

Impact of long-term industrial contamination on the bacterial communities in urban river sediments

lei zhang (✉ leizhang2014@163.com)

Chuzhou University

Demei Tu

Chuzhou University

Xingchen Li

Chuzhou University

Wenxuan Lu

Anhui Academy of Agricultural Sciences

Jing Li

Anhui Academy of Agricultural Sciences

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Abstract

Background: The contamination of the aquatic environment of urban rivers with industrial wastewater has affected the abiotic conditions and biological activities of the trophic levels of the ecosystem, particularly sediments. However, most current research about microorganism in urban aquatic environments has focused on indicator bacteria related to feces and organic pollution. Meanwhile, they ignored the interactions among microorganisms. To deeply understand the impact of industrial contamination on microbial community, we study the bacterial community structure and diversity in river sediments under the influence of different types of industrial pollution by Illumina MiSeq high-throughput sequencing technology and conduct a more detailed analysis of microbial community structure through co-occurrence networks.

Results: Although the composition of dominant bacterial phyla in different sediment samples was similar, their relative abundance was different. These dominant bacterial phyla showed significant differences in different types of industrial contaminated sediment. In addition, redundancy analysis indicated that the structure of the bacterial community in river sediments was influenced by a variety of environmental factors. TN, TP, TOC and metals (Cu, Zn and Cd) were the most important driving factors that determined the bacterial community in urban river sediments ($P < 0.01$). According to PICRUSt analysis, the bacterial communities in different locations had similar overall functional profiles. It is worth noting that the 15 functional genes related to xenobiotics biodegradation and metabolism were the most abundant in the same location. The non-random assembly patterns of bacterial composition in different types of industrially polluted sediments were determined by a co-occurrence network. Environmental conditions resulting from different industrial pollutants may play an important role in determining their co-occurrence patterns of these bacterial taxa. Among them, the bacterial taxa involved in carbon and nitrogen cycles in module I were relatively abundant, and the bacterial taxa in module II were involved in the repair of metal pollution.

Conclusions: Our data indicate that long-term potential interactions between different types of industrial pollution and taxa collectively affect the structure of the bacterial community in urban river sediments.

Background

Urban rivers are a vitally important foundation for reclaimed water utilization and the development of urban environments [1] {Francis, 2012 #2}{Francis, 2012 #2}. However, rivers flowing through cities often receive treated and untreated urban wastewater [2]. Among them, industrial wastewater has become one of the important sources of urban river aquatic environment pollution [3]. Exceeding a certain content of organic and inorganic contaminants in industrial wastewater will not only have negative consequences for aquatic ecosystems [4-6] but also pose significant health risks for water users [2]. The discharge of improperly treated wastewater into rivers or reservoirs used for agricultural irrigation may also have indirect adverse impacts on human health [7]. In addition, refractory organics and heavy metals released from industrial wastewater can cause long-term problems.

As an important part of the river system, sediments are complex habitats densely settled by various microorganisms [3]. Microorganisms are considered to be the most diverse and abundant biogroup in the world, regulating global biogeochemical cycles and affecting the functions of almost all ecosystems [8-10]. When different types of industrial wastewater are continuously discharged into the water environment, contaminants in the water can be diluted or purified. Conversely, contaminants in sediments can exist for longer periods of time [11]. Meanwhile, microbial communities in freshwater sediments are highly sensitive to changes in physicochemical status [12]. This inevitably affects the microbial community and its function in the sediments [13]. To adapt to different survival environments, microorganisms usually form a specific community structure to address various adverse effects [14]. Hence, analysis of the microbial communities in polluted river sediments can better identify potential biological indicator species as well as biomarker communities that respond to specific pollutants [15].

Many previous studies have analyzed the diversity and composition of microbial communities in rivers and determined the microbial indicators under local environmental impacts. For example, different bacterial indicators in river sediments under different environmental conditions were determined by linear discriminant analysis effect size (LEfSe) analysis [16, 17]. In addition, indicator species analysis methods were used to determine bacterial indicators in rivers under different land use types and human activity gradients [18]. However, most of these studies focused only on the interaction between river microorganisms and environmental pressure and ignored the interactions among microorganisms. In fact, microbe-microbe interactions have a crucial impact on community assembly and ecosystem function [19, 20]. In addition, the dynamics and composition of microbial communities are also greatly influenced by abiotic environmental factors [21, 22]. Therefore, understanding the interactions between microorganisms can provide new insights for further studying the structure of microbial communities in sediments under different environmental conditions.

Microbial co-occurrence networks can not only analyze microbe-microbe interactions and keystone species in the ecosystem but also explain potential intra or interspecific interactions in the water environment [23]. At present, co-occurrence networks have been applied to study complex ecosystems, such as marine bacterioplankton [24] and soil bacterial [25] or fungal communities [26]. Co-occurrence networks have intrinsic power and are useful in revealing information about community organizations, interactions among members, keystone species and their responses to different environmental conditions [27]. At the same time, the application of functional prediction technology can provide a glimpse of the overview of the functional spectrum of the microbiome, making the study of bacterial communities more detailed [28].

In fact, despite our advances in freshwater microbial ecology, most microbial research in urban aquatic environments has focused on indicator bacteria associated with feces and organic pollution [29-31]. Research on the effects of different types of industrial pollution on microorganisms in the aquatic environment of urban rivers is even more limited. Here, we study the bacterial community structure and diversity in river sediments under the influence of different types of industrial pollution by Illumina MiSeq high-throughput sequencing technology and conduct a more detailed analysis of microbial community

structure through co-occurrence networks. We hypothesized that the sediment bacterial community is influenced by the river environment, including nutritional factors and heavy metals. Industrial pollution influences the bacterial community structure in river sediment by changing the river environment. Our specific goals are (1) to explore the causes for the differences in the composition of bacterial communities in river sediments affected by different types of industrial pollution; (2) to explore the relationship between bacterial community function and habitat in river sediments under different industrial pollution conditions.

Results

Physicochemical characteristics of sediment samples

Table S1 shows the 12 physicochemical properties of different types of industrial contaminated surface water and sediments from the four sample sites (Fig. 1). The temperature and pH values of all samples were between 21.1 ~ 22.7 °C and 7.19 ~ 10.01, respectively. The TN, TP, and TOC contents of GGS (steel plant) were higher than those of other samples (Table S1). Additionally, the heavy metal concentrations in the four sites were compared. The results showed that Cu and Zn levels in GGS were the highest (Table S1). The highest contents of Pb and Cr were observed in ZMS (lighting factory), and the highest content of Cd was observed in FZS (Table S1). Based on the Chinese Soil Environmental Quality Standard (GB15618-1995), the concentration of heavy metals is divided into five classes (I, II, III, IV, V), corresponding to clean, relatively clean, normal, polluted, and moderately to heavily polluted, respectively [32]. The Cd concentration reached the class IV standard in ZMS and SPS and the class V standard in FZS (Table S2).

Diversity and richness analysis of bacterial communities

In this work, we analyzed microbial community diversity and phylogenetic structure in samples from different types of industrially polluted sediments by Illumina MiSeq high-throughput sequencing. After trimming, screening, and removing chimeras and single pieces, 505,911 valid 16S rRNA sequences were obtained. Among them, at least 31,412 valid sequences were obtained for each sample, with an average length of 417 bp. OTUs were grouped at the 97% cutoff, and diversity indexes and richness estimates were calculated for each sediment sample (Table S3). The Chao and ACE indexes of different sediment samples were compared, and the results were consistent with the above results (Fig. 2 (a); Table S3).

Simpson and Shannon index analysis results showed that FZS and ZMS had the highest diversity, followed by SPS, whereas GGS displayed significantly lower diversity (Table S3; Fig. 2 (b)). All rarefaction curves approached saturation, indicating sufficient sequencing depth (Fig. 2 (c)). Similarly, the coverage of each sample was higher than 0.99 (Table S3), which indicates that the sequencing depth was sufficient and some rare species were included.

Composition of bacterial communities

The overall characteristics of bacterial communities in different types of industrially polluted sediment samples were observed by principal coordinate analysis (PCoA) (Fig. 3 (a)). PC1 and PC2 accounted for 45.18% and 42.88%, respectively. In general, spatially adjacent samples were more closely distributed. Therefore, we found that SPS and ZMS samples had similar features. ANOSIM analysis (analysis of similarities) confirmed significant differences in bacterial community structure in different types of industrially polluted sediment samples ($R = 1$, $p = 0.001$) (Table S4).

A total of 16 major bacterial phyla were detected in sediment samples collected at different locations. Among them, *Proteobacteria*, *Actinobacteria*, *Chloroflexi*, *Acidobacteria*, *Firmicutes* and *Bacteroidetes* together accounted for 77.62% to 94.49% of OTUs in all samples, and their relative abundance in each sample was higher than 1% (Fig. 3 (b)). These dominant bacterial phyla exhibited significant differences in different types of industrially polluted sediment (Fig. 3 (c)). Further analysis revealed that there was no significant difference in the relative abundance of the dominant bacterial phyla in both SPS and ZMS sediments (Fig. S1). *Proteobacteria* had the highest average relative abundance in sediment samples from SPS and ZMS, accounting for 45.22% and 45.04%, respectively. The average relative abundance of *Firmicutes* was highest in GGS (26.59%), while the phylum with the highest average relative abundance in FZS was *Actinobacteria* (28.41%).

To better explain the structure of the microbial community in different types of industrial polluted sediments, the relative abundance and classification of OTUs were analyzed at the family level (Fig. 3 (d)). It is worth noting that some species had a large proportion in some samples and were the dominant bacteria, while in other samples, its proportion was small, or even nonexistent. Family *Propionibacteriaceae* (9.51%) and family *Synergistaceae* (8.96%) were the dominant family in GGS samples. However, they were almost undetectable in the other three groups of samples. In FZS samples to *norank_c_Acidobacteria* (13.81%) are the most common, other dominant families including *norank_o_JG30 KF-CM45* (4.04%), *norank_CKD4-96* (3.81%) and family *Nocardioideaceae* (3.77%). Among them, the family *Nocardioideaceae* was found to be much more abundant in the FZS samples than in the other samples. The most abundant families in SPS and ZMS were family *Rhodocyclaceae* (9.99%) and family *Xanthomonadales_Incertae_Sedis* (6.99%).

Significant differences in microbial communities

Biomarker analysis with linear discriminant analysis (LDA) effect size (LEfSe) was used to identify species with significant abundance differences in different types of industrially polluted sediment samples. As showed in Fig. 4, 176 bacterial clades present statistically significant differences with a LDA threshold of 3.5 (Fig. S2). The degree of enrichment was greatest in FZS for most bacteria, and a total of 54 clades showed an abundance advantage. In contrast, the degree of enrichment was smallest in the SPS samples, with only 30 clades. It is worth noting that the family *Rhodocyclaceae* (LDA = 4.92) and genus *Dechloromonas* (LDA = 4.91) in SPS not only have high relative abundance but also have significant effects on differences among groups. In contrast, although the relative abundance of the

family *Sphingomonadaceae* (LDA = 4.51) and genus *Sphingomonas* (LDA = 4.51) in FZS was low, they also had significant effects on differences among groups.

Environmental factor analysis

The correlation between environmental parameters and bacterial community composition was determined by RDA (Fig. 5). TN, TP, TOC, pH, Cu, Zn and Cd were environmental variables that had a significant effect on the relationship between the bacterial community and the environment (all $P < 0.01$). Among them, Cu and Zn were positively correlated with samples from GGS, and Cd was also positively correlated with the FZS samples. Moreover, *Firmicutes* was positively correlated with TN, TP, TOC, Cu and Zn. pH and Cd were also positively correlated with *Actinobacteria* and *Acidobacteria*. However, *Proteobacteria* and *Chloroflexi* were significantly negatively correlated with TN, TP, and TOC.

Co-occurrence network analysis

Considering the non-random aggregation pattern of microbial communities in river sediments under different types of industrial pollution, a network interface was established to better show the topological and taxonomic characteristics of microbial co-occurrence patterns (Fig. 6). According to the analysis results, 4196 edges were captured among 257 nodes that described significant correlations between species ($\rho > 0.7$, $P < 0.05$). At the same time, significant topological characteristics were derived based on calculations to determine the complex patterns of interrelationships among nodes [33]. The average path length (APL) was 2.825 edges, and the diameter was 6 edges. The clustering coefficient (CC) was 0.641, and the modularity index (MD) was 0.827, where $MD > 0.4$ indicates that there was a modular structure in the network [34].

The nodes in the network were divided into 6 bacterial phyla (Fig. 6 (a)). Among them, *Proteobacteria*, *Actinobacteria*, and *Chloroflexi* accounted for 63.2% of all nodes, and they were also dominant bacterial phyla in the community. When the node distribution was modularized, all nodes were mainly divided into six modules (Fig. 6 (b)). Each module consisted of a set of OTU nodes, and the interconnections among these nodes were more frequent than the nodes in other modules. The OTUs in module I had a higher relative abundance in FZS, and the OTUs in module II and module VI had a higher relative abundance in GGS. Module III and module V had a higher relative abundance in ZMS, and module IV dominated in SPS. The top five keystone genera with the highest number of connections were *Gaiella*, *Denitratisoma*, *Anaeromyxobacter*, *Candidatus_Microthrix*, and *unclassified_p__Chloroflexi*, and the number of related genera was not less than sixty. In addition, the betweenness centrality values of the genera *Denitratisoma*, *Anaeromyxobacter*, and *Candidatus_Microthrix* were all less than 200 (their closeness centrality range from 0.379 to 0.382). This represents a high degree and low betweenness centrality values, indicating that they can be considered central species [20, 35].

PICRUSt functional predictive analysis

PICRUSt (phylogenetic investigation of communities by reconstruction of unobserved states) analysis was conducted to predict the metabolic functions of bacterial communities. The results indicated that the major functional gene families were related to metabolism, genetic information processing, environmental information processing and cellular processes (Fig. 7(a)). Among them, the relative abundance of metabolism was the highest in all samples, followed by genetic information processing. Their average proportions in different groups were 68.94% and 10.96%, respectively. At KEGG (Kyoto Encyclopedia of Genes and Genomes) level 2, a large number of sequences in each sediment sample were allocated to carbohydrate metabolism and amino acid metabolism (Fig. 7 (a)). In addition, rivers are the primary recipient of pollutants and xenobiotics input from the watershed, most aquatic organisms, as well as bacteria, are exposed to these xenobiotics [36]. Therefore, we focused on the functional genes of xenobiotics biodegradation and metabolism. In this category, FZS had the highest average relative abundance (Fig. 7(a)). At KEGG level 3, 15 individual KEGG pathways from xenobiotics biodegradation and metabolism were chosen (Fig. 7 (b)), and the content of each pathway in FZS was higher than the other three groups. Among them, the average relative abundance of benzoate degradation was the highest, followed by aminobenzoate degradation.

Discussion

Bacteria are important for ensuring and maintaining the environmental and ecological processes of river ecosystems [37]. Here, the bacterial communities in river sediments under different types of industrial pollution were analyzed by Illumina high-throughput sequencing technology. Overall, the sediment bacterial communities were dominated by *Proteobacteria*, *Actinobacteria*, *Chloroflexi*, *Acidobacteria*, *Firmicutes* and *Bacteroidetes* (Fig. 3 (b)). Wang et al. (2016) studied the community structure of aquatic bacteria in urban rivers and obtained similar results [38]. Su et al. (2018) investigated the bacterial communities in coastal sediments and found that *Proteobacteria*, *Firmicutes*, *Chloroflexi*, *Acidobacteria*, *Bacteroidetes*, *Actinobacteria*, *Nitrospirae*, *Gemmatimonadetes* and *Planctomycetes* predominated [39]. These results showed that these sediment samples shared the characteristic profile of high bacterial rank commonly found in other aquatic ecosystems.

Although there were similarly high taxonomic level characteristics between different sampling sites, the relative abundance was different among different sites (Fig. 3 (c)). According to the data from this study, *Proteobacteria* were the most abundant in SPS (food factory) and ZMS (lighting factory) and were mainly composed of *Betaproteobacteria* and *Gammaproteobacteria* (Fig. 4). Most *Proteobacteria* groups play very important roles in the decomposition of organic matter and circulation [40]. Further research found that the family *Rhodocyclaceae* (class *Betaproteobacteria*) not only dominates in SPS but also had significant differences in abundance at different sites (Fig. 4). This strain has extensive metabolic capabilities and can degrade multiple carbon sources, such as many aromatic compounds [41]. Therefore, some members of the family are active in the degradation of recalcitrant chemicals [42]. For example, the genus *Dechloromonas* is capable of degrading a variety of complex organic pollutants (Fig. 4) [43]. Therefore, the dominance of the family *Rhodocyclaceae* in SPS may indicate that the area was rich in organic wastewater compounds.

The most dominant phylum in FZS (textile mill) was *Actinobacteria*. *Actinobacteria* have been confirmed to play a pivotal role in the carbon cycle of freshwater ecosystems [44]. In fact, the data from this study showed that TOC was the lowest in FZS, and nutrition in this sample was relatively poor (Table S1). The results were consistent with previous studies, suggesting that *Actinobacteria* are indeed active in oligotrophic environments [45]. In addition, the family *Sphingomonadaceae* was significantly enriched in FZS, and there were significant differences among different sites (Fig. 4). The family *Sphingomonadaceae* is usually found in habitats contaminated by a high proportion of recalcitrant (poly) aromatic compounds of natural or anthropogenic origin [46-48]. Moreover, the genus *Sphingomonas* in *Sphingomonadaceae* can degrade various recalcitrant compounds (Fig. 4) [49]. Thus, the members of this genus can grow vigorously in polluted environments [50]. It is worth noting that the textile industry produces a large amount of wastewater, which contains a variety of chemical compounds, such as azo dyes, heavy metals, and surfactants [51]. Therefore, the relative advantages of the family *Sphingomonadaceae* in FZS indicated that they are well adapted to the environment in FZS and possibly utilize a wide variety of nutrients to resist or withstand environmental disturbances.

Firmicutes are well known for having many members that are able to degrade very recalcitrant organic compounds [43]. Few previous studies also found a dominance of *Firmicutes* in freshwater sediments [52]. However, the abundance of *Firmicutes* was highest in GGS (steel plant). The data from this study indicated that the content of TN, TP and TOC in GGS was the highest, especially the TOC, which was 2 to 3 times that of the other three groups of samples (Table. S1). Meanwhile, RDA showed that the abundance of *Firmicutes* was positively correlated with TN, TP, and TOC (Fig. 5). This is the same result as previous studies, suggesting that as copiotrophs or fast-growing organisms, *Firmicutes* can exist in carbon-rich environments that meet their high energy requirements and maintain their growth rates [53]. Moreover, *Clostridiales* are known as metal-coping bacteria and thrive in environments rich in metal contaminants such as GGS [54] (Fig. 4; Table S1). Therefore, the enrichment of *Clostridiales* may also be one of the reasons for the largest proportion of *Firmicutes* in GGS. These results indicated that the environmental conditions may select different bacterial species, which leads to different spatial distributions of bacterial populations.

Multiple studies have shown that environmental factors, such as temperature [38], nutrients [55], pH [56], water turbidity [57] and sediment particle size [58], often affect the composition and structure of bacterial communities. On the one hand, RDA showed that TN, TP and TOC were significantly related to the composition of bacterial communities in different types of industrial polluted sediments ($P < 0.01$) (Fig. 5). TN, TP, and TOC are important factors for structuring bacterial communities in river sediments, which is consistent with other research results. In reality, microorganisms may prefer to use bioavailable forms of phosphorus, nitrogen, and carbon (e.g., PO_4^{3-} , NO_2^- , NO_3^- , NH_4^+ , etc.) and the forms may be more closely related to bacterial community composition [45, 59, 60]. Therefore, the relationship between microbial communities and detailed environmental factors needs to be studied in greater depth. On the other hand, the heavy metals Cu, Zn, and Cd were significantly correlated with the distribution of bacterial communities in different types of industrially polluted sediments ($P < 0.01$) (Fig. 5). It has been

documented that high concentrations of heavy metals can significantly reduce bacterial biomass in sediments [61]. This was consistent with our observations that the species richness and diversity in the GGS samples, which had the highest heavy metal concentrations, were the lowest (Fig. 2 (a, b); Table S1).

In summary, the differences in bacterial community composition in different types of industrially polluted sediments reflect the tolerance of OTUs to specific environments. The above abiotic factors, such as TN and TP, may directly change the composition of bacterial communities by affecting the growth of certain bacteria in the sediment. That is, changes in the physicochemical properties of river sediments caused by the input of different types of industrial wastewater drive the formation of different bacterial communities. Meanwhile, this also means that microbial communities in urban river sediments have potentially evolved phylogenetic versatilities under the long-term effects of industrial pollution.

The interrelationships among different microbial communities play a pivotal role in maintaining the structure, function and stability of microbial ecosystems [14]. In the network analysis of this study, most nodes belonged to three dominant phyla: *Proteobacteria*, *Actinobacteria* and *Chloroflexi* (Fig. 6 (a)). The keystone genera *Gaiella*, *Denitratisoma*, *Anaeromyxobacter*, *Candidatus_Microthrix*, and *unclassified_p__Chloroflexi* were the top five with the highest number of connections. This suggested that the other genera respond more strongly to the metabolites produced by these five genera [62]. Compared with other taxa in the network, keystone taxa play an important role in maintaining the network structure [22]. It is speculated that the disappearance of keystone taxa may lead to disintegration of the network [44]. Keystone taxa are by the definition the taxa essential for ensuring and maintaining stability, so their existence is by definition important for the stability of ecosystem structure and function. Additionally, the average relative abundances of the genera *Gaiella*, *Denitratisoma*, *Anaeromyxobacter*, *Candidatus_Microthrix*, and *unclassified_p__Chloroflexi* were all low (0.24% ~ 0.65%), suggesting the significance of rare genera in bacterial communities. Currently, rare genera are being increasingly recognized as crucial components of communities in biochemical processes and community assemblies [63]. Although the abundance of such genera may not have been high, more attention should be paid to them as key nodes in the microbial community [14].

In addition, the genera *Denitratisoma*, *Anaeromyxobacter*, and *Candidatus_Microthrix* were considered central species due to their high degree (> 60) and low betweenness centrality values (<200). The genus *Denitratisoma* contains denitrifying bacteria that contribute to the removal of nitrogen [64]. The genus *Anaeromyxobacter* is metal-reducing bacteria, and members of the bacteria can affect the mobility of metal contaminants [65, 66]. Moreover, previous studies have shown that the genus *Candidatus_Microthrix* helps in the removal of total nitrogen [67]. Therefore, these keystone taxa may play a pivotal role in ecological function processes.

Due to the modularity, the entire network was mainly divided into six modules (Fig. 6 (b)). Modularity may reflect habitat heterogeneity and divergent selection regimes [68]. Meanwhile, the habitat preference of microorganisms may also help determine their co-occurrence patterns [19]. Therefore, it can be reasonably found that microorganisms in different types of industrial polluted sediments tend to form

distinct modules. The main taxa in module I may be bacteria involved in the biogeochemical C- and N-cycles. For instance, the genus *Nocardioides* can utilize multiple organic compounds as carbon source [69]. The genus *Nitrospira* consists of chemically autotrophic nitrite-oxidizing bacteria [60]. The genus *Streptomyces* has been shown to participate in the nitrogen cycle [70]. Other bacteria in module I included *Microbacteriaceae*, *Rhodospirillaceae*, *Bradyrhizobiaceae* and *Streptomycetaceae*, which are also involved in C and N cycling [71]. Apparently, these bacteria had the highest abundance in FZS, indicating that carbon and nitrogen cycling associated with microorganisms occurs frequently in this area. Sulfate-reducing bacteria (SRB) in module II, including the genera *Clostridium_sensu_stricto_1*, *DeFluviicoccus*, and *Desulfobulbus*, were significantly enriched in GGS [72]. Interestingly, SRB plays a major role in repairing the environment polluted by heavy metals (Fe, Cu, Pb, Zn, etc.) [72]. Therefore, heavy metals in GGS may be one of the important factors driving these bacteria. In conclusion, the non-random assembly pattern of these bacteria indicates that the complexity of bacterial community structure and functional processes in river sediments under different types of industrial pollution seems to be dominated by environmental filtering and function-driven.

According to PICRUSt analysis, the overall functional profiles of bacterial communities in different river sediment samples were similar. Carbohydrate metabolism and amino acid metabolism were the dominant metabolic genes in the bacterial community (Fig. 7(a)). As core resources metabolism pathways, they were potential drivers of microbial community structure and function of microbial communities in rivers [36]. It is worth noting that the xenobiotics biodegradation and metabolism that we focus on had more advantage in FZS (Fig. 7(a)). The possible reasons were FZS accepted more xenobiotic compounds from industrial wastewater, and these compounds were used by bacteria as the sources of carbon, nitrogen, or energy. Some previous reports indicated that there was a correlation between the abundance of xenobiotic degradation genes and the rate of xenobiotic biodegradation [73, 74]. Therefore, biodegradation genes could be proposed as indicators of the presence of xenobiotics and their metabolites [45, 75]. Furthermore, FZS surpassed the other three groups in the functional profiles related to the degradation of 15 chemical pollutants (Fig. 7(b)). For instance, benzoate degradation and aminobenzoate degradation. Meanwhile, the enrichment of many organic pollutant-degrading bacteria was found in FZS, such as genera *Sphingomonas*, *Mycobacterium*, *Novosphingobium*, and *Bacillus* (Fig. 4) [76-78]. The predicted higher enrichment of chemical pollutant degradation pathways means that the pollution was more severe in FZS, while such functional response of bacterial community might accelerate the bioremediation of contaminated zone. In general, the PICRUSt algorithm determined the predicted functions of the microbial community, providing a general overview of the functional potential within the community. However, rarefaction of pooled DNA samples fails to capture the full extent of diversity present within the system, which is likely reflected in the predicted functional profile [79]. Furthermore, this method is influenced by phylogenetic differences between environmental samples and sequenced genomes [28]. Therefore, we propose that further studies are needed in this system that use metagenomic sequencing and marker gene studies, to fully assess gene categories.

In this study, because the pollutants at each sampling point have been discharged into the water through pipes or channels for a long time, the sampling conditions were restricted. Therefore, we only paid

attention to the impact of long-term discharge of industrial wastewater on the bacterial community of river sediments, and we did not cover the research before wastewater discharge. This may cause some limitations to our research. However, our findings represent an important step in understanding the impact of long-term industrial pollution on bacterial communities in urban river sediments, and thus contributes to the increasing knowledge of microbial ecology in the urban river sediments.

Conclusion

This study revealed the composition and structure of bacterial communities and their co-occurrence patterns in different types of industrially polluted sediments. The composition of the dominant bacterial phyla was similar in each sampling location, but the relative abundance was different, and there were significant differences among different locations. Environmental factors, including metals (Cu, Zn and Cd) and nutritional factors (TN, TP and TOC), had significant effects on the composition of bacterial communities in different types of industrially polluted sediments (all $P < 0.01$). Although the relative abundance of highly connected taxa (such as the genera *Denitratisoma*, *Anaeromyxobacter* and *Candidatus_Microthrix*) in the co-occurrence networks was low (0.24% ~ 0.65%), they may play a pivotal role in maintaining the structure and function of ecological communities. Non-random co-occurrence and ecological function-driven modular patterns occurred in the bacterial communities, providing a new perspective of microbial assembly in different types of industrially polluted sediments. Furthermore, the accumulation of multiple chemical pollutant-degrading genes at the same location means that the zone was more polluted, while such functional response of bacterial community could contribute to the bioremediation of polluted river environment. Overall, these results provide valuable information for ecological risk assessment and management of urban rivers under different types of industrial pollution, thereby helping to monitor and control the level of water environmental contamination.

Materials And Methods

Sites description and sample collection

The sampling sites were located in the Qingliu River (watershed area of 1318 km² and mainstream length of 84 km), which is a typical urban river in Chuzhou city, China [80]. The river flows through the center of the city and is surrounded by many industrial areas. Therefore, a large amount of different types of industrial wastewater is discharged along the river and is one of the main sources of pollution that causes the deterioration of the aquatic environment. Sampling points were from wastewater outlets of different types of factories near the Qingliu River (Fig. 1). They are steel plant (GGS), lighting factory (ZMS), food factory (SPS) and textile mill (FZS), respectively.

Sampling was carried out in May 2019. A Peterson sampler was used to collect surface sediment samples (<5 cm deep). Three parallel sediment samples were randomly obtained at 3 m intervals for each sampling site. The 4 sampling sites are shown in Fig. 1. The sediment samples were stored in sterile polyethylene zipper bags and transferred to the laboratory on ice within four hours. A portion of the

sediment samples was collected into a 2.5 ml sterile centrifuge tubes and stored at -80 °C until DNA extraction was performed. The other portion was immediately subjected to physio-chemical analysis at 4 °C.

Analysis of physicochemical properties

While collecting the samples, the water temperature (T), pH and dissolved oxygen (DO) were measured in situ using a YSI-6600 multiparameter controller (Yellow Springs Instruments, USA). The ammonia nitrogen ($\text{NH}_4^+\text{-N}$), total nitrogen (TP), total phosphorus (TN), and total organic carbon (TOC) contents were measured in the laboratory according to standard methods [52]. The content of metal elements copper (Cu), zinc (Zn), lead (Pb), cadmium (Cd), chromium (Cr) in sediment samples were determined by using the X7 inductively coupled plasma mass spectrometer (ICP-MS) of Thermo Corporation in the collision cell mode [81].

DNA extraction

Base on the manufacturer's instructions, Total DNA was extracted from an aliquot of 0.25 g of sediment from each sample using an MP Biomedicals Fast DNATM SPIN Kit. The extracted genomic DNA was detected by 1% agarose gel electrophoresis, and the purity and concentration were determined by a UV spectrophotometer (Eppendorf, Germany). The measured DNA sample was stored at -20 °C for subsequent use.

PCR amplification and sequencing analysis

Usearch (version 7.1 <http://drive5.com/uparse/>) was used to merged the paired-end 16S reads, trimmed primers and distal bases and removed quality-filtered sequences and singletons [82]. The RDP classifier (version 2.2, <http://sourceforge.net/projects/rdp-classifier>) Bayesian algorithm (confidence threshold of 0.7) was used to obtain the species classification information corresponding to each OTU, and OTU representative sequences were classified at a similarity level of 97% using the QIIME platform (http://qiime.org/scripts/assign_taxonomy.html) [83].

Statistical analysis

According to the OTU information, mothur (version v.1.30.1) was used to calculate the alpha diversity index (Chao 1, ACE, Shannon, Simpson) [84], and curves were generated with the R language tool [85]. Beta diversity analysis was represented by Bray-Curtis distance matrices generated from the OTU table, and statistical analysis and mapping was performed with PCoA in R language [86]. The community structure composition of different classification levels (such as phylum, genus and OTU) was obtained by taxonomic analysis. A similarity analysis (ANOSIM) of the Bray-Curtis similarity matrix of the initial pyrophosphate phosphate content was performed by the R-language vegan software package. One-way analysis of variance was performed using SPSS 19.0 and post-hoc Scheffe test was used for pairwise comparisons. The level of significance was set at 0.05. The relationship between microbial community

composition and environmental factors was explained through the RDA function of the vegan package using R language [87]. The significantly discriminant taxa in each group were determined by the linear discriminant analysis (LDA) effect size pipeline (LEfSe) program at <http://huttenhower.sph.harvard.edu/galaxy/root>, which employs the factorial Kruskal-Wallis rank-sum test to identify communities or species that have significant differences in sample partitioning between species [88]. PICRUSt software was used to predict bacterial function and metabolic pathways [28], and the bioinformatics analysis images were drawn using the Origin software. Based on the Spearman correlation, the co-occurrence networks of microbial communities were determined. Select OTUs with significant and robust correlations ($\rho > 0.7$ and $P < 0.05$). All the robust correlations identified from pairwise comparison of the genera abundance form a correlation network where each node represents one genus, and each edge stands for a strong and significant correlation between the nodes. Gephi (<http://gephi.github.io/>) was used for network visualization and modularization analysis. The topological properties of the network, including degree (the number of neighbors of a node), average path length (the average number of steps along the shortest paths for all possible pairs of network nodes), clustering coefficient (the tendency of neighbors of a node to connect with each other), betweenness centrality (the number of shortest paths going through a node) and modularity (a measure of how well a network is divided into modules) were determined by gephi [89]. Nodes with high degree and low betweenness centrality values were recognized as keystone species in the co-occurrence network [35]. The raw sequencing data have been submitted to NCBI Sequence Read Archive under the project accession code SRP242054.

Declarations

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Authors' contributions

LZ designed the experiment. Data analyses were performed by XL, data collection by WL and JL. The manuscript was draft by DT and revised by all authors. All authors have read and approved the manuscript.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

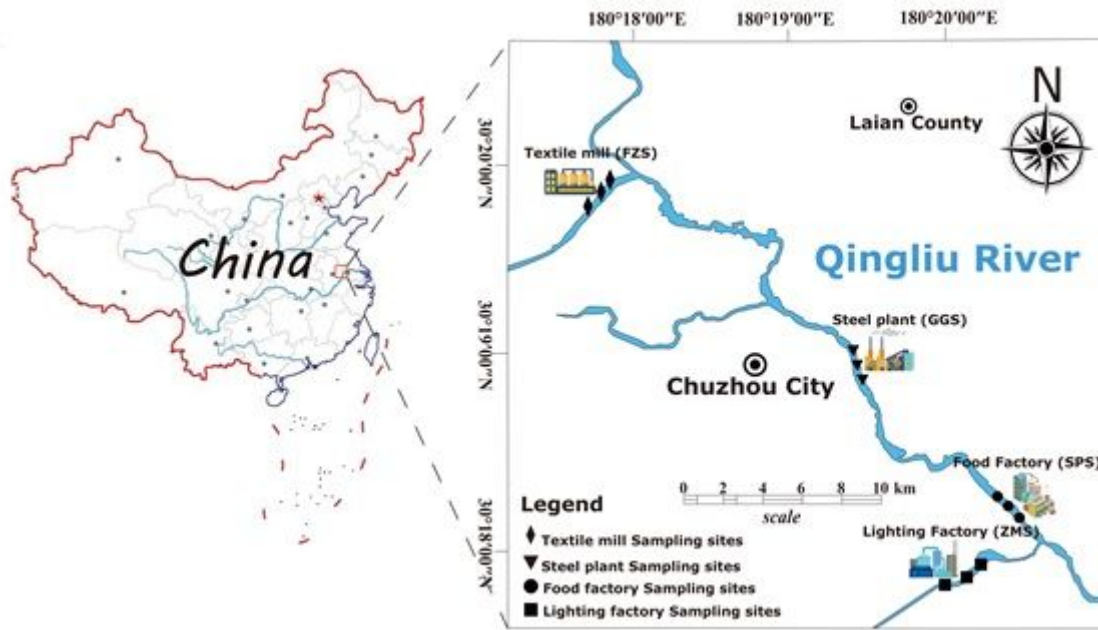


Figure 1

Location of sampling sites in Qingliu River, Anhui, China. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

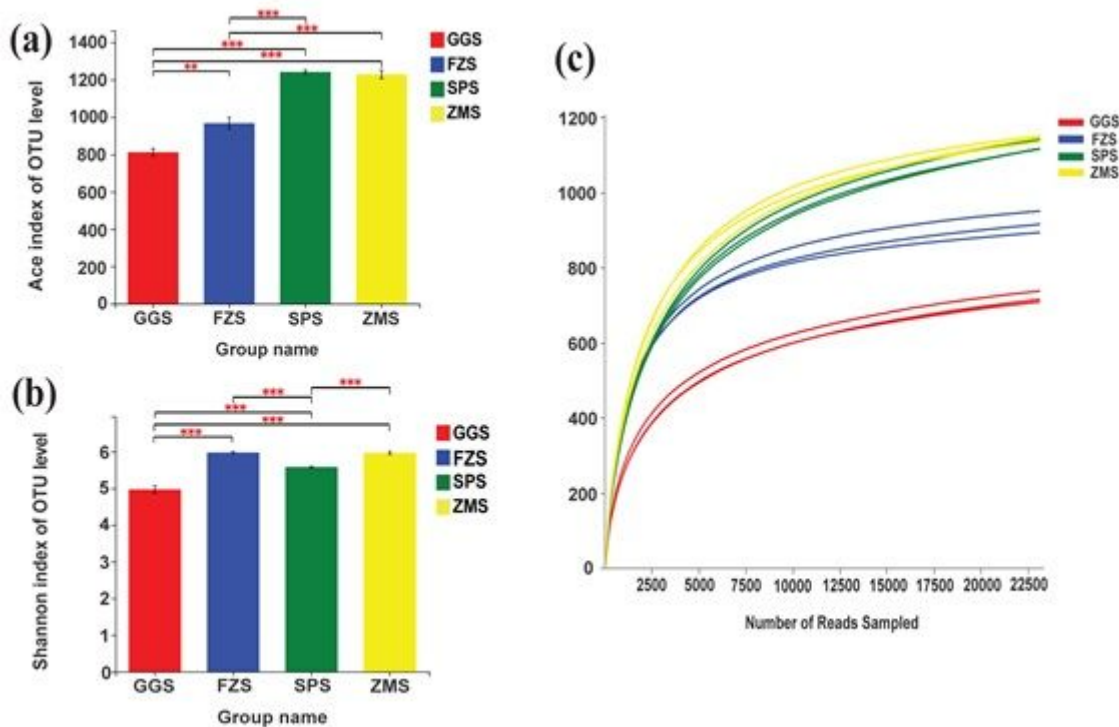


Figure 2

Alpha diversity of sediment samples from different types of industrial pollutions. (a) Richness, indicated by Ace, Ace species richness estimator: higher values represent higher diversity. (b) Diversity, indicated by Shannon, the Shannon index: higher values represent higher diversity. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. (c) Rarefaction curves of OTUs clustered at 97% sequence identity across the twelve samples.

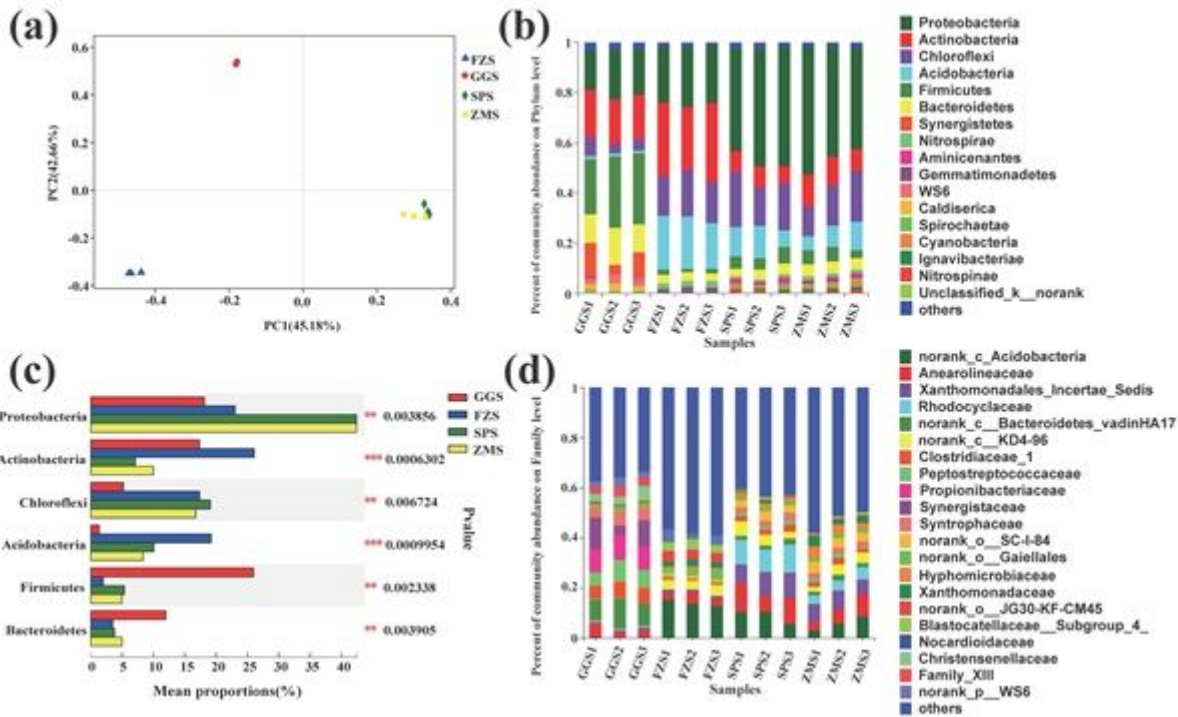


Figure 3

Principled coordinate analysis (PCoA) for the four groups of different industrial polluted sediment communities at the OTU level (a). Community composition of bacteria in the phylum level (b) and the family level (d) in sediment samples. (c) Significantly different relative abundance of dominant bacteria in four groups of different industrial polluted sediments. One-way analysis of variance (ANOVA) was used to evaluate the importance of comparisons between indicated groups. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

taxa with significant enrichment in the corresponding group and significant influence on intergroup difference. Yellow circles stand for taxa with no significant differences in the sediment.

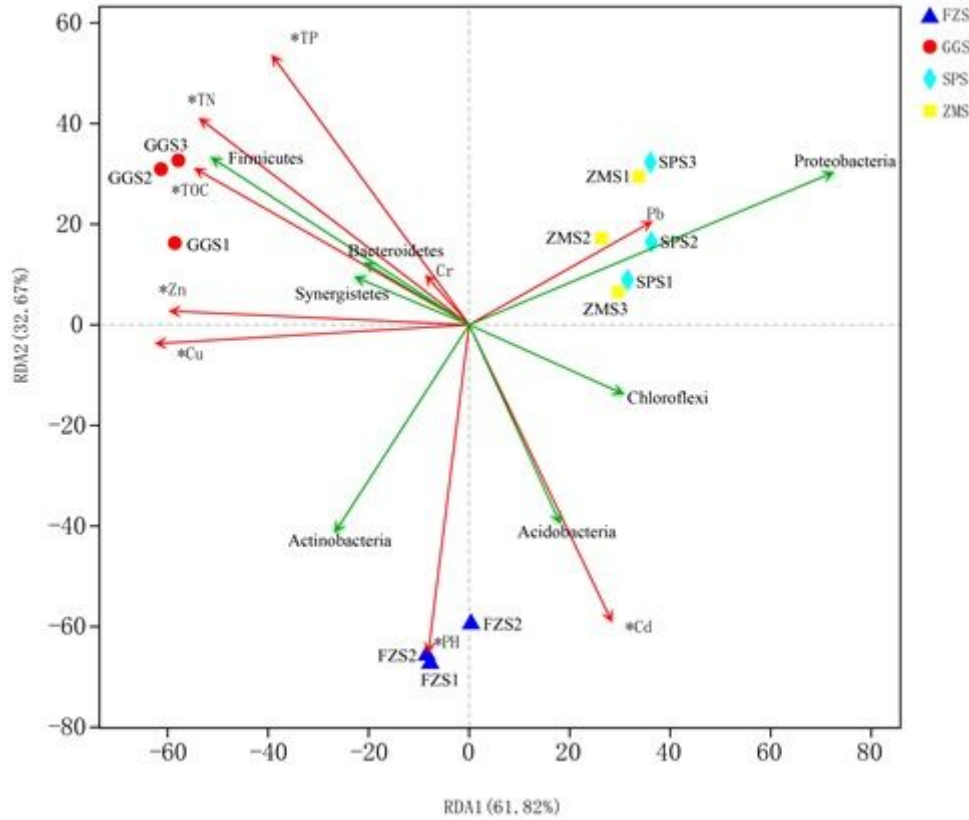


Figure 5

Redundancy analysis (RDA) of bacterial community composition in sediments and environmental factors from different types of industrial polluted sediments. (note that the significant environmental factors identified by the Monte-Carlo test are marked with an asterisk. An * represents significant correlations at $P < 0.01$).

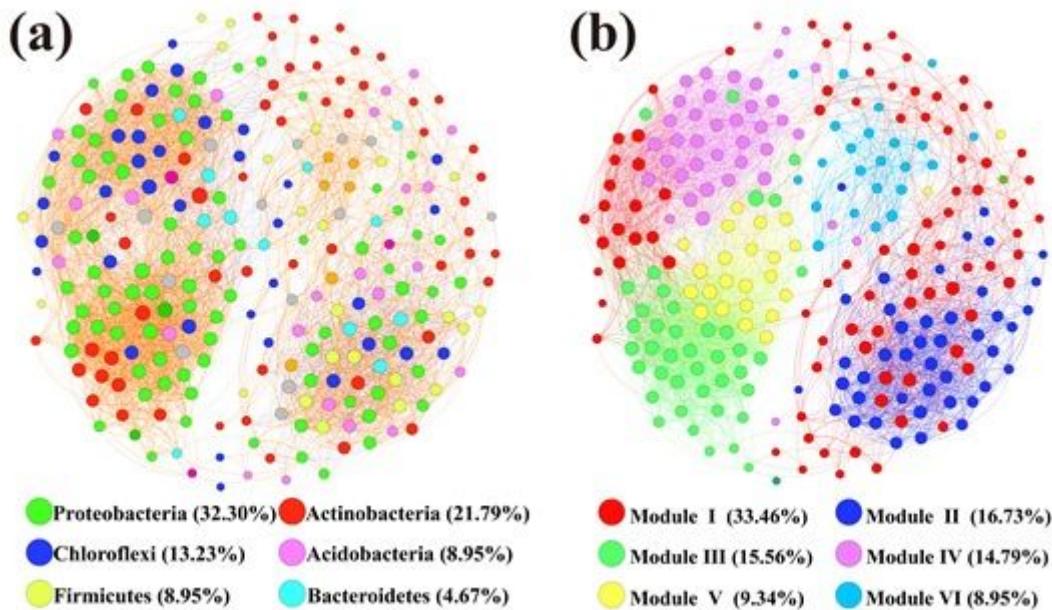


Figure 6

Co-occurrence networks of bacterial communities in different types of industrial polluted sediments based on correlation analysis. The nodes in network (a) are colored by phylum. The nodes in network (b) are colored by modularity class. The connections indicate strong (spearman's $\rho \geq 0.7$) and significant ($P < 0.05$) correlations. The size of each node is proportional to the relative abundance of specific genus.

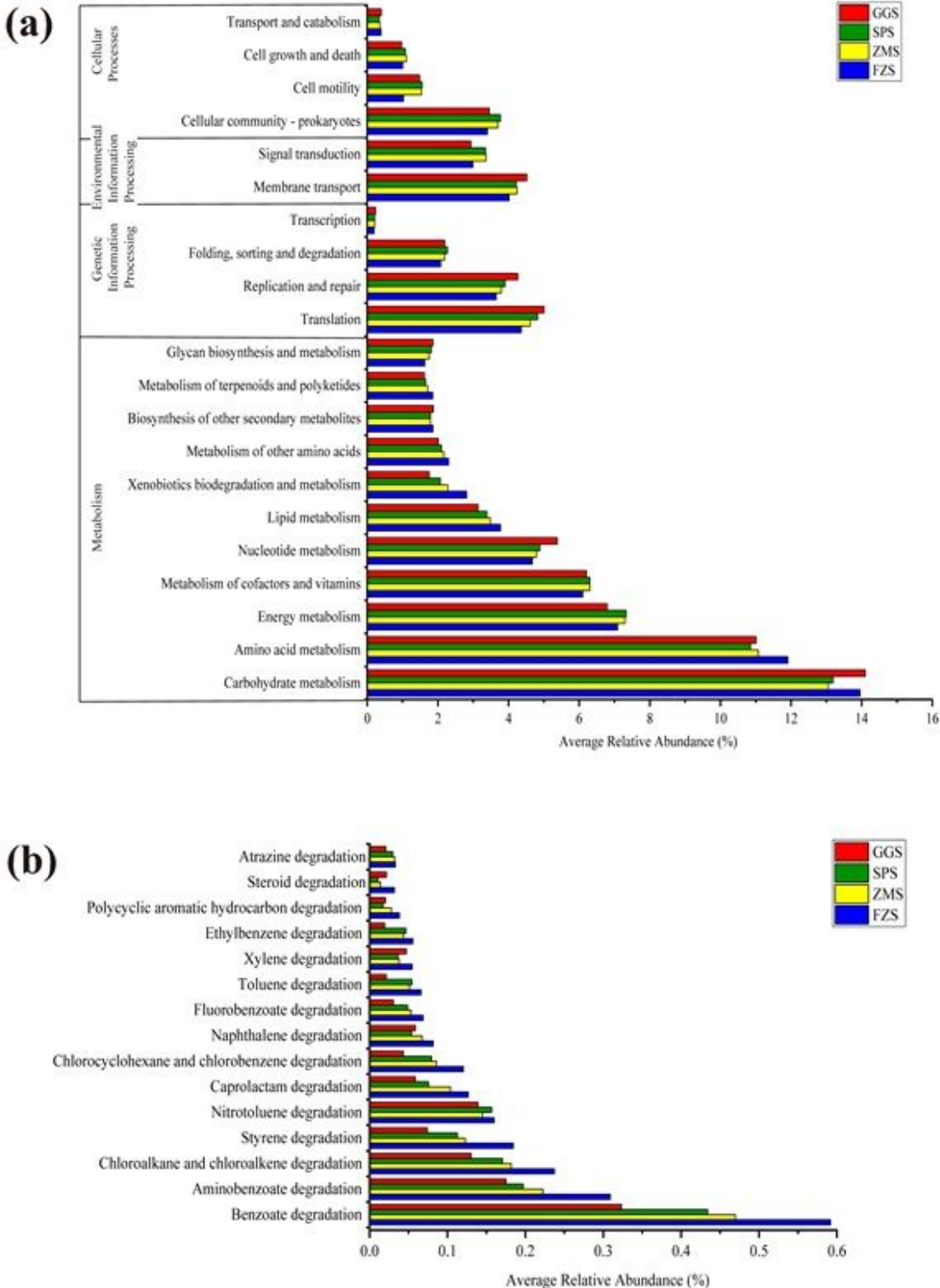


Figure 7

The comparison of bacterial community functions predicted by PICRUSt at level 2 and level 3 among different groups. (a) The predictive functions at level 2 is related to metabolism, genetic information processing, environmental information processing and cellular processes. (b) The predictive functions at level 3 is related to xenobiotics degradation and metabolism.

Supplementary Files

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