Nonpoint Source Pollution (NPSP) Induces Structural and Functional Variation in the Fungal Community of Sediments in the Jialing River, China

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Received: 12 November 2021 / Accepted: 5 April 2022 / Published online: 13 April 2022
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Abstract
Nonpoint source pollution (NPSP) from human production and life activities causes severe destruction in river basin environments. In this study, three types of sediment samples (A, NPSP tributary samples; B, non-NPSP mainstream samples; C, NPSP mainstream samples) were collected at the estuary of the NPSP tributaries of the Jialing River. High-throughput sequencing of the fungal-specific internal transcribed spacer (ITS) gene region was used to identify fungal taxa. The impact of NPSP on the aquatic environment of the Jialing River was revealed by analysing the community structure, community diversity, and functions of sediment fungi. The results showed that the dominant phylum of sediment fungi was Rozellomycota, followed by Ascomycota and Basidiomycota (relative abundance > 5%). NPSP caused a significant increase in the relative abundances of Exosporium, Phialosimplex, Candida, Inocybe, Tausonia, and Slooffia, and caused a significant decrease in the relative abundances of Cercospora, Cladosporium, Dokmaiia, Setophaeosphaeria, Paraphoma, Neosetophoma, Periconia, Plectosphaerella, Claviceps, Botrytis, and Papiliotrema. These fungal communities therefore have a certain indicator role. In addition, NPSP caused significant changes in the physicochemical properties of Jialing River sediments, such as pH and available nitrogen (AN), which significantly increased the species richness of fungi and caused significant changes in the fungal community β-diversity (P < 0.05). pH, total phosphorus (TP), and AN were the main environmental factors affecting fungal communities in sediments of Jialing River. The functions of sediment fungi mainly involved three types of nutrient metabolism (symbiotrophic, pathotrophic, and saprotrophic) and 75 metabolic circulation pathways. NPSP significantly improved the pentose phosphate pathway, pentose phosphate pathway, and fatty acid beta-oxidation V metabolic circulation pathway functions (P < 0.05) and inhibited the chitin degradation to ethanol, super pathway of heme biosynthesis from glycine, and adenine and adenosine salvage III metabolic circulation pathway functions (P < 0.05). Hence, NPSP causes changes in the community structure and functions of sediment fungi in Jialing River and has adversely affected for the stability of the Jialing River Basin ecosystem.

Keywords Jialing River · NPSP · Fungi · Community structure · Function prediction

Introduction
Rivers are important for the hydrological cycle and for material migration on Earth and have rich biodiversity [19]. With rapid economic development and the continuous increase in urbanization, China’s aquatic environments have been severely damaged, particularly in the form of river pollution, which has become the focus of aquatic environment governance in China [16]. In recent years, the Chinese government has consistently improved the quality of aquatic environments and strengthened aquatic ecological systems through policy guidance and financial support [18]. However, nonpoint source pollution (NPSP) has not yet been
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Effectively controlled. Research shows that NPSP has surpassed point source pollution as the main cause of water pollution on a global scale, especially in agricultural production in developing countries [3, 53]. Extensive agricultural production, with high input of pesticides and chemical fertilizers, and the rapid expansion of aquaculture have led to increasingly serious agricultural NPSP, and it has severely restricted the healthy development of the aquatic environment [4, 16]. Therefore, the control of agricultural NPSP not only has become the top priority for water pollution control but also has gradually become a major issue for the sustainable development of modern agriculture and society [52]. NPSP is mainly caused by dissolved or solid pollutants in the ground surface or soil, which enter the receiving water through surface runoff, soil erosion, farmland drainage, and infiltration [4, 45, 47]. NPSP has the characteristics of randomness, extensiveness, lag, and latency, which increase the difficulty of pollution prevention, detection, and control [16]. Consequently, it still needs to strengthen the basic scientific research to develop monitoring and controlling measures that are more accurate and powerful for NPSP control and to reduce the environmental impact on river basins [10].

River sediment is the main site for microbial nutrient metabolism and pollutant degradation. Microorganisms can enter water environment with pollutants and continue to spread to other areas via the water flow [38]. Zhao et al. [57] used bacteria versus fungi for predicting anthropogenic pollution in subtropical coastal sediments. Stochastic processes were found as the dominant ecological driver in shaping the community assembly of both bacteria and fungi. Moreover, environmental factors explained a considerable portion of variation in bacterial communities, while spatial factors were more influential in structuring larger body size and weak mobility fungal communities. Hence, most microorganisms are deposited gradually via attachment to materials such as soil and fallen leaves and reshape the microbial community structure in river sediments under the action of environmental selection, interspecific competition, and stochastic processes [50]. In addition, sediment microorganisms change the aquatic environment conditions through biochemical activities such as assimilation, which further affects the transformation and distribution of nutrients in the sediments and promotes the material cycling of essential elements such as carbon, nitrogen, and phosphorus in the sediments [2, 31, 56]. Meanwhile, the change of human activities on the environment and the interaction between microorganisms and environmental factors make the microbial community change significantly [40, 44]. When pollutant discharge causes changes in the physical and chemical properties of river sediments, the community structure or functions of sediment microorganisms also change accordingly [48]. Therefore, the structural and functional variations in the sediment microbial community can reflect the long-term cumulative effects of natural and anthropogenic stresses on river ecosystems, and are an important indicator of the stability of river water ecosystems [5, 23, 25]. Studying the response of fungal communities in river sediments to NPSP will help improve the level of pollution monitoring. Such research will play a positive role in promoting NPSP control and in aquatic environmental impact assessment.

As an important first-level tributary of the upper reaches of the Yangtze River, the Jialing River is an important ecological barrier for the Yangtze River Basin. A water quality monitoring dataset from the environment management department showed that among the 25 sections of Jialing River tributaries monitored in 2020, the nitrogen and phosphorus levels of 24 tributaries did not meet the national level III water quality requirements of China. These areas are dominated by agriculture, with few chemical companies. Almost all of the pollution originates from agricultural NPSP and domestic sewage. At present, the research on NPSP in this area is mainly focused on NPSP output characteristics, pollution load estimation, pollution prevention, and control technologies and management [12, 13, 22]. However, the study of the effect of NPSP on microbial ecological processes in river sediments has not been reported in detail, especially the mechanism underlying the effect of NPSP on fungal community construction in sediments is still unclear [45, 47]. Therefore, to clarify the characteristics of the sediment fungal community in the Jialing River and reveal the degree of influence of NPSP on the structure and function of the sediment fungal community, the present study employed the sediment fungi of the Jialing River as the research object by a combined usage of high-throughput sequencing technology and bioinformatic analysis methods to explore the influence of NPSP on the community composition and function of the sediment fungi. Further analysis of the interaction between the diversity of the sediment fungal community and environmental factors is expected to provide a scientific basis for ecological environment protection and NPSP control in the Jialing River Basin.

Materials and Methods

Study Area and Sample Collection

The study area was located in the mid-downstream section (Nanchong Section of Sichuan Province) of the Jialing River (32°10’ to 33°30’, 105°50’ to 106°10’ (Fig. 1). The Jialing River originates from the Qinling Mountains in Shaanxi Province. Its main stream flows through Shanxi Province, Gansu Province, Sichuan Province, and Chongqing City and flows into the Yangtze River at Chaotianmen, Chongqing City. With a total length of 1345 km, the Jialing River is an important tributary of the Yangtze River, with the largest
basin area, second only to the Yalong River in length and second only to the Minjiang River in flow. The average water quality of the Jialing River (Nanchong Section of Sichuan Province) is stable at level II (this level of water is suitable for the key protection area of centralized domestic drinking water, the habitat of aquatic organisms, and the spawning ground of fish and shrimp) of the national environmental quality standards for surface water. But the water quality of many tributaries of the Jialing River is poor, including rivers with severe NPSP, such as the Xichong River and Dong River, the water quality standards of which fluctuate between level III (this level of water is suitable for secondary protection area of centralized domestic drinking water, fish and shrimp migration channel and aquaculture area) and IV (this level of water quality is suitable for industrial water and entertainment water that is not in direct contact with human body) all year round, and occasionally level V (this level of water quality is suitable for agricultural irrigation and water areas with ordinary waterscape).

The study sites were located in the estuary area where the Dong River and Xichong River flow into the Jialing River (Fig. 1). Both tributaries are typical NPSP rivers, with many villages on both sides and no industrial enterprises. In December 2020, the sampling sites of the NPSP tributaries were set up in the Dong River and Xichong River at a distance of 3 km from their confluence with the Jialing River, the sampling sites of the NPSP main stream were set up at a distance of 5 km downstream of the main stream from the confluence of the Xichong River and Dong River with the Jialing River, and the sampling sites of the non-NPSP main stream were set up at a distance of 5 km upstream of the main stream from the confluence of the Xichong River and Dong River with the Jialing River. One river sediment sample was collected at each site, and 1 sample each was collected 100 m upstream and downstream from the sampling point [55]. A total of 18 sediment samples were collected. The NPSP tributary samples collected in the Dong River were named A1-1, A1-2, and A1-3, and the NPSP tributary samples collected in the Xichong River were named A2-1, A2-2, and A2-3. The non-NPSP mainstream samples collected from the Dong River Estuary were named B1-1, B1-2, and B1-3, and the non-NPSP mainstream samples collected from the Xichong River Estuary were named B2-1, B2-2, and B2-3. The NPSP mainstream samples collected from the Dong River Estuary were named C1-1, C1-2, and C1-3, and the NPSP mainstream samples collected from the Dong River Estuary were C2-1, C2-2, and C2-3.

A mud picker was used to collect sediment samples and deionized water to clean the mud picker between sampling sites. The sediment samples were divided into two parts. One part weighed approximately 0.05 kg after removing plants and rocks and was stored in an incubator with dry ice.
and sent to the laboratory for extraction of sediment fungal DNA. The other sample weighed approximately 1 kg and placed in a sterile valve bag and stored in an electronic refrigerat at 4 °C. It was taken back to the laboratory for determining the physical and chemical properties [57].

**Determination Method**

**Physicochemical Properties of the Sediments**

The pH of the sediment was measured with a pH analyser (HQ30d, HACH, U.S.A.) using a soil-to-water ratio of 1:2.5. The sediment total nitrogen (TN) and total carbon (TC) concentrations were determined using an elemental analyser (VarioEL III, Germany). Fresh sediment samples were oven-dried at 60 °C for 24 h and then ground and sieved through a 150-μm mesh. Each sediment sample (25 mg) was placed in the combustion furnace of the elemental analyser, and the manufacturer’s instructions were followed to determine sample elements and their ratios [42]. The sediment total phosphorus (TP) content was measured using alkali fusion-molybdenum antimony spectrophotometry [15]. The sediment total organic carbon (TOC) content was measured using the nondispersive infrared method and an automated TOC analyser (Shimadzu, TOC-VCPH, Japan). Sediment samples were treated with HCl solution to remove inorganic carbon and then placed with the platinum salt catalyst in the combustion furnace of the automated TOC analyser following the manufacturer’s instructions [49]. The available nitrogen (AN) content was determined using the AN proliferation method as described by [45, 47]. The carbon-to-nitrogen ratio (C/N) was the ratio of total sediment carbon to total nitrogen [27].

**Sediment Fungal DNA Extraction**

Total microbial community DNA was extracted from triplicate sediment samples (each weighing 0.1 g) using the E.Z.N.A.® Soil Fungi DNA Kit (Omega Bio-tek, Norcross, GA, USA) following the manufacturer’s instructions [41]. Then, 1% agarose gel electrophoresis was used to check the quality of the extracted DNA. The concentration and purity of the DNA were determined by a NanoDrop 2000. Each sample was prepared in triplicate for PCR amplification.

**PCR Amplification and Sequencing**

The fungal internal transcribed spacer (ITS) variable region was amplified by PCR using the primers ITS1F (5’-CTT GGT CAT TTA GAG GAA 3’) and ITS2R (5’-TCC TCG CTT ATT GAT TG-3’) [7]. The 20-μL PCR system contained 4 μL of 5 × FastPfu Buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each primer (5 μM), 0.4 μL of TransStart FastPfu DNA Polymerase (TransGenAP221–02), 0.2 μL of BSA, and 10 ng of template DNA [35]. Amplification was performed in an ABI GeneAmp 9700 thermocycler (ABI, Carlsbad, CA) with the following temperature profile: 95 °C for 3 min; 33 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 45 s; and a final extension at 72 °C for 10 min. The amplicons of the same sample were mixed and recovered by 2% agarose gel electrophoresis, and then, the products were purified by the Axyprep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) and detected by 2% agarose gel electrophoresis. A QuantaDNAFluorometer (Promega, USA) was used to detect and quantify the recovered products. The NEXTFLEX Rapid DNA-Seq Kit was used to extract PCR products from agarose gels. Sequencing was performed to build the database by using the Illumina MiSeq platform [12, 13]. Raw data files of the fungal ITS were submitted to the NCBI Sequence Read Archive under BioProject ID: PRJNA812388.

**Sequence Splicing and Annotation**

Raw sequencing data were analysed using the qiime2 process (v2020.2). Fastp software (v0.19.6) was used to process the original sequence data, and then Flash software (v1.2.7) was used to splice the optimized data. The dada2 denoising and analysis process was used to remove duplicates and generate a table with amplicon sequencing variants (ASVs) [11]. The RDP classifier Bayesian algorithm was used to perform taxonomic analysis according to the minimum number of sample sequences with a confidence threshold of 0.7 and compare each sequence with the UNITE database (Unit 8.0/its Fungi). The results of species classification annotation were obtained.

**Statistical Analysis**

Several α-diversity indexes (Ace, Chao, Shannon, and Simpson) were calculated to describe the richness and diversity of ASVs at the different sites. Venn diagrams were made using R language tools. Variations in the fungal community compositions of sediments with different sites were evaluated by the principal component analysis (PCA). Based on the results of species annotation, a Circos diagram and community histogram were used to analyse the species composition. Linear discriminant analysis effect size (LEfSe) was used to identify the species characteristics that best explained the differences between two or more groups of samples and the degree of influence of these characteristics on the differences between groups [20]. The functions of sediment fungi were predicted and analysed using the FUNGuild and PICRUSt2 tools according to nutrition mode and metabolic circulation pathway [33]. A redundancy analysis (RDA) was performed in R using the Vegan package with fungal
species abundance and environmental chemical data. SPSS software (IBM SPSS Statistics 22 for Windows) was used to calculate Pearson correlation coefficients and to perform one-way ANOVA.

Results

Differences in the Physicochemical Properties of Sediments at Different Sites

The physical and chemical properties of sediments in the Jialing River are affected by many factors, such as water and soil loss in the riparian zone, pollution discharge, and river disturbance. Therefore, the physical and chemical properties of sampling points in different river sections exhibited certain differences. Table S1 (supplementary material) shows that the pH of the sediments at site B was higher than that at the other sites ($P < 0.05$). Compared with that in B, the AN content in A and C was significantly increased ($P < 0.05$). The TC content varied from 5.72 to 15.37 g·kg$^{-1}$, and the TN content varied from 0.36 to 1.02 g·kg$^{-1}$. These two physicochemical parameters showed the same change trends at the different sites; both showed that the NPSP tributary samples and NPSP mainstream samples were significantly higher than the non-NPSP mainstream samples. The TOC content varied from 4.68 to 12.11 g·kg$^{-1}$, and the C/N value varied from 8.11 to 15.92. There were certain differences in the three physicochemical parameters at different sites, but there was no obvious change trend.

DNA Sequencing Data and ASV Distribution of Fungi in Sediment Samples from Different Sites

A total of 1,294,031 quality controlled sequences were detected in 18 sediment samples through high-throughput sequencing, with a total base number of 284,476,801 bp, an average sequence number of 71,891, an average base number of 15,804,267 bp, and an average base length of 219.9 bp. The sequences were analysed by ASV taxonomy according to 100% similarity, and 11,184 ASVs were obtained. As shown in Fig. 2, the distribution numbers of ASVs at the 6 sampling sites decreased in the order $A1 > C1 > C2 > B1 > A2 > B2$, and the numbers of ASVs detected were 4003, 2297, 3922, 2216, 1617, and 2602, respectively.

The numbers of ASVs of the sediment fungal communities from sites A and C of the same estuary were similar (Fig. 2a–b). The number of shared ASVs between A and C (1372 shared by A1 and C1 and 773 shared by A2 and C2) is shown in the Venn diagram. A small number of ASVs shared between B and other sites (671 shared by A1 and B1, 624 shared by B1 and C1, 360 shared by A2 and B2, and 352 shared by B2 and C2) are also shown. This shows that the distribution of sediment fungal ASVs in the main stream of the Jialing River is significantly affected by the NPSP tributary and is affected by other environmental factors.

As shown in Fig. 2d–f, the numbers of ASVs shared between each pair of sites (749 shared by A1 and A2, 474 shared by B1 and B2, and 825 shared by C1 and C2) and unique to each site (3254 unique to A1, 1467 to A2, 1823 to B1, 1143 to B2, 3097 to C1, and 1777 to C2) are shown in the Venn diagram (Fig. 2d–f). The fungal communities of the two NPSP tributaries exhibited certain differences, and the distribution of fungal ASVs in the sediments of the main stream of the Jialing River showed changes.

Diversity Analysis of the Fungal Community in Sediments from Different Sites

α-Diversity Analysis of the Fungal Community in Sediments from Different Sites

Several α-diversity indexes were calculated to describe the richness and diversity of sediment fungal ASVs at the different sites (Table 1). The independent t-tests used to explore the differences between the six sites indicated that the richness of the sediment fungal community in C1 and A1 was significantly higher than that in other sites ($P < 0.05$). Similarly, the richness of the sediment fungal community in C2 was significantly higher than that in A2 and B2 ($P < 0.05$). This result indicates that the NPSP tributaries contributed to changes in the fungal diversity in the Jialing River sediments.

β-Diversity Analysis of the Fungal Community in Sediments from Different Sites

The correlations of and differences in the sediment fungal communities among the six sampling sites were compared by PCA of the Pearson distance algorithm at the species level (Fig. 3). The cumulative explained variance of the first axis and the second axis reached 83.64%, 92.01%, and 61.97%. Figure 3a shows the PCA of fungal communities with 12 samples from the confluence of Dong River tributaries, and Fig. 3b shows the PCA of fungal communities with 12 samples from the confluence of Xichong River. The fungal communities at different sampling sites in the same estuary showed no obvious overlap with each other and could be separated from each other. Analysis of similarities (ANOSIM) of the groups showed that the fungal communities in sites A1 and C1 shared high similarity and were significantly different from the fungal communities in B1 ($P < 0.05$), while the fungal communities at A2, B2, and C2 were significantly different ($P < 0.05$). In addition,
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Fig. 3c shows the PCA analysis and plot with all the samples. Twelve samples from B2, A1, B1, and C1 overlap visually due to the influence of pixels and explained variance. This shows that the fungal communities of B2 and Dong River Estuary sediments shared a certain similarity, and the fungal community diversity of C2 exhibited higher variation, which was affected by the Xichong River (P < 0.05).

Fig. 2 Number of sediment fungal ASVs in each sampling site, specific to a sampling site, or shared by two or three sampling sites. The number of ASVs within each sampling site was calculated after merging the sequences obtained from the three replicates from each sampling site, which were analysed by high-throughput sequencing after PCR. A1 and A2 were NPSP tributary sites, B1 and B2 were non-NPSP mainstream sites, and C1 and C2 were NPSP mainstream sites.

Table 1 Comparison of the α-diversity index of sediment fungi at different sampling sites. Different lowercase letters denote significant differences between sampling sites at P < 0.05. The maximum values in each column are denoted by “a”. α-Diversity indexes were calculated at the ASV level.

| Site | Ace       | Chao         | Shannon–Wiener | Simpson         |
|------|-----------|--------------|----------------|-----------------|
| A1   | 1717.33 ± 283.83a | 1717.33 ± 283.83a | 6.23 ± 0.28ab | 0.0100 ± 0.0073b |
| B1   | 1033.67 ± 71.23bc | 1033.67 ± 71.23bc | 5.25 ± 0.13bc | 0.0218 ± 0.0075b |
| C1   | 1753.33 ± 77.36a | 1753.33 ± 77.36a | 6.38 ± 0.08a  | 0.0051 ± 0.0008b |
| A2   | 985.33 ± 214.76bc | 985.33 ± 214.76bc | 3.93 ± 1.33d  | 0.1874 ± 0.1833a |
| B2   | 747.33 ± 66.29c  | 747.33 ± 66.29c  | 4.50 ± 0.17 cd | 0.0431 ± 0.0015b |
| C2   | 1209.00 ± 46.87b | 1209.00 ± 46.87b | 4.44 ± 0.36 cd | 0.1029 ± 0.0240ab |
Community Structure and Indicator Species of Fungi in Sediments from Different Sites

Fungal Community Structure Characteristics of Sediments from Different Sites

Taxonomic analysis of ASVs at the phylum level (Fig. S1, supplementary material) showed that the sediment fungi from all the sites belonged to 14 known phyla. Among them, Rozellomycota, Ascomycota, and Basidiomycota were the main fungal phyla at each sampling site (relative abundance > 5%). The relative abundance of Chytridiomycota was higher than 5% in B1 and B2 and lower than 5% in other sites. Mortierellomycota, Zoopagomycota, Olpidiomycota, Kickxellomycota, Glomeromycota, Blastocladiomycota, Calcarisporiellomycota, Mucoromycota, Basidiobolomyco- cota, and Monoblepharomycota were rare fungal phyla at each sampling site (relative abundance < 5%).

Taxonomic analysis of ASVs at the genus level (Fig. S2, supplementary material) showed that the sediment fungi from all the sites belonged to 778 genera. Among them, Cladosporium, Paraphaeosphaeria, Saitozyma, Pseudovertium, Trichoderma, Epicoccum, Penicillium, Plectosphaerella, Didymella, Talaromyces, Tausonia, Botrytis, Acaulopage, and Podospora were known dominant fungal genera at each site. Most of the fungi belonged to rare genera or genera that could not be classified and named.

Analysis of Indicator Fungi in Sediments from Different Sites

The LEfSe analysis results show that the characteristics of the fungal communities in the sediments differed among sites (Fig. 4). At the phylum level, the average relative abundance of Chytridiomycota accounted for 4.19%, which was an indicator fungal phylum with significant differences in B, while the average relative abundance of Rozellomycota accounted for 5.39%, which was an indicator fungal phylum with significant differences in C. At the genus level, the average relative abundance of Cercospora, Cladosporium, Dokmaia, Setophaeosphaeria, Paraphoma, Neosetophoma, Periconia, Plectosphaerella, Claviceps, Botrytis, and Papiliotrema accounted for 3.01%, 4.21%, 3.15%, 3.16%, 3.01%, 3.08%, 3.42%, 3.72%, 3.07%, 3.51%, and 3.48% respectively.
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These fungal genera were indicators with significant differences in the sediments of B. The average relative abundance of Acrocalymma, Emericellosis, Trichoderma, Podospora, Ciboria, and Apiotrichum accounted for 3.19%, 3.22%, 3.50%, 3.50%, 3.35%, and 3.16%, respectively. These fungal genera were indicators with significant differences in the sediments of A. The average relative abundance of Exosporium, Phialosimplex, Candida, Inocybe, Tausonia, and Sloopia accounted for 3.16%, 3.19%, 3.38%, 3.28%, 3.51%, and 3.42% respectively. These fungal genera were indicators with significant differences in the sediments of C. In addition, there were some unclassified and unnamed fungi in the sediments of various sites.

Fungal Functional Groups of Sediments in Different Sites

The FUNGuild microecological analysis tools were used to predict the utilization of similar environmental resources based on fungal communities. As shown in Fig. S3 (supplementary material), the detected sediment fungi were classified into three types (symbiotrophs, pathotrophs, and saprotrophs) according to their absorption and utilization of environmental resources. The fungi were classified into 12 functional groups, including litter saprotrophs, soil saprotrophs, wood saprotrophs, bryophyte parasites, lichen parasites, rhododendron ericoid mycorrhizae, ectomycorrhizae, animal pathogens, dung saprotrophs, plant pathogens, endophytes, and fungal parasites. The 12 functional types were very evenly distributed among various plots, and NPSP did not have a significant impact on the different environmental resource utilization mechanisms.

PICRUSt 2 software was used to predict and analyze the functional metabolic pathways of sediment fungal communities (Table 2). All the samples contained a total of 75 metabolic circulation pathways. Among them, 25 main metabolic circulation pathways showed significant differences among different types of sites (relative abundance of functional gene sequences > 1%). The levels of the metabolic circulation pathways PWY-7288 (fatty acid), PWY66-409 (super pathway of purine nucleotide salvage), and PWY-5189 (tetrapyrrole biosynthesis II) in B were significantly higher than those in the other sites (P < 0.05). The levels of PWY-7007 (methyl ketone biosynthesis) and PWY-7208 (nucleobases salvage) in A were significantly lower than those in the other sites (P < 0.05). The levels of the metabolic circulation pathways NONOXIPENT-PWY (pentose phosphate pathway), PENTOSE-PWY (pentose phosphate pathway), and PWY-6837 (fatty acid beta-oxidation V) in C were significantly higher than those in the other sites (P < 0.05). The levels of PWY-7118 (chitin degradation to ethanol), PWY-5920 (super pathway of heme biosynthesis from glycine), and PWY-6609 (adenine and adenosine salvage III) in the C group were significantly lower than those in the other groups (P < 0.05).

Relationship Between the Physicochemical Properties of Sediments and Fungal Community Diversity

The correlation analysis between the sediment physicochemical properties and sediment fungal α-diversity showed (Table S2, supplementary material) that the Ace index and Chao index were significantly negatively correlated with sediment pH (P < 0.05) and significantly positively correlated with the sediment TN and AN levels (P < 0.05).

The results of redundant analysis of sediment fungal community structure and sediment physical and chemical properties at the species level (Fig. 5) show that the cumulative explained variation of the two axes reached 75.95%, reflecting more than 70% of the sediment fungal community change characteristics and influencing factors. The results of the displacement test showed that the sediment pH ($r^2 = 0.6189, P = 0.002$), TP content ($r^2 = 0.4486, P = 0.016$) and AN content ($r^2 = 0.3321, P = 0.049$) were the key environmental factors that led to structural variation in the fungal communities.

Discussion

Effects of NPSP on Fungal Communities in Sediments of the Jialing River

NPSP is caused mainly by pesticides and fertilizers used in agricultural production and the discharge of rural domestic sewage. This type of pollution is associated with a large number of organic pollutant particles and terrestrial environmental microorganisms, which have a great impact on the microbial diversity of water sediments [29, 37, 45, 47]. To date, some studies have explored the impact of human activities on river microbial community diversity and the underlying diffusion mechanism. These studies have shown that environmental microbial diversity is closely related to human production and life activities, and human activities.
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Han et al. [18] found that human pollution discharge can lead to a significant increase in the diversity of planktonic fungal communities in the main stream of the Laohe River. Guo et al. [17] revealed that higher chemical oxygen demand (COD) concentrations can inhibit microbial diversity and richness. Adding bamboo charcoal in anaerobic environment can reduce COD concentrations and improve microbial diversity. It can be understood as increasing organic matter input is crucial for improving the diversity of microbial community in the anaerobic environment, which is basically consistent with the conclusion of this study. The two tributaries in the study area both flow through a large area, including both urban and rural areas, and receive urban sewage discharge, crop straw, fertilizers, and pesticides, leading to NPSP being the most important form of pollution for the two tributaries. The diversity of fungal communities in the sediments of the Jialing River after the two tributaries merged into the main stream was significantly higher than that before merging, and the changes in the fungal diversity of the sediments in the main stream tended to be consistent. This phenomenon could be attributed mainly to microbial attachment to the particulate pollutants from the land environment and gradual deposition of these microbes in the estuary. Changes in the diversity of the microbial communities in sediments occurred through environmental selection, interspecies competition, and random diffusion mechanisms [21, 51]. At the same time, the research results of Zhao et al. [57] further verified that stochastic processes were found as the dominant ecological driver in shaping the community assembly of both bacteria and fungi. Moreover, NPSP caused an increase in nutrients in the river water, and gradual deposition in the estuary improved the nutrient conditions and oxygen flux in the sediments, which significantly increased the diversity of the fungal community in the sediments [14].

The RDA results for A1 and C1 showed partial overlap. This indicated that the influx of NPSP tributaries had

| Pathway Description | A          | B          | C          |
|---------------------|------------|------------|------------|
| PWY-7288 Fatty acid | 0.0337 ± 0.0037c | 0.0412 ± 0.0014a | 0.0337 ± 0.0016b |
| GLYOXYLATE-BYPASS Glyoxylate cycle | 0.0272 ± 0.0014a | 0.0243 ± 0.0005c | 0.0260 ± 0.0004b |
| PWY-7184 Pyrimidine deoxyribonucleotides de novo biosynthesis I | 0.0214 ± 0.0003b | 0.0236 ± 0.0009a | 0.0223 ± 0.0010ab |
| PWY-7111 Pyruvate fermentation to isobutanol | 0.0206 ± 0.0004ab | 0.0202 ± 0.0002b | 0.0206 ± 0.0003a |
| PWY-7228 Superpathway of guanosine nucleotides de novo biosynthesis I | 0.0192 ± 0.0010b | 0.0203 ± 0.0004a | 0.0194 ± 0.0007ab |
| NONOXIPENT-PWY Pentose phosphate pathway | 0.0183 ± 0.0011b | 0.0182 ± 0.0004b | 0.0206 ± 0.0026a |
| PWY-7007 Methyl ketone biosynthesis | 0.0164 ± 0.0040b | 0.0209 ± 0.0018a | 0.0196 ± 0.0003a |
| PWY-7197 Pyrimidine deoxyribonucleotide phosphorylation | 0.0183 ± 0.0013b | 0.0199 ± 0.0006a | 0.0188 ± 0.0006ab |
| PWY-7208 Superpathway of pyrimidine nucleobases salvage | 0.0178 ± 0.0007b | 0.0185 ± 0.0003a | 0.0185 ± 0.0004a |
| VALSYN-PWY 3-Valine biosynthesis | 0.0180 ± 0.0007a | 0.0177 ± 0.0002b | 0.0185 ± 0.0001a |
| PWY-5067 Glycogen biosynthesis II | 0.0174 ± 0.0011b | 0.0183 ± 0.0001a | 0.0171 ± 0.0007ab |
| PANTO-PWY Phosphopantothenate biosynthesis I | 0.0168 ± 0.0006ab | 0.0164 ± 0.0002b | 0.0171 ± 0.0004a |
| PENTOSE-PWY Pentose phosphate pathway | 0.0161 ± 0.0003b | 0.0161 ± 0.0002b | 0.0168 ± 0.0008a |
| PWY-7118 Chitin degradation to ethanol | 0.0169 ± 0.0021a | 0.0171 ± 0.0011a | 0.0139 ± 0.0004b |
| PWY-7411 Superpathway of phosphatidate biosynthesis | 0.0159 ± 0.0005a | 0.0151 ± 0.0004b | 0.0161 ± 0.0006a |
| PWY-6837 Fatty acid beta-oxidation V | 0.0131 ± 0.00025b | 0.0150 ± 0.00099b | 0.0175 ± 0.0021a |
| PWY-7282 4-Amino-2-methyl-5-phosphomethylpyrimidine biosynthesis | 0.0157 ± 0.0008a | 0.0150 ± 0.0006ab | 0.0147 ± 0.0001b |
| PWY66-422 α-Galactose degradation V | 0.0151 ± 0.0004a | 0.0145 ± 0.0004b | 0.0151 ± 0.0003a |
| PWY-7210 Pyrimidine deoxyribonucleotidases biosynthesis from CTP | 0.0156 ± 0.0018a | 0.0138 ± 0.0011b | 0.0137 ± 0.0012b |
| PWY-5920 Superpathway of heme biosynthesis from glycine | 0.0134 ± 0.00077a | 0.0120 ± 0.0009a | 0.0128 ± 0.0002b |
| PWY66-409 Superpathway of purine nucleotide salvage | 0.0115 ± 0.00020b | 0.0140 ± 0.0009a | 0.0119 ± 0.0010b |
| PWY-5189 Tetrapyrrole biosynthesis II | 0.0125 ± 0.00010b | 0.0103 ± 0.0011a | 0.0117 ± 0.0001b |
| PWY-6609 Adenine and adenosine salvage III | 0.0120 ± 0.0016a | 0.0123 ± 0.0004a | 0.0097 ± 0.0015b |
| PWY-6317 Galactose degradation I | 0.0122 ± 0.0016a | 0.0100 ± 0.0007b | 0.0104 ± 0.0015b |
| PWY-7385 1,3-Propanediol biosynthesis | 0.0119 ± 0.0013a | 0.0099 ± 0.0009b | 0.0104 ± 0.0008b |
The study showed that the dominant fungi in the Jialing River sediments disturbed by NPSP belonged to Rozellomycota, while the dominant fungi in the non-NPSP sites belonged to Ascomycota. Ascomycota and Basidiomycota are the dominant phyla in most natural environments. However, the composition of fungi in different research environments has certain specificities, and Rozellomycota may be more closely related to human activities [6]. In addition, NPSP caused a significant increase in the relative abundances of *Exosporium*, *Phialosimplex*, *Candida*, *Inocybe*, *Tausonia*, and *Slooffia*. Most of them belong to animal and plant pathogens. The increase of such fungal community indicates that the environmental quality of sediments has decreased, which poses a serious threat to the security of regional ecosystems.

The physical and chemical properties of sediments are the main factors affecting the diversity of fungal communities in sediments. Generally, nutrients such as carbon, nitrogen, and phosphorus affect the growth and metabolism of microorganisms. A result of research showed that organic carbon is the main environmental factor affecting the fungal community in the sediments of the Poyang Lake Estuary, while the abundance and diversity of the fungal community have no significant correlation with environmental factors such as sediment pH, organic carbon, organic nitrogen, and C/N [23, 25, 33]. However, pH, TP, and AN are the main environmental factors affecting fungal communities in Jialing River sediments in this study. In addition, other researchers view the remarkably higher antibiotics, nitrate-nitrogen, and water temperature to play essential roles in shaping the structure and function of microbial communities [40, 44]. Difference in research results may be related to differences between the research areas. The sites studied by Wang Peng and others were set in the same area, and the pH value was relatively stable. In contrast, the pH of the sites in this study fluctuated greatly under the disturbance caused by NPSP, which affected the community diversity of sediment fungi. Nutrients such as carbon, nitrogen, and phosphorus usually function in accordance with the “barrel principle”, indicating that the lack of nutrients in the area may be the first limiting factor affecting the diversity of the microbial community [1]. In addition, many studies show that metal elements such as K, Mn, and Na were the main factors explaining the structural changes and composition of microbial flora [24]. The different metal substances have different effects on fungal communities under different environmental conditions. Objectively, NPSP is bound to increase the content of metal substances in river sediments. This also leads to the diversified and uncertain impact of NPSP on the microbial community of river sediments.
Effect of NPSP on the Fungal Function of Sediments in the Jialing River

To date, most studies on fungal functions have focused on the type of resource utilization and have classified fungi as saprotrophs, pathotrophs, or mycoheterotrophs [34]. Few studies have carried out detailed analysis of the fungal metabolic pathways, especially in research on the impact of NPSP on sediment fungal function, which is completely insufficient. Studies by Zheng et al. [55] have shown that the planktonic fungi in the Danjiangkou Reservoir area are mainly pathotrophic, saprotrophic, and pathotrophic-saprotrophic. Among them, the proportion of plant pathogens and animal pathogens is relatively high, and there are potential ecological risks. This is very similar to our research results. In this study, the ITS genome of the fungi in the sediments of the Jialing River was sequenced, and FUNGuild function prediction analysis was performed. The results showed that the functions of sediment fungi are mainly saprotrophic and include two types of nutrient metabolism: symbiotrophic and pathotrophic. The fungi with the three types of nutrient metabolism can be divided into 12 functional types: litter saprotrophs, soil saprotrophs, wood saprotrophs, bryophytic parasites, lichen parasites, ericoid mycorrhizal fungi, ectomycorrhizal fungi, animal pathogenic fungi, dung saprotrophs, plant pathogens, endophytes, and fungal parasites. Many fungal groups have one or more of the above nutrient metabolism functions. At the same time, we did not find that NPSP had a significant impact on certain nutrient metabolism functions. This also showed that although the environment has undergone major changes, the nutrient metabolism function of fungi has remained relatively intact, and fungi can play an important role in maintaining the structure and function of the ecosystem [26].

We used the PICRUSt2 tool to further analyze the metabolic pathways of sediment fungi in the Jialing River. The results showed that the fungi in the sediments of the Jialing River had 75 metabolic circulation pathways. Aerobic respiration I, aerobic respiration II, fatty acids, glyoxylate cycle, de novo biosynthesis of adenosine ribonucleotide, TCA cycle II, D-myo-inositol, etc., were the most important metabolic circulation pathways of sediment fungi. NPSP directly or indirectly affected 25 of the metabolic circulation pathways. NPSP significantly improved the functions of the pentose phosphate pathway, pentose phosphate pathway, and fatty acid beta-oxidation V ($P < 0.05$) and inhibited the functions of metabolic circulation pathways such as chitin degradation to ethanol, super pathway of heme biosynthesis from glycine, and adenine and adenosine salvage III ($P < 0.05$). The effects of NPSP on these metabolic circulation pathways are highly complex. It is very likely that human production and life activities cause antibiotics and pharmaceuticals to enter water bodies with NPSP, which has a toxic effect on certain fungal communities and inhibits the corresponding fungal metabolic circulation pathways [30]. It is also possible that after a pollutant enters the water body, its toxicity is weakened or it is converted into easily degraded compounds [28]. The microorganisms in the sediments transform or decompose exogenous biological substances, leading to increased performance in exogenous substance biodegradation and metabolic functions [8, 32]. At present, the relevant research is very limited, and the database itself has certain limitations. It is recommended that additional study directions should be considered and may be combined with metagenomic sequencing methods to perform an in-depth study of fungal functions in the sediment environment.

Ecological Measures and Management Suggestions for Controlling the NPSP

NPSP has become a key issue restricting the sustainable development of China’s rural areas. The main contradiction lies in the relationship between rural economic development and aquatic environmental protection [52]. The results of the present study clearly showed that NPSP caused changes in the structure and function of fungal communities in river sediments and posed a potential threat to the ecological environment [29]. Hence, it is urgent to strengthen research on rapid identification, monitoring and evaluation, and comprehensive treatment technologies [9]. Studies have shown that the treatment of NPSP is divided into three parts: reduction at the source, runoff control, and end treatment [39, 43]. Ecological treatment methods are the cheapest and most effective measure to control NPSP. Therefore, to reduce NPSP and promote the health and stability of the ecological environment, it is recommended to speed up the development of ecological agriculture production methods based on transformation and comprehensive utilization of rural domestic sewage and waste resources, the optimization of agricultural planting layouts and fertilization structures, and the implementation of ecological farming. In addition, based on the results of this study, the identification of NPSP environmental microbial indicators should be further studied. The monitoring and evaluation of environmental microbial indicator species at the source of pollution should be developed to scientifically manage NPSP discharge [16]. Ecological measures for NPSP prevention and control involve interconnected production, life, and ecological functions. The function of ecological control measures is closely related to the rationality of optimizing land use types [21]. In terms of NPSP runoff control, the construction mode and location of the waterway intersection zone, ecological ditches, and vegetation buffer zone should be optimized to give full play to the filtering function. In terms of terminal treatment, large wetland habitat areas should be constructed to exert degradation, adsorption, and metabolism functions and to ensure
that NPSP is effectively controlled before entering natural rivers [54]. Finally, identifying the structure and characteristics of various pollution sources and formulating comprehensive control strategies are the key to comprehensive pollution-based NPSP control [45, 47]. It is recommended to summarize the land use types, land use characteristics, and multi-functional composition of various ecological measures in combination with cases and documents. The classification of the different functions of ecological measures at different scales provides a theoretical basis for the optimal design of NPSP ecological measures.

Conclusions

In this study, we obtained novel insights into the impact of NPSP on the diversity and function of fungal communities in river sediments. The results showed that the dominant phylum of sediment fungi was Rozellomycota, followed by Ascomycota, Chytridiomycota, Basidiomycota, Mortierellomycota, and Zoopagomycota (relative abundance > 1%). NPSP had a significant promoting effect on Rozellomycota, Saccharomycetes, Microaascales, Saccharomycetaceae, Branch02, Branch03, etc., and a significant inhibitory effect on Chytridiomycota, Dothideomycetes, Capnodiales, Glomerellales, Xylariales, Chaetothyriales, etc. (P < 0.05). NPSP caused significant changes in the physicochemical indicators of Jialing River sediments, such as pH and AN, changes in which significantly increased the abundance of fungal species and caused significant changes in the fungal community β-diversity. pH, TP, and AN are the main environmental factors affecting bacterial communities in Jialing River sediments. The functions of sediment fungi mainly involve 3 types of nutrient metabolism (symbiotrophic, pathotrophic, and saprotrophic) and 75 metabolic circulation pathways. NPSP significantly improved the NONOXIPENT-PWY, PENTOSE-P-PWY, and PWY-6837 metabolic circulation pathways functions (P < 0.05) and inhibited the PWY-7118, PWY-5920, and PWY-6609 metabolic circulation pathway functions (P < 0.05). The structure and function of the fungal community in the sediments of the Jialing River changed under the combined effect of NPSP and the physicochemical conditions of the sediments. A good aquatic environment has an important role in stabilizing, maintaining, and protecting the fungal community in sediments.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00248-022-02009-5.

Acknowledgements We thank for the American Journal Expert agent (AJE) to help with the manuscript revision.

Author Contribution Fei Xu: writing—original draft preparation; Lanping Zhu: reviewing, checking, and editing; Yuqin Xue: investigation; Kunhe Liu: software, data curation; Fubin Zhang: software, data curation; Tuo Zhang: conceptualization, supervision, funding acquisition.

Funding This study was financially supported by the Science Research Program of China West Normal University (Project No. 19E061), the Tianfu Scholar Program of Sichuan Province (Project No. 2020–17), the Environmental Protection Research Program of the Yangtze River (Project No. CJZDZY347), and the National Science Foundation of China (NSFC) (Project No. 41907132).

Data Availability The data involved in this article have been included in the “Results” section and the supplementary information.

Code Availability Not applicable.

Declarations

Competing Interests The authors declare no competing interests.

Ethics Approval Not applicable.

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