling in freshwater systems, which could facilitate the minimization of the impacts of N_2O emissions on global warming.

Microbiota play active roles in the conversion of N in freshwater ecosystems (Mosier et al., 1998). Generally, organic N is transformed into molecular N via two pathways. First, ammonium-N (NH_4^+-N) is oxidized into nitrate-N (NO_3^--N) or nitrite-N (NO_2^--N) by nitrifying bacteria; second, NO_3^--N or NO_2^--N is reduced to gaseous N by denitrifying bacteria (Shen et al., 2021). In addition, N fixation refers to the reduction of N_2 molecules to NH_3 or organic N by N-fixing bacteria (Ryu et al., 2020). In freshwater environments, nitrification, denitrification, and N fixation processes mediated by distinct microbiota establish the N cycle alternately or simultaneously. The denitrification process produces N_2O as an intermediate. N_2O emissions are higher in areas with intense anthropogenic disturbance than in areas with minor disturbance (Zhao et al., 2021).

Microbiota as the foremost decomposers in nature drive the decomposition of biological remains and maintain biogeochemical cycling (Zaan et al., 2010 and Xu et al., 2014). Microbiota colonize suitable environments (Harrison et al., 2014) and respond sensitively to environmental stimuli, such as toxic substances, sewage treatment, and natural self-purification (Pei et al., 2018 and Wang et al., 2021). In addition, aquatic bacterial diversity is influenced by evapotranspiration, elevation, and temperature (Zhang et al., 2021). A large number of denitrifying bacteria exist in high-N wastewater (Pai et al., 1999; Liu et al., 2020). In the past, denitrification was mainly considered to occur in anaerobic or hypoxic conditions; however, recent studies have reported that some bacteria can also perform denitrification under aerobic conditions (Wan et al., 2009). According to Alessandra (2017), N_2O emissions are released from the hyporheic–benthic zone and benthic–water column zone in the Kalamazoo River, Michigan, USA.

Anthropogenic disturbance disrupts the intensity, frequency, and timing of natural disturbance regimes that maintain the ecological integrity of river ecosystems (Cabezas et al., 2009). River ecosystem characteristics are often governed by interactions between hydrological connectivity and local environmental conditions. Such interactions, coupled with intensive agricultural and urbanized land uses, severely alter river hydrodynamic and biogeochemical gradients (Valipour et al., 2012 and Campo et al., 2014). Despite the impacts of

anthropogenic disturbance on microbiota have been explored in marine and terrestrial ecosystems (Archer et al., 2020), it remains unclear how different land-use types influence microbial community structure in freshwater ecosystems.

Numerous factors influence microbe-mediated N cycling and denitrification in aquatic ecosystems. For example, saltwater intrusion can alter the interactions between biotic and abiotic components in tidal wetland ecosystems and therefore influence denitrification rates (Neubauer et al., 2019). Long-term sulfide inputs in freshwater lake sediments enhance chemoautotrophic denitrification, rather than dissimilatory NO_3^--N reduction into NH_4^+-N (Ypa et al., 2021). However, there is still a dearth of studies assessing the impacts of different land-use types on denitrification efficiency and associated microbial communities in freshwater river ecosystems.

The present study evaluated the impacts of different land-use types on the characteristics of river environment, microbial community structure, and denitrification-related functions in a freshwater ecosystem. The results of the present study could offer novel insights into microbe-mediated denitrification in freshwater rivers under anthropogenic disturbance.

Methods

Study area and sampling

The study area covers the Weihe River, Hanjiang River, and their tributaries in the Qinling Mountains in Southern Shaanxi province, China (Fig. 1). The Weihe and Hanjiang Rivers run on the north and south sides of the Qinling Mountains, respectively. Weihe River passes through Baoji, Xianyang, and Xi'an, which are densely populated cities. Hanjiang River passes through the cities of Hanzhong and Ankang. The rivers have numerous tributaries at high elevations, which are characterized by low anthropogenic disturbance in the Qinling Mountains.

In October 2017 (autumn) and April 2018 (spring), water and sediment samples were collected from 52 sites in 12 rivers (Fig. 1, Table S1). The sampling sites span the north mainstem (Weihe River), south mainstem (Hanjiang River), and 10 tributaries (Luofu River, Bahe River, Shidi River, Shitou River, Heihe River, Jinqian River, Jinshui River, Xuhe River, Xushui River, and Yuehe River) in Qinling Mountains. At each sampling site, water samples were collected from 0.5-m depths with aseptic polyethylene bottles. The samples for chemical analysis (2 L each) were preserved at 4°C before being transported to the laboratory. The samples for DNA-based analysis (4 L each) were filtered through 0.22-μm mixed cellulose ester membranes (Millipore, Bedford, MA, USA) within 24 h. The membranes were stored at –80°C until DNA extraction. Each sediment sample was collected for microbial analysis.

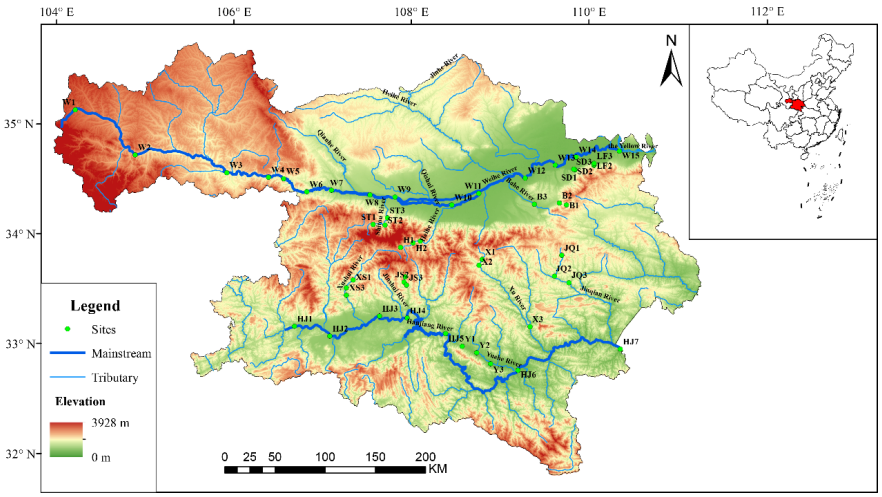

Fig. 1 Location of the study area and 52 sampling sites in the river ecosystems in the Qinling Mountains. Green dots represent the sampling sites in numerical order as summarized in the Supporting Information (Table S1). W, Weihe River; HJ, Hanjiang River; LF, Luofu River; B, Bahe River; SD, Shidi River; ST, Shitou River; H, Heihe River; JQ, Jinqian River; JS, Jinshui River; X, Xuhe River; XS, Xushui River; and Y, Yuehe River

Environmental and land-use data collection

The environmental factors, i.e., water temperature (T), electrical conductivity (Cond), dissolved oxygen (DO), pH, turbidity (Turb), total N (TN), NO_3^--N, O_2^--N, NH_4^+-N, and total phosphorus (P; TP), were determined according to the methods of Zhao et al. (2020). Sediment TP and TN were determined using a UV-2450 ultraviolet-visible spectrophotometer (Shimadzu, Kyoto, Japan), and sediment total organic carbon was determined using a TOC-L analyzer (Shimadzu).

Catchment-scale land-use variables, i.e., the area proportions of forest land, shrub land, grassland, agricultural land, urbanized land, open water, and "others", were obtained at each sampling site using ArcGIS v10.3 (ESRI Inc., Redlands, CA, USA). Each site was delineated using the soil and water assessment tool (SWAT; Jiang et al., 2021), with a spatial resolution of 30 m. The outputs were converted to a basin polygon for each site, which included the entire drainage area upstream of the site. For each sub-watershed, the land-use data used included available Landsat, Sentinel 2, and ASTER images. The images were interpreted and expressed as area proportions of six major land-use types.

Genomic DNA extraction, library construction, and sequence analysis

A Water DNA Kit (Bioteke, Beijing, China) was used to extract genomic DNA from water samples, and a NucleoSpin Soil Kit (Macherey-nagel, North Rhine-Westphalia, Germany) was used to extract genomic DNA from sediment samples, according to the protocols provided by the manufacturer. The extracted DNA was quantified with a Qubit Fluorometer (Thermo Fisher, Waltham, USA) and a Qubit dsDNA BR Assay Kit (Invitrogen, Carlsbad, CA, USA), and the DNA quality was checked by 1% agarose gel electrophoresis.

The V4–V5 variable regions of the bacterial 16S rRNA genes were amplified with degenerate PCR primers, 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 907R (5'-CCGTCAATTCMTTTRAGTTT-3') (Shan et al., 2015). Both forward and reverse primers were tagged with Illumina adapter, pad, and linker sequences. PCR amplification was performed in a 50-μL reaction containing 30 ng of template DNA, fusion PCR primers, and PCR master mix. PCR cycling conditions were as follows: 95°C for 3 min, 30 cycles of 95°C for 45 sec, 56°C for 45 sec, 72°C for 45 sec, and a final extension at 72°C for 10 min. The PCR products were purified using Agencourt AMPure XP (Beckman-Coulter, Brea, CA, USA) beads and eluted in Elution buffer. DNA libraries were qualified using an Agilent 2100 bioanalyzer (Agilent Technologies, Palo Alto, CA, USA). The validated libraries were used for sequencing on an Illumina MiSeq platform (Illumina, San Diego, CA, USA) following the standard pipelines, which generated 2 × 250-bp paired-end reads. All raw sequences from this study have been stored in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) database under the BioProject number PRJNA 774972 and the accession number SUB10569930.

The raw FASTQ files were processed and analyzed using the QIIME2 v2019.02 (*https://qiime2.org*) platform (Hall et al., 2018). Quality controls, annotations, and statistical calculations were implemented using the standard QIIME2 pipeline (Bokulich et al., 2018). Demultiplexing was conducted to determine the sample sources of each sequence. The sequences were then denoised to obtain amplicon sequence variants (ASVs, which are operational taxonomic units with a sequence similarity of 100%) (Gonzalez et al., 2019) using DADA2 (Prodan et al., 2020). The obtained ASVs were taxonomically classified using the Greengenes 16S rRNA gene database (Bolyen et al., 2019). Before subsequent analysis, all chloroplast, mitochondrial, archaeal, and eukaryotic sequences were removed. To minimize the influence of unequal sequencing efforts, the ASV table was rarefied for each sample. All ASVs with relative abundance <0.01% were also removed to produce the ASV abundance table of each sample (Sogin et al., 2006).

Data analysis

To evaluate the differences in land-use type among groups, land-use data were visualized by principal coordinates analysis (PCoA) based on Bray-Curtis distances. Pearson's correlation coefficients were used to evaluate the impacts of different land-use types on the environmental factors. Both analyses were performed using the "*vegan* " package in R software (versions 4.1.2) (R Core Team, 2021).

Microbial alpha diversity was calculated using the "*vegan* " package in R software (versions 4.1.2) (R Core Team, 2021). The phylogenetic tree was constructed using the unweighted pair group method with arithmetic mean (UPGMA) based on Bray-Curtis distances with the "*ggtree* " package in R software (versions 4.1.2) (R Core Team, 2021) and visualized with iTOL(versions 6) (https://itol.embl.de). Microbial community composition was visualized using the nonmetric multidimensional scaling ordination (NMDS) method based on Bray-Curtis dissimilarities. Analysis of Similarities (ANOSIM) was used to evaluate the degree of separation in microbial communities between groups. Furthermore, a regression analysis was used to determine the Bray-Curtis dissimilarity of microbial communities between sample pairs; subsequently, the environmental factors and land-use types were plotted against the community dissimilarity. The impacts of land-use type and environmental factors on the Bray-Curtis dissimilarity were evaluated using the Mantel's test and Pearson's rank correlation.

Microbial functional groups were predicted using the FAPROTAX database (Louca et al., 2016), which is suitable for functional annotation prediction of biogeochemical cycle processes (especially the carbon [C], hydrogen [H_2], N, P, sulfur [S] cycles, and other element cycles) in environmental samples (e.g., oceans and lakes). The FAPROTAX database was established based on published and validated literature (Sansupa et al., 2021). The relative abundances of predicted functional groups were subjected to heatmap analysis using the "*pheatmap* " package in R software (versions 4.1.2) (R Core Team, 2021). To assess the impacts of environmental factors and land-use types on microbial functional groups, the heatmap and Pearson's rank correlation were visualized based on Bray-Curtis dissimilarities. Linear discriminant analysis (LDA) effect size (LEFSe) analysis was conducted on the functional groups to identify biomarkers of anthropogenic disturbance.

Results

Distribution of land-use types and dynamics of environmental factors

PCoA biplots show that the land-use types of the Weihe River were markedly different from those of the Hanjiang River and Qinling tributaries, with the first axis explaining 87.88% of the variation in land-use type (Fig. 2a). In Weihe River, agricultural land was the primary land-use type, accounting for 61.22±12.58% of total land area. In addition, the area proportion of urbanized land in Weihe River was significantly higher than those in the other two regions (Fig. 2b). Forest was the main land-use type in the Hanjiang River and Qinling tributaries, accounting for 60.88±5.85% and 64.91±12.48% of total land area, respectively. According to the distributions of different land-use types, the river areas were dominated by agricultural or forest land. Specifically, the Weihe River area mainly comprised agricultural land, grassland, and urbanized land; the Hanjiang River area mainly hosted forest, agricultural, and shrub land; and the Qinling tributary area was dominated by forest and shrub land.

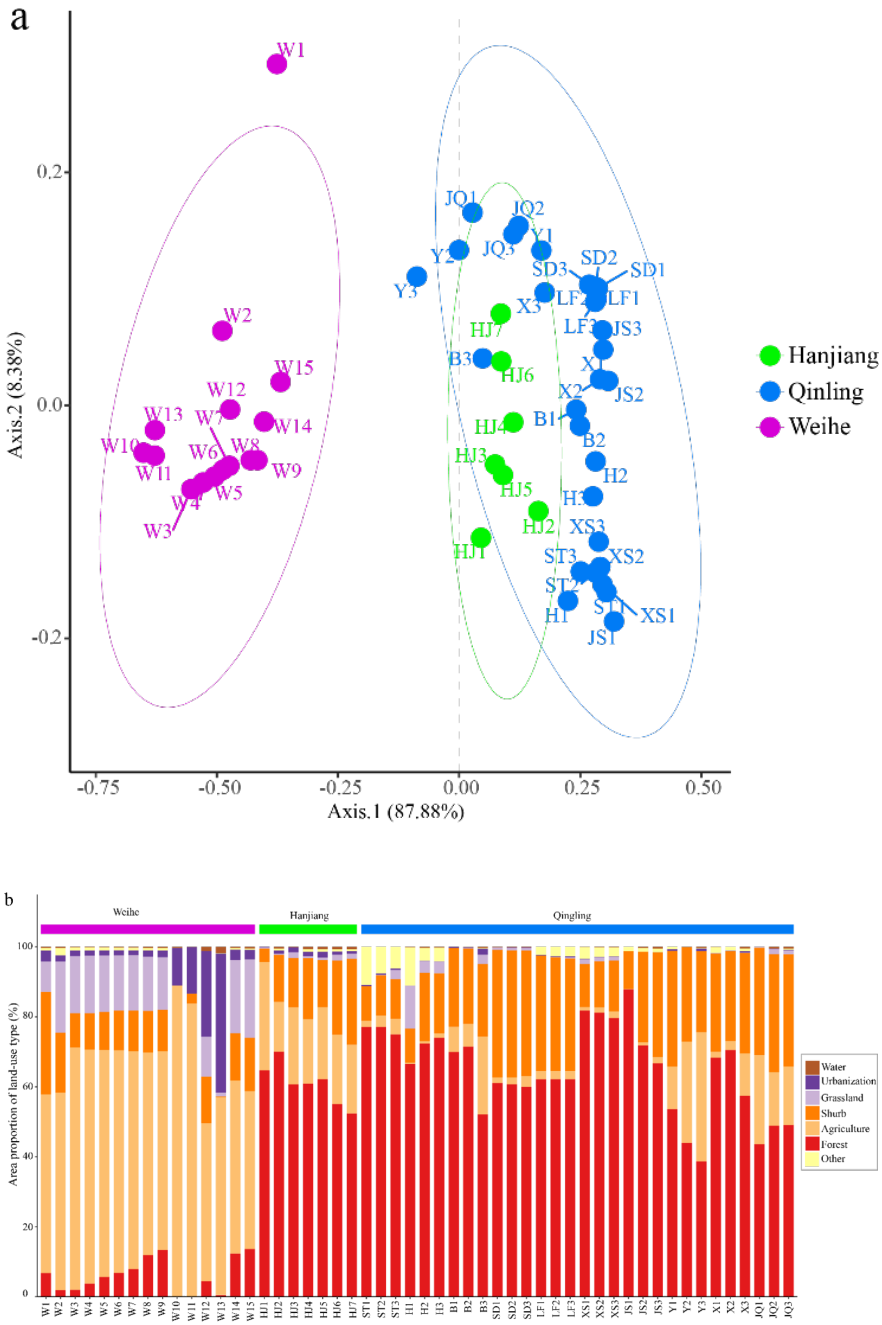

Fig. 2 Distributions of different land-use types in the study area. (a) Principal coordinates analysis (PCoA) biplot and (b) area proportions of major land-use types in three study regions.

The environmental factors in the different rivers over two seasons (autumn and spring) are visualized in Fig. S3. In both seasons, Turb, Cond, TN, TP, NH_4^+-N, and NO_3^--N in water samples from Weihe River were significantly higher compared with those of the Hanjiang River and Qinling tributaries. Pearson's correlation coefficients were used to analyze the impacts of different land-use types on the environmental factors. The proportions of agricultural land, grassland, urbanized land, and open water areas exhibited significant positive correlations with Turb, Cond, TN, TP, NH_4^+-N, and NO_3^--N. However, with an increase

in the proportion of forest land area, all environmental factors exhibited decreasing trends, excluding DO (Fig. S3).

Community diversity and microbiota composition

Overall, 7,463,182 raw sequence reads were obtained from 104 water and sediment samples. After quality filtering, 1,450,613 clean reads were obtained and then clustered into 1352 ASVs. To determine whether the sampling depth was sufficient to give an overview of the microbiota, rarefaction curves were generated for the number of ASVs per individual or species (Fig. S1). The alpha diversity indices of microbiota in water and sediment are presented in Fig. S2. Taking into account the richness, Shannon, Simpson, Pielou, Chao, and ACE indices, microbial alpha diversity was higher in Hanjiang River than in the Weihe River and Qinling tributaries. In Weihe River, all the alpha diversity indices in autumn were lower than those in spring.

The microbiota in water samples comprised 14 bacterial phyla and 73 bacterial families. Proteobacteria, Bacteroidetes, Actinobacteria, and Firmicutes were identified as the dominant phyla, accounting for >98% of all sequence reads. In addition, there were 16 dominant families (including Comamonadaceae, Flavobacteriaceae, Cytophagaceae, Moraxellaceae, and Enterobacteriaceae), constituting >84% of all sequence reads (Fig. S4a). The microbiota in sediment samples comprised 14 bacterial phyla and 75 bacterial families. Proteobacteria, Bacteroidetes Firmicutes, Planctomycetes, Actinobacteria, Acidobacteria, and Cyanobacteria were identified as the dominant phyla, amounting to >96% of all sequence reads. In addition, there were 23 dominant families (including Comamonadaceae, Flavobacteriaceae, Xanthomonadaceae, Rhodobacteraceae, Rhodocyclaceae, and Sphingomonadaceae), constituted >81% of all sequence reads. A notable finding was that large numbers of *Acinetobacter*spp. occurred at the W9, W10, and W11 sites, which mainly fell under agricultural and urbanized land uses in spring (Fig. S4).

The ASVs from the water and sediment samples were clustered into distinct groups between autumn and spring following UPGMA clustering based on Bray-Curtis distances (Fig. S4). The ASVs from water samples of most sites in Weihe River were clustered into one group in autumn. The cluster distances of ASVs in Qinling tributaries and Hanjiang River were close. Similar results for ASV clusters were obtained for sediment samples (Fig. S4b). NMDS ordination results showed significant differences in the compositions of microbial communities between different river areas (water in autumn: ANOSIM $R = 0.265$,$P = 0.001$, *stress* $= 0.093$; water in spring: ANOSIM $R = 0.243$,$P = 0.001$, *stress* $= 0.104$; sediment in autumn: ANOSIM$R = 0.294$, $P = 0.001$, *stress* $= 0.145$; sediment in spring: ANOSIM $R = 0.362$, $P = 0.001$, *stress* $= 0.122$). The UPGMA tree and NMDS clustering results of microbiota in water samples were consistent with the PCoA results of land-use types in Weihe River, Qinling River, and Hanjiang tributaries (Fig. 3, Fig. 2a).

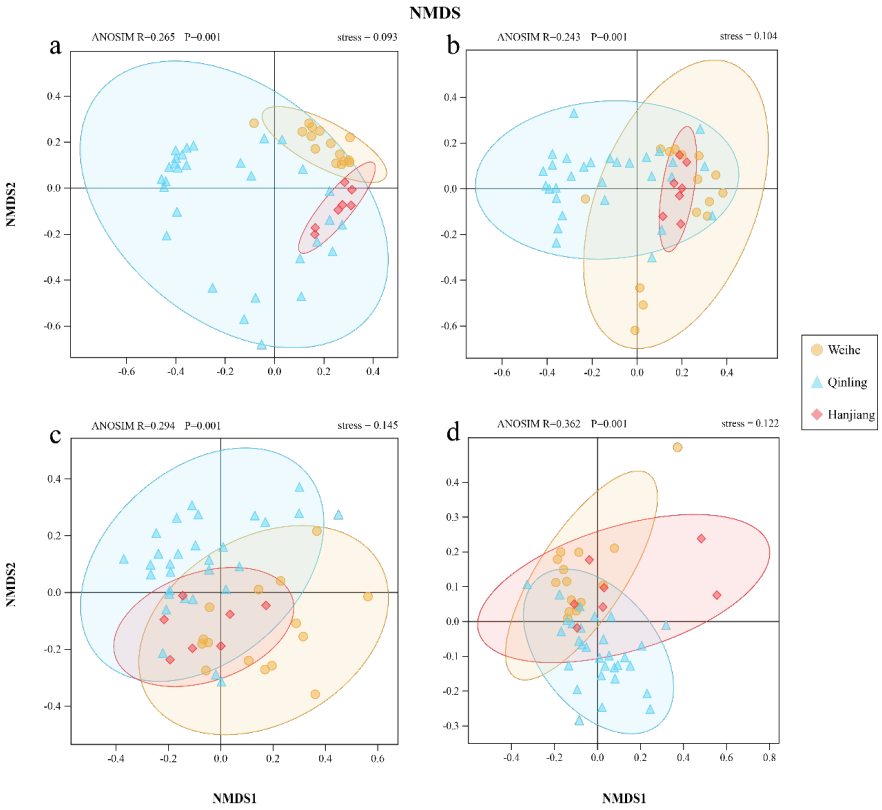

Fig. 3 Compositions of microbial communities along anthropogenic disturbance gradients in the river ecosystems of Qinling Mountains. Non-metric multidimensional scaling (NMDS) ordination based on Bray-Curtis dissimilarity showing microbial community variation in water (a: autumn, b: spring) and sediment (c: autumn, d: spring) samples under different land-use types.

We used Mantel's test to determine the impacts of environmental factors and land-use types on microbial community composition in the study area. NH_4^+-N, Cond, T, TN, and NO_3^--N showed significant correlations ($P < 0.01$, Mantel's $r > 0.1$) with microbial community composition in water. N and P contents increased with an increase in the proportion of agricultural land; however, both elements tended to decrease with an increase in the proportion of forest land, which further affected microbial community composition. In addition, the proportions of agricultural land, forest, and grassland areas were significantly correlated (Mantel's $r > 0.2$, $P < 0.01$) with sediment microbial community composition (Fig. 4). Based on results of microbiota community composition analyses in river water and sediment over the two seasons, different land-use types had considerable impacts on microbial community composition in river water and sediment (Fig. S5a-b).

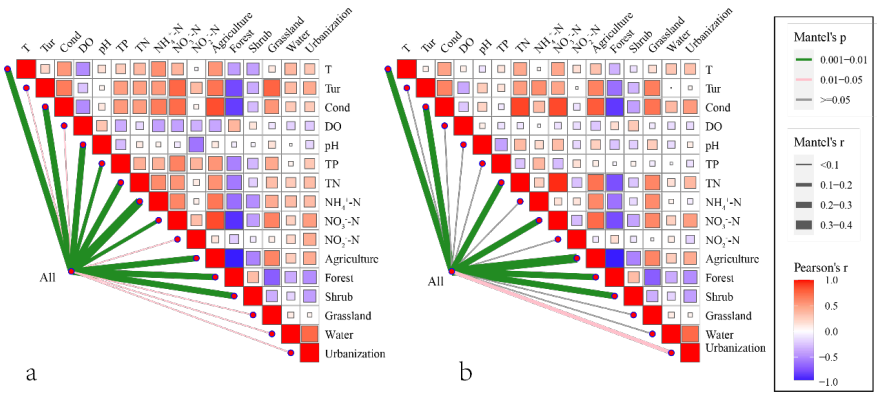

Fig. 4 Environmental factors and land-use types shaping microbial community composition in the river ecosystems during (a) autumn and (b) spring. Pairwise comparisons of environmental factors and land-use types are shown at the upper-right, with a color gradient representing Spearman's correlation coefficients. Microbial community composition was correlated to each environmental factor or land-use type based on the partial Mantel's test. The line width represents the partial Mantel's r statistic for the corresponding correlation, and the line color represents the significance based on 999 permutations. T, water temperature; Turb, water turbidity; Cond, electrical conductivity; DO, dissolved oxygen; TP, total phosphorus; TN, total nitrogen; NH_4^+-N, ammonium nitrogen; NO_3^--N, nitrate nitrogen; NO_2^--N, nitrate nitrogen.

Microbial functional groups under different land-use types

To study the influence of environmental factors and land-use types on microbial functioning in different river areas, microbial functional groups were predicted based on 16S rRNA gene sequences using FAPROTAX database. Subsequently, Pearson's correlations between microbial functional groups and environmental factors or land-use types were analyzed. Numerous functional groups were associated with nitrate denitrification, nitrite denitrification, denitrification, N respiration, N fixation, and iron (Fe) and manganese (Mn) respiration in the water of river areas dominated by agricultural land (Weihe River). The predicted functions were significantly positively correlated with multiple environmental factors (e.g., NH_4^+-N, TP, TN, NO_3^--N, and NO_2^--N) and land-use types (agricultural and urbanized land), whereas they had negative correlations with DO, pH, forest land, and shurb land.

There were few denitrification-related functional groups in the water of river areas dominated by forest land (Hanjiang River and Qinling tributaries). However, numerous functional groups were associated with aerobic anoxygenic phototrophy, oxidation, nitrite ammonification, nitrate ammonification, nitrification, aerobic nitrite oxidation, anoxygenic photoautotrophy, and aerobic ammonia (NH_3) oxidation in the water of Hanjiang River. The functional groups associated with N fixation were significantly positively correlated with NO_2^--N, shrub land, and forest land, but negatively correlated with DO, NH_4^+-N, pH, T, agricultural land, grassland, and urbanized land. Furthermore, there were few functional groups associated with N and P cycles in the water of Qinling tributaries. The relative abundances of N metabolism-related functional groups were significantly higher in the water of Weihe River than in other areas, whereas the relative abundances of carbon metabolism-related functional groups were distinctively higher in the water of Hanjiang River than in the other areas (Fig. 5).

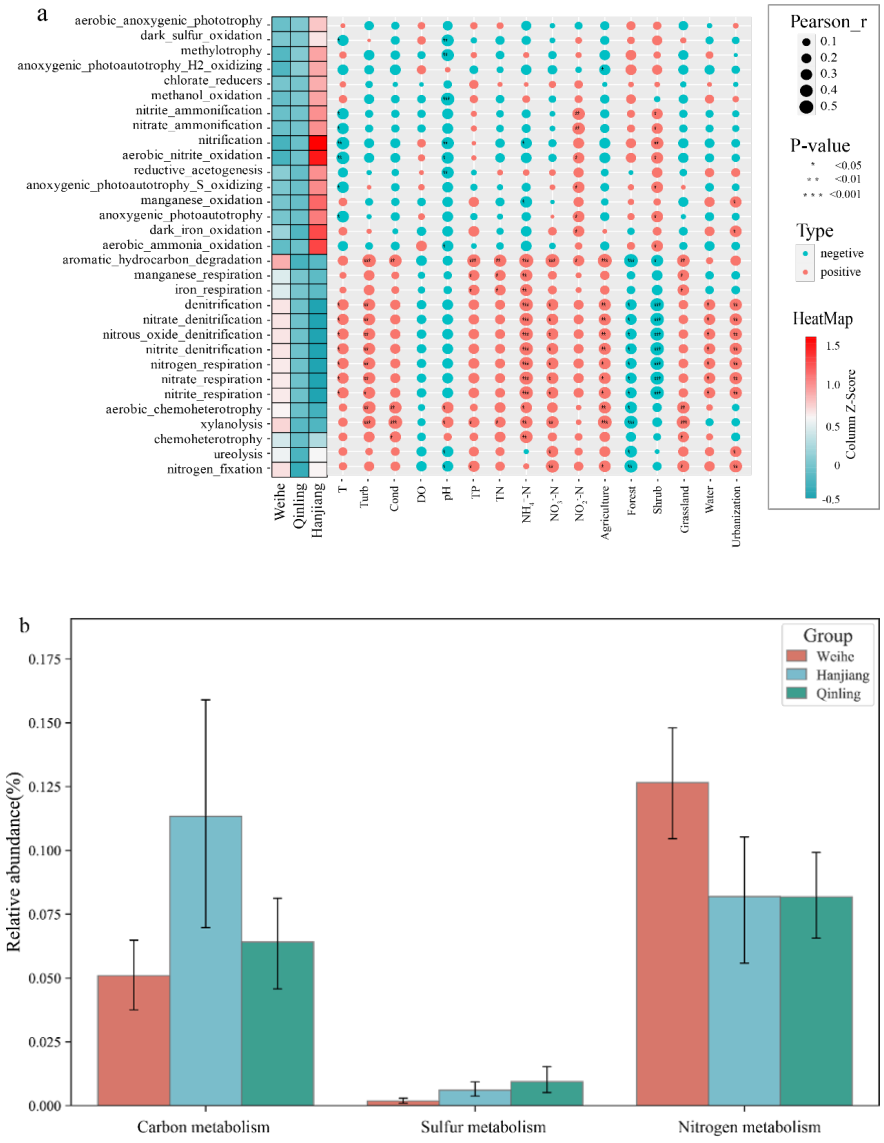

Fig. 5 Functional versus taxonomic groups of microbiota in the water of river ecosystems. (a) Z-scores achieved by standardization of relative abundances of functional groups (heatmap) within each functional group (heatmap, averaged across each functional group). Rank correlations between environmental variables and relative abundances of functional groups. Blue and red colors indicate negative and positive correlations, respectively. *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$. (b) Key functions of microbiota under different land-use types.

The functional prediction results showed that the microbial functional groups in sediment samples were more diverse than those in water samples. A comparative analysis across the three river areas revealed numerous microbial function groups associated with N respiration, nitrate respiration, denitrification, and human pathogens in the sediment of Weihe River. In addition, there were multiple microbial function groups associated with fermentation, methanogenesis, aerobic nitrite oxidation, and human pathogenic gastroenteritis in the sediment of Hanjiang River. Furthermore, there were some microbial functional groups associated with aerobic chemoheterotrophy, photoautotrophy, and intracellular parasites in the sediment of Qinling tributaries (Fig. S6).

To determine whether the functional groups with differences in relative abundance could serve as biomarkers of anthropogenic disturbance in freshwater environments, an LEfSe analysis was performed at the functional group level (LDA $>$ 2, $P < 0.05$). N cycling was the main function in the Weihe River sediment, with denitrification and organic degradation being the main functions in autumn, and ammonification and metal respiration being the main functions in spring (Fig. 6a-b). Fermentation and oxidation-related functions were dominant in the Hanjiang River sediment. Phototrophy and oxidation were the main functions in the sediment of Qinling tributaries. Overall, N metabolism was the primary function in the river areas under different land-use types, and the relative abundance of microbial functional groups associated with N metabolism was significantly higher in the sediment of Weihe River than those in the other areas in autumn (Fig. 6c-d).

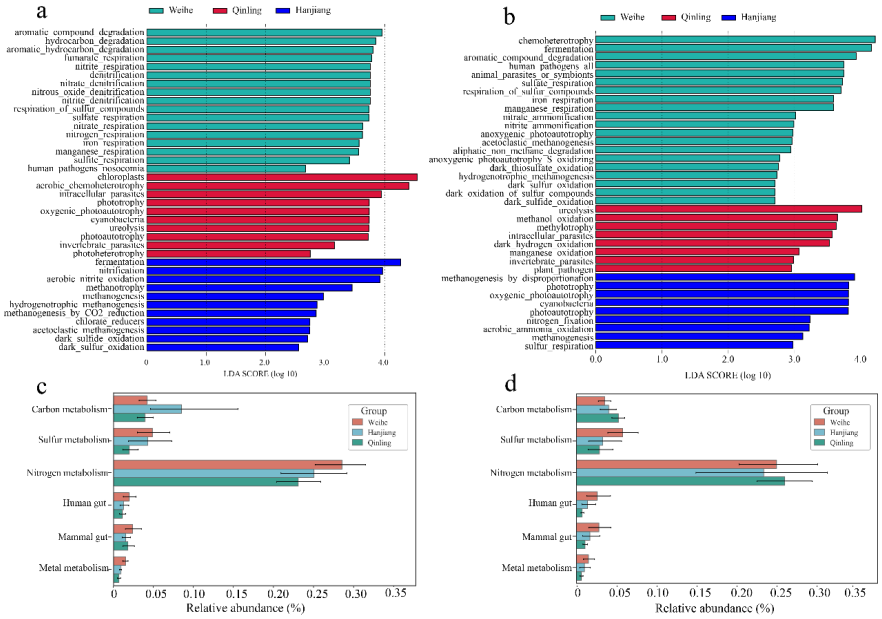

Fig. 6 Linear discriminant analysis (LDA) effect size (LEfSe) analysis (LDA $>$ 2, $P < 0.05$) of the relative abundances of microbial functional groups in the sediment of three river areas under different land-use types during (a) autumn and (b) spring. Relative abundances of key functional groups in (c) autumn and (d) spring.

Discussion

Land-use type influence environmental characteristics of freshwater rivers

Previous studies exploring the impacts of land-use type on N and P dynamics have focused on soil environments, with a few studies conducted in freshwater river ecosystems (Enanga et al., 2011 and Leblanc, 2008). In the present study, environmental data were obtained from freshwater rivers under disturbance by different land-use types. We observed that land-use type could induce shifts in the ecological environments of rivers. For example, Cond, TN, NH_4^+-N, and NO_3^--N in water samples increased with an increase in the proportion of agricultural land (Fig. 4). The sensitivity of the environmental factors to land-use type may indicate increases in soil erosion and agricultural or municipal sewage discharge. As a tributary of the Yellow River and Loess Plateau, Weihe River is associated with severe soil erosion (Wei et al., 2010).

Agricultural land use increases water demand, whereas rapid urbanization and population growth exacerbate water pollution (Song et al., 2015). Through Pearson's correlation analysis, we observed that Turb, Cond, TN, TP, NH_4^+-N, and NO_3^--N in water samples all significantly increased with an increase in disturbance

by agricultural and urbanized land use (Fig. 4). Urban and agricultural land border river basins and runoff from such land areas loads nutrients into the adjacent canals, and ultimately flow into Weihe River.

Anthropogenic land uses reduce microbial diversity and alter microbiotacomposition in rivers

Based on 16S rRNA gene sequencing, we observed that agricultural land use could induce microbial diversity loss and community compositional changes in river water and sediment. The alpha diversity of microbiota in water samples was lower in the river area mainly under agricultural land use. We speculated that due to soil erosion in agricultural land, abundant N was released into water (Li et al., 2017), negatively affecting microbial diversity, which is consistent with the finding of a former study that reported changes in microbial diversity with an increase in N concentration in a freshwater river (Tai et al., 2013). Microbial community composition in sediment samples showed trends similar to those observed in water samples, which could be attributed to the long-term fusion of microbiota between river water and sediment (Kristensen, 1984).

We also explored the impacts of land-use type on microbial community composition in the river ecosystems. The NMDS and UPGMA clustering results showed that microbiota in water samples were congregated in the river area dominated by agricultural land. Notably, high levels of*Acinetobacter* spp. occurred at a few agricultural and urbanized land sites in spring.*Acinetobacter* spp. reportedly utilize a wide range of organic compounds as sole sources of carbon and energy (Hommel et al., 2014), so that they can degrade a variety of organic pollutants (Zhang et al., 2021; Hommel et al., 2014), which is consistent with the environmental characteristics of our study area; in spring, TN and NO_3^--N concentrations in water samples were as high as 6.94±0.97 (mg/L) and 5.22±1.32 (mg/L), respectively, which were 14.3% and 40.3% higher than those in autumn. The increased N nutrients could stimulate*Acinetobacter* spp. proliferation. In many previous studies, N has been considered to have a prominent impact on microbial community composition in freshwater environments (Xing et al., 2020 and Maritz et al., 2019).

We subsequently studied the relationship between environmental factors and microbial community composition using the Mantel's test. According to the results, both TN and NO_3^--N concentrations in water samples increased with increases in proportions of agricultural and urbanized land areas; furthermore, microbial community composition was shaped by specific environmental factors. In contrast, the proportions of forest and shrub land areas were negatively correlated with N concentrations, and shaped microbial community composition. He et al. (2016) also observed that land-use type considerably influenced microbial community composition in a karst underground river.

Denitrification-related microbial functional groups in river water are shaped by anthropogenic land uses

As decomposers, microbiota play vital roles in N and P cycles in ecosystems (Dijkstra et al., 2012). Soil erosion can increase N and P in water (Ma et al., 2016). When N concentrations increase in the water environment, nitrifying and denitrifying bacteria could colonize in large numbers (Geets et al., 2007). Here, we observed that the N concentrations in river water increased with increases in proportions of agricultural and urbanized land area (Fig. 4). We speculate that anthropogenic land uses may stimulate nitrification and denitrification-related microbial functional groups. The results of functional gene prediction showed that the microbial functional groups were associated with denitrification, N cycle, and Fe and Mn respiration in the river areas under mainly agricultural and urbanized land uses, with their relative abundances positively correlated with TN, NH_4^+-N, NO_3^--N, and NO_2^--N concentrations in river water (Fig. 5).

Through metagenomic analysis, Wang et al. (2022) found that denitrifying bacterial community abundance was markedly improved near a sewage plant. In the present study, the impacts of anthropogenic disturbance on denitrifying bacteria in the freshwater river ecosystems were variable. The processes of N respiration, nitrate respiration, and nitrite respiration constitute denitrification, which is accompanied by the production of gaseous N such as nitric oxide (NO), N_2O, and N_2 (Torralbo et al., 2017). Potential strategies of reducing the emission of N_2O, a GHG, have attracted considerable attention (López et al., 2013, Rafique et al., 2014). Considering N pollution and GHG emissions, it is essential to reduce TN, NH_4^+-N, NO_3^--N, and NO_2^--N concentrations in river areas disturbed by agricultural and urbanized land use activities.

Microbial responses to environmental change are complex (Scheuerl et al., 2020). Recently, Xun et al. (2021) found that bacterial communities with higher phylogenetic diversity tend to be more stable, implying that microbiota with higher biodiversity are more resistant to disturbance. In the present study, we predicted the microbial functions involved in the synthesis of major and trace elements required for bacterial growth. In the river area mainly under forest land, the major functional groups were related to methylotrophy (Tami et al., 2015), methanol oxidation (Pastawan et al., 2020), anoxygenic photoautotrophy S oxidation, anoxygenic photoautotrophy H_2 oxidation, aerobic anoxygenic phototrophy (Sasikala et al., 1995), oxidation, nitrite ammonification (Howie-Esquivel et al., 2010), nitrate ammonification, nitrification, aerobic nitrite oxidation, anoxygenic photoautotrophy, aerobic NH_3 oxidation, and microelement (Mn and Fe) oxidation. Most of the functions are involved in the synthesis of organic compounds with complex structures to support bacterial growth. The relative abundances of carbon metabolism-related functions were also higher in the river area under mainly forest land use, compared with that under mainly agriculture and urbanization (Fig. 5b). Therefore, microbial community structures under agricultural sites were simpler than those under forest sites in the river ecosystems.

In the present study, microbial community composition and functional groups were influenced not only by land-use types but also by elevation in the river areas of the Qinling tributaries. The alpha diversity of microbiota in river water was the lowest and the microbial functional groups were the fewest in the Qinling tributaries. Due to the associated low temperature and nutrient concentrations (Fig. S3), high elevations are not conducive for the normal growth of microbiota (Zhang et al., 2013). Consequently, severe natural disturbance can also reduce microbial stability in freshwater river waters.

Land-use disturbance by agriculture and urbanization has long-term impacts on microbial functional groups in river sediment

It is generally considered that microbial community functions in sediment are more diverse than those in the water column. Sediment microbiota mainly participate in organic decomposition, N and P cycling, and water pollution remediation in rivers (Kallmeyer et al., 2012, Meng et al., 2019 and Wu et al., 2021). Long-term natural evolution and human activities shape microbial community composition in sediment (Huang et al., 2021, Hung et al., 2021 and Marshall et al., 2019). In the present study, we observed that the functional composition of sediment microbiota was altered by different land-use types in the freshwater rivers. There were numerous functional groups associated with denitrification and human (e.g., multiple denitrifications, human pathogens, human gut metabolism, mammal gut metabolism, and organic synthesis) in the river ecosystems, mainly under agricultural and urbanized land use (Fig. S6). However, only organic synthesis and nitrification-related functional groups were observed in the river ecosystems under mainly forest land use, with few functional groups associated with human and denitrification.

Considering the environmental factors and microbial community composition, land-use type shapes microbial functional groups in rivers. Furthermore, the human-related microbial functional groups detected in the sediment provide strong evidence of the impact of anthropogenic land use on microbial functional groups in river sediment (Fig. 6c-d). Similarly, microbial functional groups have been applied in the measurement of microbial water quality responses to land-use type using fecal indicator bacteria and molecular source tracking in rivers and near-shore surface waters (Verhougstraete et al., 2012).

In the present study, the major functional groups of sediment microbiota were similar to those predicted in river water; the relative abundances of functional groups associated with C metabolism and metal metabolism were lower in sediment in river ecosystems under mainly agricultural and urbanized land uses than under forest land use, with opposite trends observed for functional groups associated with N metabolism and human or mammal gut metabolism. Through LEfSe analysis, we identified the microbial functional groups that were characteristic of river sediment (Fig. 6a-b). Denitrification and human-related microbial functional groups were relatively abundant in the sediment of rivers mainly under agricultural and urbanized land uses, whereas few human-related microbial functional groups were observed in the sediment of rivers mainly under forest land use. Overall, the results indicate that the microbial functional groups in freshwater river sediment were influenced by land-use type.

Conclusions

The present study investigated the impacts of different land-use types on denitrification-related microbial communities and associated functional groups in freshwater rivers. Compared with natural land uses, anthropogenic land uses (agriculture and urbanization) increased N concentrations in river water, altering microbial community structure. The anthropogenic land uses also shaped microbial functional groups and stimulated microbially-mediated denitrification in the river basins. The results of the present study could facilitate the estimation of land-use type impacts on freshwater ecosystems through the analysis of microbial functional groups. These conclusions will provide scientific references for the impact of anthropogenic land on ecology of freshwater rivers and land quality improvement in the Qinling Mountains. We recommend further studies based on quantitative PCR, metagenomics, and metatranscriptomics approaches to verify our findings. Furthermore, it is necessary to determine the threshold N concentrations for denitrification efficiency in ecosystems other than freshwater rivers, which can facilitate the control of denitrification or N_2O emissions caused by anthropogenic disturbance.

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Conflict of Interest Statement

The authors declare no conflict of interests

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