The Effect of G-C₃N₄ on Bacterial Community in Sediment of Xiang River Under Tetracycline Pressure

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Abstract

As photocatalysts applied more and more often to treat pollutants by photocatalytic reactions, they may enter the environment via water spreading. Although there are some investigations about their influence on different organisms, little is known about its impact on the ecological microenvironment. To understand how photocatalysts effect sediment ecological microenvironment in the process of pollution remediation, the impact of typical photocatalyst g-C₃N₄ (Graphitic carbon nitride) on riverbed sediment community polluted by typical antibiotic tetracycline (TC) was investigated. The sediment samples were exposed to different concentrations of TC, g-C₃N₄ or TC/g-C₃N₄ (exposed to 60, 120, 180 mg/L TC, or 25, 75, 125 mg/kg g-C₃N₄, or 25, 75, 125 mg/kg g-C₃N₄ plus 60, 120, 180 mg/L TC, respectively), and sediment bacterial community were analyzed by Illumina sequencing. The results indicated that the dominant bacterial phyla in the samples were *Acidobacteriota*, *Proteobacteria*, *Actinobacteriota*, *Chloroflexi*. The diversity and richness of microorganisms in riverbed sediment were increased a little bit by g-C₃N₄ with different concentrations, which reached the highest value when exposed to 75 mg/kg g-C₃N₄. g-C₃N₄ lightly increased the percentage of relative abundance of *Cyanobacteria*. The bacterial communities' structure of the samples treated with TC, g-C₃N₄ or TC/g-C₃N₄ were distinguishable. g-C₃N₄ alone had little effect on microbial structure, while TC/g-C₃N₄ had medium influence and TC had great impact on it. Under TC stress, g-C₃N₄ slowed down the growth of *Cyanobacteria* to some extent and restored the changes of bacterial community structure caused by TC, and reduced the residual TC in water body, thus eliminating the side effects of TC. The result shown that g-C₃N₄ could significantly reduce the residue of TC in riverbed sediment, without affecting the microbial ecology in the environment.

1. Introduction

Antibiotics are extensively used for human therapy, animal farming and for agricultural purposes (Martinez, 2009). Currently, antibiotics have been found at diverse residual concentrations in riverbed sediment, aquaculture, groundwater and so on (Archundia et al. 2017; Batt et al. 2006; Liu et al. 2018). In China, large quantities of various antibiotics are widely produced and used. Tetracycline (TC) is one of the typical antibiotics in livestock practice due to its high pharmacological activity and solubility in water (Chopra et al. 2001), and it is widely used for human therapy, animal husbandry, and aquaculture, which is ranked second worldwide and first in China for production and usage (Chopra et al. 2001). Significant concerns have been raised over the presence TCs in aquatic environments (Ji et al. 2009). TC had significant negative effects on soil microbial community function and obviously effects Shannon's diversity, evenness of bacteria (Kong et al. 2006). It can influence the microorganisms in the environment, inducing the emergence of dominant microbial populations and changing microbial community structure. It had been documented that under exposure of TC, the activity of nitrifying bacteria was prevented and an important *Nitrifier*, *Nitrospira*, was inhibited (Du et al. 2018; Shu et al. 2015), and *Bacteroidetes* and *Acidobacteria* were increased, while *Actinobacteria* and *Firmicutes* decreased under antibiotics exposure (Uddin et al. 2019). TC also residues would significantly inhibit sediment N and C cycling rates and reduce the abundance of functional microbial groups (He et al. 2021).

Since TC was poorly biodegradable and toxic for microorganisms (Yahiat et al. 2011), various techniques have been developed to removal it from water, such as physical adsorption (Hu et al. 2019; Wu et al. 2019b), biodegradation (Wei et al. 2019), chemical oxidation (Yang et al. 2019; Zhu et al. 2019a) and photocatalytic degradation (Ye et al. 2019; Zheng et al. 2019). Photocatalytic degradation was widely considered as potential method since it used light as energy and could mineralize TC. $\text{g-C}_3\text{N}_4$ was a commonly used photocatalyst as it had some advantages, such as energy conservation, sufficiently efficient, stable, inexpensive and capable of harvesting light (Wang et al. 2009). $\text{g-C}_3\text{N}_4$ was widely applied in wastewater treatment, due to its low cytotoxicity and photoactivity with visible light (Luo et al. 2017), inspiring researchers to use it for the remediation of TC pollution (Yan et al. 2020). Previous studies have shown that $\text{g-C}_3\text{N}_4$ displayed enhanced photocatalytic activity for TC degradation (Hong et al. 2016; Xue et al. 2015). Direct contact of $\text{g-C}_3\text{N}_4$ and bacterial cells was indispensable for the cell inactivation (Deng et al. 2017), and h^+ was demonstrated as the dominant reactive species which could make the bacteria cells inactivated (Li et al. 2017). $\text{g-C}_3\text{N}_4$ not only exhibited striking bactericidal but also showed high efficiency in breaking down and preventing formation of new biofilms in vitro (Wang et al. 2017).

Sediment microorganisms played an important role in the benthic food web and the biogeochemical cycle of water ecosystems as they changed with environmental conditions and reflected the pollutant status of water sediment via variations in their abundance, diversity, and structure (Yang et al. 2013). However, although there were many studies about the impact of TC on microorganism, there was still little information regarding $\text{g-C}_3\text{N}_4$ application in sediment, especially for TC pollution control and the potential ecotoxicity.

In this study, in order to investigate the potential ecotoxicity of $\text{g-C}_3\text{N}_4$ in the application of pollution remediation, TC was used as a typical environmental pollutant to explore the effect of $\text{g-C}_3\text{N}_4$ on bacterial community structure in riverbed sediment while it photodegraded TC. The outcomes from this study may provide important information on the feasibility of applying $\text{g-C}_3\text{N}_4$ photocatalysis for control of organism pollution.

2. Materials And Methods

2.1 Soil treatment

The riverbed sediment sampling for this study was conducted on July 2, 2020, and it was collected from the Xiang River (112°94'71"E, 28°14'23"N) in Changsha, Hunan, China. The soil was collected from a depth of 10–20 cm by five-point sampling method. After removing plant residues, the sediment sample kept in crushed dried ice boxes for transportation to the laboratory. The collected samples were homogenized and considered as fresh riverbed sediment (labelled as H), and divided into two parts for 16S rRNA sequencing and experimental design, respectively. The background physicochemical properties of the homogenized sediment samples, including pH, Total nitrogen (TN), Total phosphorus (TP), Total

potassium (TK), Soil organic matter (SOM), Ammonium nitrogen ($\text{NH}_4^+\text{-N}$), Nitrate nitrogen ($\text{NO}_3^-\text{-N}$) and TC concentration, were detected as illustrated in Table S1. The concentration of TC was detected by high performance liquid chromatography (HPLC), and these results showed that the residues of TC in the riverbed of the middle Xiang River was low (Table S1).

2.2 Target pollutant (TC) and photocatalyst ($\text{g-C}_3\text{N}_4$)

TC ($\text{C}_{22}\text{H}_{24}\text{N}_2\text{O}_8\cdot\text{HCl}$, USP grade) was purchased from Shanghai Aladdin Biochemical Technology (Shanghai, China). $\text{g-C}_3\text{N}_4$ was prepared by literature reports by direct polycondensation of melamine (Hou et al., 2020). After being loaded into a porcelain boat with a lid, and placed in a tube furnace, 5 g melamine was heated at 550 °C for 4 h under N_2 condition (rate: 5 °C/min), and then cooled naturally to room temperature. The obtained $\text{g-C}_3\text{N}_4$ was ground thoroughly into powder and stored at room temperature for further use.

2.3 Sediment exposure experiment

10000 mg/L TC solution was prepared by dissolving 1g of TC in 100 mL sterile water. 100 mg of H were paced into 500 mL flasks with 100 mL water. 10000 mg/L TC solution and a certain amount of $\text{g-C}_3\text{N}_4$ powder were added into the flasks to achieve the final exposure concentration of TC (60, 120, 180 mg/L), and the target exposure doses of $\text{g-C}_3\text{N}_4$ (25, 75, 125 mg/kg), and then stirred for 3 min for even mixture. The mixture containing 60, 120, 180 mg/L TC was marked as T_L , T_M , T_H , and containing 25, 75, 125 mg/kg $\text{g-C}_3\text{N}_4$ was labelled as P_L , P_M , P_H , respectively. The sediment without TC or $\text{g-C}_3\text{N}_4$ was utilized as the control. Each treatment was prepared for three replicated (CK, T_L , T_M , T_H , P_L , P_M , P_H , T_LP_L , T_LP_M , T_LP_H , T_MP_L , T_MP_M , T_MP_H , T_HP_L , T_HP_M , T_HP_H). The treatments samples were kept static in a homeothermic incubator at 30 °C $\pm$ 1 °C under 2000 lux illumination with a light dark period of 12:12 h. After 30 days, each set of treatment was respectively collected together and homogenized, 1–2 g of the homogenized sediment samples were stored at -80 °C for the analysis of bacterial community structure and diversity.

2.4 HPLC analysis for TC

The extraction method of TC in riverbed sediments was described in detail in the supplementary material. After filtering through 0.22 μm microporous membrane and centrifuging at 13000 $\text{r}\cdot\text{min}^{-1}$ for 10 min, 20 μL sediment precipitate was applied to detected TC concentration at 360 nm by high-performance liquid chromatography (HPLC). The HPLC system (Shimadzu corporation, Kyoto, Japan) was equipped with the Symmetry® C18 column (length $\times$ I.D.: 25 cm $\times$ 4.6 mm; 5 μm particles, Shimadzu corporation, Kyoto, Japan). Chromatographic conditions were as follows: liquid A, mobile phase 0.1% TFA ddH₂O, liquid B, 0.1% TFA ACN (acetonitrile). The flow rate was 1 $\text{mL}\cdot\text{min}^{-1}$, the equilibrium solution was 10% B solution, and the gradient rose to 30% B solution within 20 minutes.

2.5 DNA Extraction and Illumina MiSeq Sequencing

The treatments samples and H were used for the extraction of Microbial community genomic DNA using FastDNA Spin Kit (Omega Bio-tek, Norcross, GA, U.S.) for Soil according to manufacturer's instructions.

The DNA extract was checked on 1% agarose gel, and DNA concentration and purity were determined with NanoDrop 2000 UV-vis spectrophotometer (Thermo Scientific, Wilmington, USA). The hypervariable region V3-V4 of the bacterial 16S rRNA gene were amplified with primer pairs 338F (5'-ACCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTAAT-3') by an ABI GeneAmp 9700 PCR thermocycler (ABI, CA, USA). In this study, the high-throughput sequencing of all samples was completed by Shanghai Majorbio Bio-pharm Technology Co., Ltd.

2.6 Statistical analysis

The Shannon index and the number of observed OTUs of the bacterial community were calculated using Mothur software (version v.1.30.1, <http://www.mothur.org>). Principal coordinate analyses (PCoA) were used to compare the beta diversity of the bacterial communities between samples based on the Bray Curtis distance matrix in the R software package using the ape and vegan libraries. Redundancy analysis (RDA) was performed using the function rda of package vegan in R to test the significance of the relationship between TC or g-C₃N₄ and microbial community dynamics. Linear discriminant analysis (LDA) together with effect size measurements analysis (LEfSe) (<http://huttenhower.sph.harvard.edu>) was considered to recognizing of the biomarkers among groups.

3. Results And Discussion

3.1 Diversity of bacteria communities in riverbed sediment

In all studied 17 soil samples, 779,643 high-quality sequences were totally obtained from the V3-V4 region of 16S rRNA gene sequences clustered in 7,214 bacterial OTUs. The rarefaction curve of bacteria in the samples showed that when the number of reads reached about 3000, the Shannon indexes of all samples tended to be flat (Fig. 1a), and the curves tended to be flat, indicating that our sequencing depth was sufficient and can truly reflect the bacterial community in the sediment sample.

Understanding biological diversity is very important as it associate the function and stability of ecosystems (Yu et al. 2020). The bacterial diversity of the riverbed sediment associated with untreated and treated sediments (TC, g-C₃N₄ and TC/g-C₃N₄) analyzed to identify whether different treatments shaped the environmental microbiome (Fig. 1b). The treatment way had a little influence on the bacterial richness. The richness (Chao 1) of bacterial communities of H was 3901.70 lower than that of CK (4227.06), and lower than those of samples exposed to TC (4326.21–5068.42), g-C₃N₄ (4563.89–4939.65) and TC/g-C₃N₄ (4641.95–4962.17), respectively. However, the exposure of TC, g-C₃N₄ and TC/g-C₃N₄ almost not changed the diversity (Shannon index) of bacterial communities. Meanwhile, the Shannon in the samples containing TC/g-C₃N₄ remained comparatively stable in the range 6.81–6.92.

Based on Bray-Curtis distance, PCoA was applied to examine the beta diversity of the samples to study the differences of bacterial community structure among them (Fig. 1c). The first two axes (PCoA1 and PCoA2) explained 19.07% and 16.37% of the total variance in the sediment bacterial communities,

respectively. A clear separation was observed among three sample groups exposed to TC, g-C₃N₄ and TC/g-C₃N₄. The difference between H and the samples treated by TC/g-C₃N₄ was littler than that between H and the sediments treated by TC alone, but larger than that between H and the samples handled with g-C₃N₄ alone. It might conclude that major changes in bacterial diversities across the samples were attributed to the difference treatments, g-C₃N₄ alone had little effect on microbial structure, while TC/g-C₃N₄ had medium influence and TC had great impact on it.

Five-Way Venn diagrams were constructed to understand the unique and shared OTUs among different treatments in each riverbed sediment (Fig. 1d). There was a high specific OTUs number in each sample group and the shared OTUs (1,898) accounted for 26.31% of the total OTUs (7,214). The number of specific OTUs in TC/g-C₃N₄ treatment group (713) was highest, followed g-C₃N₄ treatment group (228) and TC treatment group (181), suggesting that the specific richness of bacterial communities in samples exposed to TC/g-C₃N₄ was the higher than those exposed to TC and g-C₃N₄. These indicated that TC/g-C₃N₄ might be capable of increasing the existence of unique species which precisely affected the bacterial communities in sediment, increasing the Chao 1 index as depicted in Fig. 1b.

The TC in soil posed a high risk for bacterial communities (Pan et al. 2016), according to other researchers studies, TC could be used as C sources by surviving bacteria, produce TC-resistant bacteria, increasing the bacterial diversity of soil (Grenni et al., 2018; Ma et al., 2020; Ullah et al. 2019). The TC in environment could significantly restrain the microbe in its resistance spectrum, thus the bacteria adapting to TC gradually replaced those not acclimated the environment with TC (Zhang et al. 2013).

Photocatalyst could produce some active groups, for example •O₂⁻ and •OH, which could act on microorganism and substances in environment, thus changed the diversity of bacteria. Hou et al. (2020) observed that g-C₃N₄ had good biocompatibility in the environment and had a positive effect on the sediment environment. Therefore, g-C₃N₄ could increase the richness of bacterial communities in the sediment, which was similar to the effect caused by other photocatalyst, such as TiO₂ (Li et al. 2020). Up to now, a large number of studies on g-C₃N₄ composite photocatalyst inactivation of pathogenic bacteria, such as *Staphylococcus aureus*, *Salmonella typhimurium*, *Escherichia coli* (Heo et al. 2019; Tang et al. 2019).

Once external substances were added into sediment, there would be some corresponding changes in the environment, in order to cope with the varieties, the genetic characteristics, physiological and biochemical processes of some soil microorganisms will be changed. Thereby, some microbe was inhibited in sediment, while others in the environment may be stimulate to growth, which enhanced the generation and evolution of the microbes that had adapted to the conditions, corresponding promoted the number of them, and increasing the microbial diversity. At the same time, nutrient composition of sediment was changed by adding external material to the sediment, the structure of the bacterial community would be changed accordingly, thus varying the beta diversity. However, the changes in diversity induced by g-C₃N₄ was basically stable and similar to those induced by other photocatalysts, including biochar (Wu et al.

2019a), TiO_2 (Li et al. 2020) implying that it had little influence on species diversity and might be safer for environment.

3.2 Composition of bacterial communities determined by Illumina MiSeq

According to the annotation and abundance of OTU, a relative abundance table for phyla was obtained (Table S2). The number of bacteria phyla hosted in H and CK was 36 and 40, respectively. In T_L , T_M , T_H , there was 41, 43, 43 bacterial phyla, respectively; In P_L , P_M , P_H , there was 45, 43 and 44 bacterial phyla, respectively; And in T_LP_L , T_LP_M , T_LP_H , T_MP_L , T_MP_M , T_MP_H , T_HP_L , T_HP_M , T_HP_H hosted 42, 44, 43, 42, 44, 43, 42, 44, 43 bacterial phyla, respectively. The number of bacteria phyla in different treatment groups was similar, and in the same treatment group, the number was basically unchanged, implying the composition of bacteria phyla might not be effected obviously in the experiments.

The distribution of bacteria phyla in sediments handled by different ways were demonstrated in Table S3. The dominant phyla in all samples were *Acidobacteriota*, *Proteobacteria*, *Actinobacteriota*, *Chloroflexi*, and their share was about 70%. Uddin et al. (2021) found that the above four phyla were account for about 60% of soil bacteria by analyzing the effect of four different antibiotics on paddy soils. Though the influence of $\text{g-C}_3\text{N}_4$ on sediment had rarely examined till now, the four dominant phyla in our research were agree with their studies. According to other relevant reports, these four bacteria phyla accounted for a large proportion in water and soil (Chopra et al. 2001). and they were also the dominant phylum in sediment, although the abundance of each phyla was various in different sediment (Welz et al. 2018). Due to the high proportion of the advantage bacterium group in the sediment, they had an important effect on maintaining the stability of community structure in different treatments.

The effect of TC on bacterial community structure in sediment was illustrated in Fig. 2a and Table S3. Compared to CK, the abundance of some microorganism were differ from the samples treated by TC, especially the dominant bacteria, such as *Actinobacteriota*, *Acidobacteriota* and *Firmicutes*, that is TC had a greater impact ($p < 0.05$) on them. However, between different concentrations of TC, the changes of relative bacteria abundance at phyla level was insignificant ($p > 0.05$) between different concentrations of TC. The average abundance of *Actinobacteriota*, *Acidobacteriota* and *Firmicutes* was significantly decreased in TC treatment ($p < 0.001$), and its abundance was 17.33%, 14.14% and 6.04%, respectively, however, in CK, the relative abundance was 25.05%, 16.07% and 8.68%, respectively, so these three phyla were regarded to be susceptible to TC application. The results were consistent with other reports, Alexandrino et al. (2017) also found that *Actinobacteriota* and *Firmicutes* were sensitive to TC.

The influence of $\text{g-C}_3\text{N}_4$ on bacterial community structure in sediment was demonstrated in Fig. 2b and Table S3. Compared with the CK, the presence of $\text{g-C}_3\text{N}_4$ significantly inhibited ($p < 0.001$) the growth of *Firmicutes* and *Actinobacteriota* in riverbed sediment. But the growth of *Acidobacteriota* (20.89%) was significantly improved ($p < 0.001$) by $\text{g-C}_3\text{N}_4$, and the average abundance was increased about 4.82% in comparison of CK. In the P_L treatment group, the biomass of *Cyanobacteria* (0.88%) was significantly

lower ($p < 0.001$) than that of CK (3.50%), this was indicated that $\text{g-C}_3\text{N}_4$ might inhibited the growth of *Cyanobacteria*. Based on the above results, it could be concluded that *Firmicutes*, *Actinobacteriota*, *Acidobacteriota* and *Cyanobacteria* were susceptible to $\text{g-C}_3\text{N}_4$ exposure. *Cyanobacteria* was photoautotrophic organisms in aquatic ecosystems and ubiquitous in riverbed sediment, as well as a typical microorganism in the identification and detection of environmental pollution (Teta et al., 2019). *Acidobacteria* had the potential to degrade polymeric carbonaceous complexes and actively participate in the cycling of organic matter, and it played an important role in maintaining the structural stability of microorganism in the environment (Kalam et al. 2020). *Cyanobacteria* proliferate in large quantities, which could affect the microbial structure in the environment (Chen et al. 2020). Our results showed that $\text{g-C}_3\text{N}_4$ could promoted *Acidobacteriota*, inhibited *Cyanobacteria*, and was beneficial to environmental pollution remediation. However, The growth of *Cyanobacteria* was effected by $\text{g-C}_3\text{N}_4$ concentration, its abundance of P_H treatment (6.34%) was higher than that of P_L treatment (0.88%), it might due to the automatically agglomerate of $\text{g-C}_3\text{N}_4$ at high concentration, inactivating its function.

The impact of TC/ $\text{g-C}_3\text{N}_4$ on bacterial community structure in sediment was illustrated in Fig. 2c and Table S3. Compared with *Actinobacteriota* (25.05%) and *Firmicutes* (8.68%) in the CK treatment group, the average abundance of *Actinobacteriota* (16.48%) and *Firmicutes* (4.97%) was significantly decreased ($p < 0.001$) in TC/ $\text{g-C}_3\text{N}_4$ treatment. But, compared with the CK treatment groups (16.07%), the average abundance of *Acidobacteriota* increased significantly ($p < 0.05$) in TC/ $\text{g-C}_3\text{N}_4$ treatment groups (20.71%). When the same does of $\text{g-C}_3\text{N}_4$ was added, the biomass of *Cyanobacteria* treated with low concentration of TC ($\text{T}_\text{L}\text{P}_\text{L} = 4.96\%$, $\text{T}_\text{L}\text{P}_\text{M} = 4.14\%$, $\text{T}_\text{L}\text{P}_\text{H} = 3.81\%$) was higher than that treated with high concentration of TC ($\text{T}_\text{H}\text{P}_\text{L} = 3.71\%$, $\text{T}_\text{H}\text{P}_\text{M} = 2.76\%$, $\text{T}_\text{H}\text{P}_\text{H} = 2.16\%$). Meanwhile, the biomass of *Chloroflexi* (11.79% – 13.73%) and *Gemmatimonadota* (3.23% – 5.32%), which were the dominant microorganism in the sample, could be maintained in a stable range with the addition of both TC and $\text{g-C}_3\text{N}_4$, and the biomass of them were similar to that of H group, respectively. *Acidobacteria* was very important for the environment to keep the structural stability of microorganism as it potentially involved in the degradation of polymeric carbonaceous complexes and the cycling of organic matte (Kalam et al. 2020). Therefore, it was regarded as a kind of potential microorganism for environmental bioremediation and biotechnological applications (Kielak et al. 2017). Our study indicated that $\text{g-C}_3\text{N}_4$ could significantly slow down the growth of *Cyanobacteria* and promote the growth of *Acidobacteriota* in the environment under TC pressure, thus it might beneficial for sediment to remediate TC pollution and haven't adverse effect on environment.

3.4 Taxonomy-based comparisons of microbiota groups

To identify the biomarkers in sediment samples, the microbial communities in different treatments was compared based on taxonomy, and the results were showed in (Fig. 3), in which LEfSe was applied to determine each group that was revealed in cladograms and histogram of LDA scores of 2.5 or more. The larger the score of LDA was, the more remarkable the difference caused by species abundance was. As depicted in the figure, there were obvious changes in dominant bacteria in sediment treated with TC, g-

C₃N₄ and TC/g-C₃N₄ at the level of phylum, class, order, family and genus level. In the sediment treated with TC, more than 20 microbes including *Actinobacteriota*, *Desulfuromonadia*, *Myxococcia* were significantly enriched ($p < 0.05$). Similarly, 8 groups of bacteria including *Brocadia*, *Entotheonellia*, *Dependentiae* were obviously enriched ($p < 0.05$) in riverbed sediment treated with g-C₃N₄. However, in the sediment treated with TC/g-C₃N₄, only 4 microbes were significantly enriched ($p < 0.05$), such as *Babeliae*, *Subgroup_25* and c_unclassified_p__*Actinobacteriota*. These indicated that the selected biomarkers could clearly distinguish ($p < 0.05$) these three treatments and the difference caused by TC was the most, orderly followed by g-C₃N₄ and TC/g-C₃N₄. TC mainly affected the bacteria with high abundance, while g-C₃N₄, TC/g-C₃N₄ mainly influenced the low abundance ones. The results suggested that g-C₃N₄ might mitigate the side effect caused by TC on sediment bacteria by reducing the difference of microbes in the environment.

3.5 TC residue and Changes in species at genus level caused by TC and g-C₃N₄

In order to study the effect of TC residue on bacteria community structure, we analyzed the TC concentration in sediment with different treatment, as well as the relative dominant bacterial genus (Fig. 4). As illustrated by Fig. 4a, there were no TC residues were detected out in CK, P_L and P_H, suggesting that the original TC residue (0.22 mg/kg) came from H were degraded in these samples. In T_H, T_HP_L and T_HP_H, the concentration of TC s was 22.68, 14.42 and 7.83 mg/kg respectively. Most of the TC added into the sediment samples were degraded by riverbed sediment, since there were only 22.68 mg/kg were existed in T_H after 30 days. The TC concentration in T_HP_L was 8.26 mg/kg lower than that in T_H, and higher than that in T_HP_H, indicating that in samples, g-C₃N₄ was also able to degrade TC and the degradation ability was improved by its concentration. g-C₃N₄ could effectively degrade TC in the water environment by some active groups, including •O₂⁻ and •OH. (Guo et al. 2017; Zhu et al. 2019b). According reports, under the irradiation of visible light, the g-C₃N₄ could degrade about 40–90% TC in 30–120 min in water (Guo et al. 2019; Panneri et al. 2017), which was much higher than that in our experiment. This phenomenon might attribute to two facts: Firstly, the light intensity under our experimental condition was weak, so only a small number of photon were available for the degradation. Secondly, the composition of the sediment system was complex, which effected the light transmittance and further effected the photocatalytic activity of g-C₃N₄.

Figure 4b and Table S4 demonstrated the dominant bacterial genus in different treated sediment. Compared with CK group, under the pressure of TC, the abundance of *Bacillus* (*Firmicutes*) decreased by 3.27%, while the biomass of RB41 (*Acidobacteriota*) only increased by 0.57%. The biomass of *Bacillus* (*Firmicutes*) in P_L (2.21%) and P_H (2.47%) was lower than that of CK (5.06%), however, the biomass of RB41 (*Acidobacteriota*) in P_L (2.91%) and P_H (2.00%) was higher than that of CK (0.89%). Compared with the T_H group, the biomass of RB41 in T_HP_L and T_HP_H group was significantly increased ($p < 0.001$) to 3.22% and 2.77%, respectively, and the abundance of *Bacillus* in T_HP_L and T_HP_H group was increased.

Under TC exposure, g-C₃N₄ was beneficial to enrichment of norank_f__*Vicinamibacteraceae* and norank_f__*Gemmatimonadaceae*, belonging to the bacteria phyla of *Acidobacteriota* and *Gemmatimonadota*, respectively

Previous research had showed that RB41 was actively participated in the carbon cycle in sediment (Ito et al. 2019), RB41 was the dominant and sensitive microorganism in contaminated soil and played a positive role in environmental ecosystem (Ai et al. 2018; Shen et al. 2018). Based on the fact that its abundance increased in the treatment of TC and g-C₃N₄, it should be sensitive to them, and more to g-C₃N₄. *Bacillus* could fully degrade organic matter and eutrophic substances in sewage (Shen et al. 2020), at the same time, *Bacillus* was also a kind of beneficial bacteria that was beneficial to soil microecological stability. It could inhibit or kill pathogenic bacteria in the environment (Nicholson et al. 2002). It had been reported that antibiotics induced oxidative stress and inhibited the growth of *Bacillus* cells (Sannasimuthu et al. 2020). Based on the fact that compared to CK, the degree of decline in the content of *Bacillus* in T_H was greater than that of samples contained g-C₃N₄, it was suggested that TC had obvious toxic effect on *Bacillus*, while g-C₃N₄ could reduce the toxic effect of TC on *Bacillus*. Hence, one inference was that the g-C₃N₄ might reduce the effect of TC on *Bacillus* in sediment.

In our experiment, we founded that the addition of g-C₃N₄ would reduce TC residue in sediment, and this trend was strengthened with the increase of the concentration of g-C₃N₄ (Fig. 4a). These results indicated that 1) g-C₃N₄ could degrade some organic substances, including TC, and changed the nutrient composition of sediment, thus affecting the structure of microorganisms; 2) g-C₃N₄ could act on some microorganisms, change the growth of sensitive bacteria, thus affecting their ecological niche in the sediment. Based on the effect of g-C₃N₄ on dominant bacteria RB41 and *Bacillus*, it was indicated that g-C₃N₄ could improve the ability of sediment remediation and reduce the toxic effect of TC on beneficial bacteria, which was beneficial to the ecological health of sediment.

3.6 Redundancy analysis (RDA) of bacterial community at the phylum level and class level

The relationships between TC, g-C₃N₄ and bacterial community was analyzed based on RDA score plot and depicted in Fig. 5. The impacts of each factor (TC or g-C₃N₄) on bacterial community were represented by the length of arrows, and the cosine angle between arrows illustrated their relationship (smaller angle indicated more significant correlation). As shown in Fig. 5a, at the phylum level, axis 1 and 2 of the RDA plots explained up to 19.37% and 3.45%, respectively. Specifically, TC positively affected ($p < 0.05$) *Chloroflexi*, *Proteobacteria*, *Myxococcota*, *Gemmatimonadetes*, *Actinobacteria* and *Firmicutes* in riverbed sediment. *Cyanobacteria* had no significant correlation ($P < 0.05$) with TC. Previous research had shown that *Firmicutes* and *Bacteroidetes* were significantly positively correlated to total antibiotics, while *Acidobacteria* was significantly negatively correlated to them (Gao et al. 2020). Studies had indicated that *Proteobacteria* and *Actinobacteria* were dominated community of the TC resistant members (Pala-Ozkok et al. 2019), and *Proteobacteria* taken a great part in pollutant degradations and environmental

remediation (Xu et al. 2020). The concentration of g-C₃N₄ in riverbed sediment was negatively correlated ($P < 0.05$) with *Actinobacteria*, *Myxococcota*, *Firmicutes* and *Gemmatimonadetes* and positively correlated ($p < 0.01$) with *Acidobacteriota*, *Proteobacteria*, *Cyanobacteria* and *Chloroflexi*.

The RDA analysis for the relationship between TC or g-C₃N₄ and bacteria at genus level was illustrated in Fig. 5b, the first axis explained 17.73% of the total variance, whereas the second axis accounted for 4.48% of the total variance. It is noteworthy that TC has significant correlation ($p < 0.05$) with *Gaiella*, *Nocardioides* and *Bacillus*. Previous studies had also shown that *Gaiella* at genus level were significantly positively correlated to the total antibiotics (Gao et al. 2020). *Nocardioides* were crucial for bioremediation, notably, dehalogenation and denitration (Ito et al. 2019). However, g-C₃N₄ exhibited negative correlations ($p < 0.05$) with *Gaiella*, *Nocardioides* and *Bacillus*. *Gaiella* was the sensitive genera that was negatively correlated to antibiotics perturbation (Uddin et al. 2019), g-C₃N₄ had significant positive correlation ($p < 0.05$) with RB41, which was positively correlated with soil carbon content and actively participated in the carbon cycle in soil environment (Ito et al. 2019). g-C₃N₄ had significant positive correlation ($p < 0.05$) with RB41, which was positively correlated with soil carbon content and actively participated in the carbon cycle in soil environment.

Using Spearman correlation analysis, the relationship between the top 30 bacterial genus and TC or g-C₃N₄ were examined to further investigate the influence of TC or g-C₃N₄ on bacteria community (Fig. 2s). According to the heatmap, TC and g-C₃N₄ significantly correlated with the major phyla and genus. *Acidobacteriota* was the main phyla positively affected by g-C₃N₄ ($p < 0.01$), *Chloroflexi* was the main phyla affected by TC ($p < 0.05$). RB41 ($p < 0.05$) and *Ellin6067* ($p < 0.001$) were the main genus significantly associated with g-C₃N₄. These results were consistent with the results obtained with the RDA.

Conclusion

To our knowledge, this was the first-time research on the potential ecological toxicity of typical photocatalyst g-C₃N₄ by analyzing the change of sediment microbial community under TC exposures. The bacterial community and TC residue in the sediment under different conditions were examined. According to the results, compared with CK, TC, g-C₃N₄ and TC/g-C₃N₄ treatments could increase microbial richness in the riverbed sediment, but kept microbial diversity basically stable. Beta diversity analysis showed that TC had greater influence on bacterial community structure, while g-C₃N₄ and TC/g-C₃N₄ had less influence on it. These indicated that g-C₃N₄ had less effect on species diversity and might be safer for environment. TC could inhibit the growth of *Actinobacteriota*, *Acidobacteriota* and *Firmicutes* in the riverbed sediment. g-C₃N₄ could significantly slow down the growth of *Cyanobacteria* and promote the growth of *Acidobacteriota* in the environment under TC exposure, thus it might be beneficial for sediment to remediate TC pollution and reduce its adverse effect on environment. The LEfSe analysis showed that there were 20 classes, 8 classes and 4 classes of biomarkers in the sediments treated by TC, g-C₃N₄ and TC/g-C₃N₄, respectively, g-C₃N₄ might mitigate the side effect caused by TC on sediment bacteria by

reducing the difference of microbes in the environment. Meanwhile, in sediment g-C₃N₄ could decrease TC residues and promoted the mass reproduction of RB41 as well as inhibited the toxic effect of TC on *Bacillus*. So, g-C₃N₄ would not adversely affect the ecological function of the reversed sediment, and was an environmentally friendly photocatalyst. In conclusion, g-C₃N₄ might be expected to be used for TC pollution remediation in the environment.

Declarations

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Data Availability

Data are available by contacting Wu Yaohui (Wyh752100@163.com) and Hu Xuemei (1778897206@qq.com)

Consent to publish: All authors have approved the paper and agree with its publication.

Author Contributions:

Xuemei Hu: Conceptualization, Methodology, Data curation, Writing- Original raft.

Kuan Peng: Investigation, Methodology.

Yijun Chen: Methodology, data curation.

Shuguang Liu: Validation, Writing- Reviewing and Editing, Supervision.

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Xiaoyong Chen: Writing- Reviewing and Editing, Supervision.

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Compliance with ethical standards

Conflict of interest: The authors declare no competing interests.

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Figures

Figure 1

Diversity and richness of the bacteria communities (a) Bacteria relative abundance of OTUs identified in the 17 soil samples, H, CK refer to 0 day of sediment, 30 days of sediment, respectively; TL, TM, TH refer to the system contain 60, 120, 180 mg/L TC, respectively; PL, PM, PH refer to the system contain 25, 75, 125 mg/kg g-C₃N₄, respectively. (b) Shannon diversity index and Chao 1 of bacteria, (c) Bacterial communities structure analysis by principal coordinates (PCoA), (d) Taxonomic diversity of bacterial

communities , venn diagrams showed the shared and specific OTUs of bacterial, H, CK, T, P and TP, refer to 0 day of sediment, 30 days of sediment, sediment treated with different concentrations of TC at 30 days, sediment treated with different concentrations of g-C3N4 at 30 days and sediment treated with different amounts of TC/g-C3N4 at 30 days, respectively.

Figure 2

Bacterial communities structure analysis by principal coordinates at the phyla level, only top (relative bacterial abundance > 1.5%) bacterial taxa are shown. (a) Microbial community structure of sediment exposed to g-C3N4 at 30 days, (b) Microbial community structure of sediment exposed to TC at 30 days, (c) Microbial community structure of sediment treated with different amounts of TC/g-C3N4 at 30 days. TL, TM, TH refer to the system contain 60, 120, 180 mg/L TC, PL, PM, PH refer to the system contain 25, 75, 125 mg/kg g-C3N4, respectively.

Figure 3

Structures of soil microbiota (a) Cladogram demonstrate the relationships among microbiota taxonomic units from the bacteria level of phylum down to the level of genus, the color of green (T), red (P), and black (TP) refers to sediment treated with different concentrations of TC at 30 days, sediment treated with different concentrations of g-C3N4 at 30 days and sediment treated with different amounts of TC/g-C3N4 at 30 days, respectively. (b) Histogram of LDA scores of taxonomic units, The taxa with the absolute LDA scores > 2.5.

Figure 4

Changes of community structure during the treatment of TC with g-C₃N₄. (a) The residual condition of TC in samples with different treatments. CK refer to the 30 days of sediment; TH refer to the sediment system contain 180 mg/L TC; PL, PH refer to the sediment system contain 25, 125 mg/kg g-C₃N₄, respectively. (b) Community abundance percentage of typical samples at genus leve.

Figure 5

Redundant analysis (RDA) of TC and g-C₃N₄ with different concentrations in sediment and bacterial community structure in sediment. Arrows indicate the direction and magnitude of environmental factors associated with bacterial community structure. (a) RDA of eight highest relative abundance of bacteria at phylum level. (b) RDA of eight highest relative abundance of bacteria at genus level. H, CK, T, P and TP, refer to 0 day of sediment, 30 days of sediment, sediment treated with different concentrations of TC at 30 days, sediment treated with different concentrations of g-C₃N₄ at 30 days and sediment treated with different amounts of TC/g-C₃N₄ at 30 days, respectively.

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