Ecological risk assessment of glyphosate and its possible effect on bacterial community in surface sediments of a typical shallow Lake, northern China

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ABSTRACT

Glyphosate is a widely used herbicide worldwide and its prevalent presence in aquatic ecosystems poses a threat to living organisms. This study evaluated potential ecological risk of glyphosate to sediment-dwelling organisms and assessed the probable effect of glyphosate on structure and predicated function of sediment-attached bacterial communities from a large shallow lake in northern China based on 16S rRNA high-throughput sequencing. Results suggested that glyphosate showed a medium to high concentration (up to 8.63 mg/kg) and chronic risk to sediment-dwelling organisms (10% samples exhibiting medium to high risk quotient), especially in sites nearby farmland and residential areas in August. Bacterial community identification based on 16S rRNA sequence indicated some species of dominant phylum Proteobacteria and Campilobacterota (e.g., Steroidobacteraceae, *Thiobacillus,* Gallionellaceae, Sulfurimonadaceae) were stimulated while some species of dominant phylum Actinobacteriota, Acidobacteriota and Firmicutes (e.g., Nocardioidaceae, Microtrichales, Vicinamibacteraceae, *Paenisporosarcina*) were inhibited by glyphosate accumulation. The stimulating species were related to sulfur-oxidizing, sulfate-, iron-, or nitrate-reducing bacteria; The inhibiting species were related to plant bacterial endophytes, polyphosphate-accumulating organisms (PAOs) and denitrifers. Correspondingly, promoted bacterial metabolic functions of "sulfite respiration", "nitrogen respiration", "aromatic compound degradation" and "nitrification" but suppressed "cellulolysis", "manganese oxidation", "anoxygenic photoautotrophy S oxidizing" and "nitrate denitrification" were predicated on functional annotation of prokaryotic taxa. Although these results could only partly suggest the impacts of glyphosate on the bacterial communities due to the lack of actual results from control experiments, the identified Steroidobacteraceae could be thought as a bioindicator in the future mechanism study for the ecological effect and bioremediation of glyphosate. This work intends to arise the concern about the depletion of biodiversity and bacterial metabolic functions with contribution of glyphosate in part in eutrophic lakes.

1. Introduction

Since its commercial introduction in 1974, glyphosate ($C_3H_8NO_5P$) has become the dominant herbicide worldwide with a broad-spectrum activity that was introduced for weed control in agricultural production fields (Duke and Powles, 2008). Even though once considered as environmentally safe and more closely approximates to a perfect herbicide than any other (Duke and Powles, 2008), the large scale and intensive use of glyphosate and its accumulation in the environment and edible products have resulted in increasing concerns in recent years about harmful side effects of glyphosate for soil and water quality, diversity and stability of soil, plants and animal guts, and plant, animal, and human health (Bruggen et al., 2018). Widenfalk et al. (2008) highlighted that freshwater sediment microbial communities could be affected by glyphosate (i.e., 0.15 mg/kg). However, the effects were complicated regarding to different environmental background and

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inconsistency microbial responses at various levels of community organization (Widenfalk et al., 2008). Perhaps detectable or clear shifts instead of unsensitive or insignificant responses came out when key microbial taxa under certain environmental conditions (including a specific range of glyphosate concentration) were identified (Zabaloy et al., 2017). The effects of glyphosate on the aquatic sediment microflora are critical because many microbial functions are important to nutrient cycling, pollutant degradation, environmental quality, and ecosystem sustainability (Vera et al., 2012).

Brovini et al. (2021) reviewed glyphosate concentrations in freshwater ecosystems worldwide based on 73 articles from 21 countries. They reported that most countries (90%) did not have restrictive legislation for aquatic glyphosate concentrations and a moderate to high risk to aquatic organisms in 95% of the countries investigated. Glyphosate has been detected in surface waters and sediments (the main sink for glyphosate in water bodies) in recent years due to land run-off (Stachowski-Haberkorn et al., 2008). Several studies have documented adverse effects on non-targeted aquatic organisms (Gandhi et al., 2021). Although *Limnoperna fortunei* could decrease the level of glyphosate but the depletion of glyphosate enhanced the production of $P-PO_4^{3-}$ and $N-NH_4^+$, which may lead to eutrophication in natural water ecosystem (Gattás et al., 2020). Vera et al. (2010) found that diatoms were far more sensitive to glyphosate in periphyton populations than cyanobacteria, and subsequently, a change in the diversity of these populations resulted over a period of time. García et al. (2022) found monoisopropylamine salt of glyphosate (GIPA) decreased the abundance of a phycocyanin-rich (PC-rich) picocyanobacteria (Pcy) cytometric population and GIPA further altered Pcy composition, despite no apparent effect of GIPA was detected on this community structure. Saxton et al. (2011) identified that glyphosate caused an increase in the abundance of *Planktothrix* spp. and a decrease in *Microcystis* spp. abundance in different lake and bay water, demonstrating positive influences on phytoplankton able to tolerate and use glyphosate as a nutrient source while negative influences on even killing phytoplankton those less tolerant. Interestingly, Pizarro et al. (2016) compared the modification of glyphosate on phytoplankton and bacterioplankton community structure in clear and turbid freshwater systems and discovered that glyphosate treatment induced a trend toward higher bacteria and picoeukaryotes abundances in clear waters, while decreased bacteria abundance but increased the abundance of picocyanobacteria in turbid waters. This finding revealed that the glyphosate impact was different in oligo and eutrophic conditions. Beside the increase in the abundance of picocynobacteria, researches showed that glyphosate-containing herbicides prevented photosynthesis in freshwater cyanobacteria and presented a safety danger for populations of tiny aquatic species rotifers, invertebrate freshwater mussels, and carp fish, etc. (Szarek et al., 2000; Pérez et al., 2007; Bringolf et al., 2007; Guilherme et al., 2009; Vera et al., 2012). Consequently, harmful impacts on aquatic and terrestrial food chains and responses of local-scale carbon and nutrient cycling to increasing glyphosate contamination combined with the aggravated eutrophication level need to be further addressed.

With a total area of 366 km^2 and an average annual water storage volume of 1.32 billion m^3 , Baiyangdian Lake is known as the "Pearl of northern China" and the largest freshwater lake in Hebei province, acting as one of the most important water tank and supplying ecosystem services and biodiversity for northern China. Since Song dynasty (960–1279), the reclamation of Baiyangdian Lake had begun to build farmland and village, and immigrant population by the lake for border defense. Large water area had been reclaimed and diked to fragmented flat croplands, rice fields, livestock farms, residential area, duck/goose/fish/shrimp/crab/lotus/reed ponds and so on. In the 1950 s, Baiyangdian Lake had an area of 567 km^2, and the residents mainly depended on handmade reed mat and aquatic products for their livelihoods (Shu, 2009). However, with the increasing population (from 190,000 in 1983–430,000 in 2009), reclamation and land uses (lake area shrunk from 567 km^2 in the 1950 s to 366 km^2 at present), and the decreasing

water storage and deteriorating water quality (bearing water use and wastewater discharge of 36 water villages within the lake, 62 lakeside villages, and other surrounding cities and townships) after 1960, especially since the 1980 s, environmental problems have become more and more serious in Baiyangdian Lake, such as eutrophication, terrestrialization, contamination and biodiversity decline (Chen et al., 2008; Xie et al., 2010; Hu et al., 2010). It would be a model freshwater lake ecosystem to assess the potential ecological risk and detect the glyphosate effect on bacterial community composition and probable responses of predicted bacterial metabolic functions based on functional annotation of prokaryotic taxa to increasing glyphosate contamination in combination with eutrophication.

Therefore, the aim of the present study was (1) to reveal the occurrence and spatial and temporal variation of glyphosate concentration in sediment of Baiyangdian Lake; (2) to evaluate the accumulation level and potential ecological risk of glyphosate to aquatic environment; (3) to detect the possible effect of glyphosate accumulation on bacterial community composition and predicted function; (4) to identify relevant key microbial performance reflecting the detectable effects of glyphosate on Baiyangdian Lake ecosystem. The results of this study may provide initially direct evidence from field for the effects of glyphosate accumulation on sediment-attached bacterial community succession and support bases for the future mechanism discovery.

2. Materials and methods

2.1. Sample collection

Sediments samples with triplicates were collected during the Sept.30th ~ Oct.6th in 2020, from fifty-six sites within the Baiyangdian Lake (38°43′–39°02′N, 115°38′–116°07′E) (Fig. 1). Since the glyphosate concentration in the lake sediments had not been surveyed before, the sampling sites were selected randomly in each water areas with different aquatic plants (e.g., *Phragmites australis, Typha angustifolia, Nelumbo nucifera, Potamogeton crispus, Ceratophyllum demersum, and Chara sp.*), water depths (1 ~ 6 m), distances from villages and environmental background based on a random spatial sampling method. In order to monitor the glyphosate changes during growing season, eight typical sites (No. 3, 19, 25, 33, 41, 45, 47, 55) with densely distributed emergent (*Phragmites australis*) and submergent (*Potamogeton crispus*) macrophytes were chosen to collect sediment samples with respective triplicates in two plant communities in April 18th and August 18th of 2021(Figs. 2d and 2e). In order to discover the spatial variation of glyphosate concentration along the water flow from the downtown nearby site (No.48) to the eastern water-gate (site 32), sediment samples with triplicates were collected from twenty-nine sites on November 26th of 2021(Fig. 2f).

2.2. Glyphosate determination

For glyphosate determination, 15 g air-dried sediment sample from each site (mixture of triplicates) was firstly extracted by 0.1 M KH_2PO_4 and filtrated through filter paper (0.45 mm cellulose acetate membrane). The derivatization was treated as follows: 0.25 ml of 5% borate buffer and 0.30 ml of FMOC-Cl (1.0 mg/ml) were added to 1 ml of sample (extract of sediment sample). After 4 h of reaction at room temperature, solution was kept in fridge until analyzed. The derivatized product (Gly-FMOC) was analyzed by HPLC (Thermo Fisher Scientific, System UltiMate 3000, detector 206 UV). The chromatographic column was AR- C18 (5 μm particle size, L × I.D. 250 mm × 4.6 mm), and the column temperature was 35 °C. The flow rate was 1.0 ml/min and the mobile phase was time-programmed using acetonitrile (A) and 0.2% v/v phosphoric acid aqueous solution (B). The percentage of organic mobile phase (A) was changed linearly as follows: 0 min, 35%; 8 min, 95%; 15 min, 35%; 20 min, 35%. The injection volume was 20 μl, using UV detection at 206 nm. The method was adapted and optimized based on
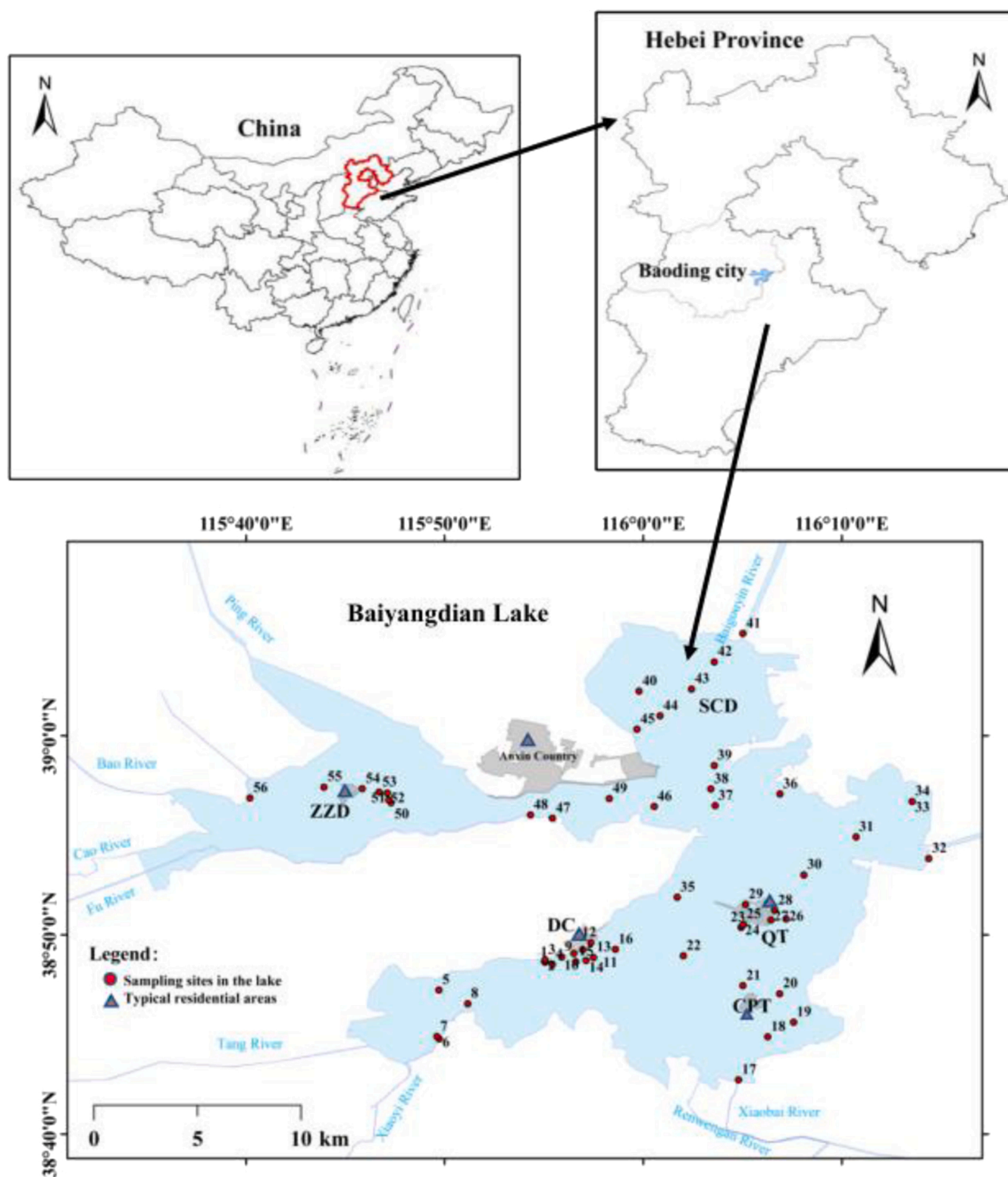
Fig. 1. Study area and sampling sites in Baiyangdian Lake (CPT, DC, QT, SCD and ZZD are abbreviation of the names of the sampled village or lake area. CPT, Caiputai lake area; DC, Duancun village; QT, Quantou village; SCD, Shaochedian lake area; ZZD, Zaozhadian lake area).

Peruzzo et al. (2008). The limits of detection (LOD) and recovery rates in the glyphosate testing were 0.001 mg/kg and 80~90%. Glyphosate determination was repeated for three times. For all samples, three readings were almost the same (the first two or three digits of the response values were the same) and the medium value among three readings was adopted.

2.3. Ecological risk assessment

2.3.1. Chronic risk in sediment-dwelling organisms
Chronic risk in sediment-dwelling organisms was assessed based on RQ (Risk Quotients) analysis according to Vryzas et al. (2011) (Eq. (1))
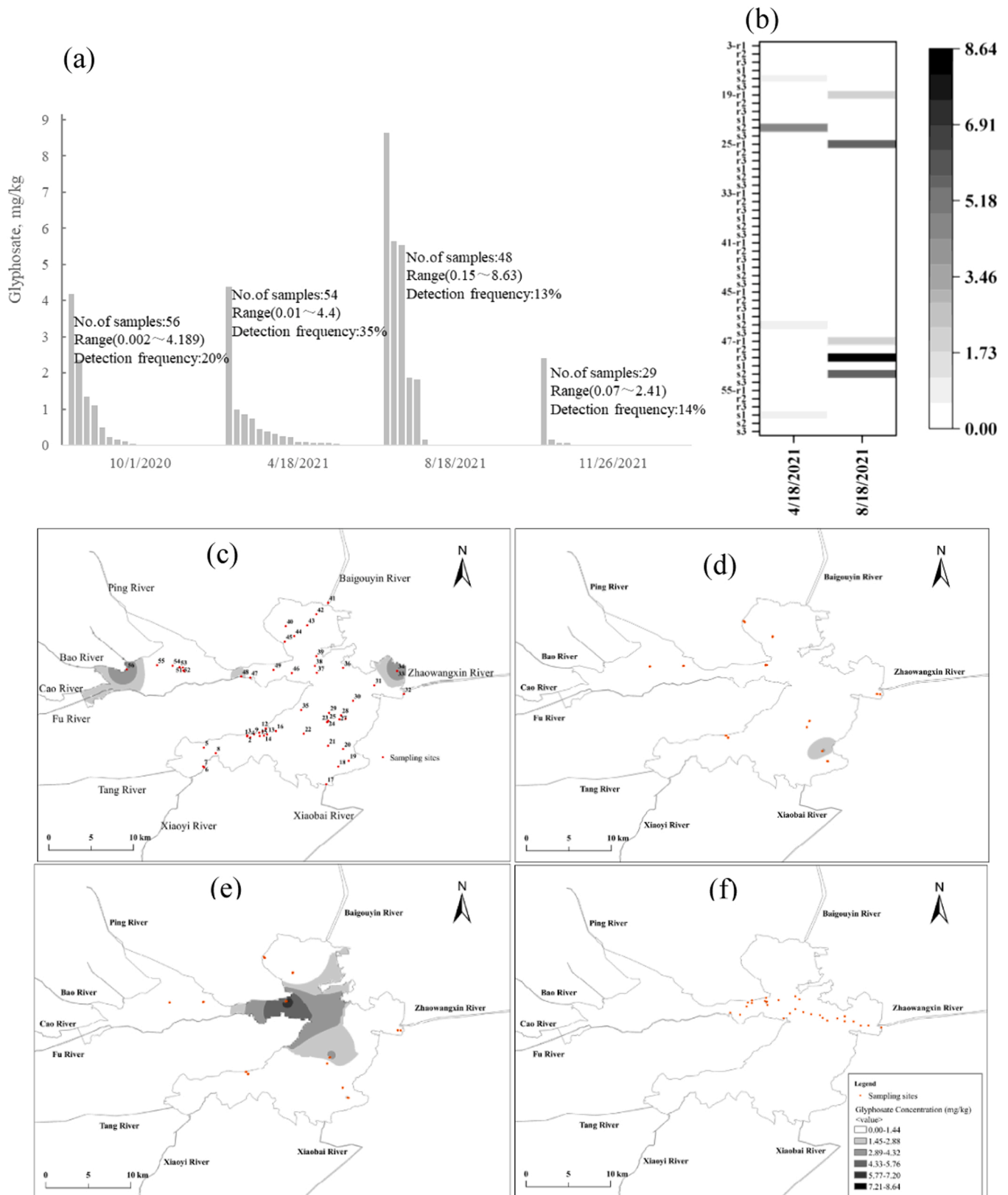
Fig. 2. Temporal and spatial distribution of glyphosate in the Baiyangdian Lake. (a) Seasonal variation of glyphosate concentration in sediment of Baiyangdian Lake. (b) Glyphosate concentration in sediments from *Pragmites australis* (r) and submergent (s) plant communities in April and August. Typical sites 3, 19, 25, 33, 41, 45, 47, 55 were randomly chosen to monitor the planted sediments. The r1, r2, r3 and s1, s2, s3 were triplicates of the sampled sediments. (c ~ f) Spatial glyphosate distribution map for multi-seasons based on ArcGIS interpolation methods.

$$RQ = \frac{C}{PNEC} \qquad (1)$$

where C is the measured glyphosate concentration in sediment and PNEC is the predicted non-effect concentration. PNEC was calculated according to Eq. (2):

$$PNEC = \frac{CC}{AF} \qquad (2)$$

where CC is the critical concentration and AF is an assessment factor. In sediment, the CC was set as the no-observed effect concentration (NOEC) in aquatic invertebrate. Because the NOEC of glyphosate for the sediment-dwelling organisms was unavailable, the NOEC for the soil-dwelling earthworm *Eisenia fetida* (1250 mg/kg) was adopted as CC. The AF was set following the methodology proposed by Papadakis et al. (2015), being 1000 if no NOEC value was available. The AF was applied to overcome the uncertainty associated with the accuracy, inherent variability, model errors and lack of toxicity data. RQ < 0.01 indicates negligible ecological risk, 0.01 < RQ < 0.1 indicates low ecological risk, 0.1 < RQ < 1 indicates medium expected risk, and RQ > 1 indicates high ecological risk.

2.3.2. Acute risk in sediments

For acute toxicity in sediments, it was necessary to convert the pesticide concentration in sediments to pore water pesticide concen-

tration, using Eq. (3), according to Schwarzenbach and Westall (1981):

$$Cpw = \frac{C_s}{K_d} \qquad (3)$$

where C_{pw} is the pore water glyphosate concentration, C_s is the glyphosate concentration measured in sediments and K_d is the linear sorption coefficient. The K_d values were calculated using Eq. (4):

$$K_d = K_{oc} \times f_{oc} \qquad (4)$$

where K_{oc} is the organic carbon-water partitioning coefficient for glyphosate and f_{oc} is the fraction of total organic carbon in sediments (Table S1 Supplementary data). The K_{oc} was calculated according to Eq. (5):

$$\log K_{oc} = a \times \log K_{ow} + b \qquad (5)$$

where K_{ow} is the octanol-water partitioning coefficient. The values of the constants a and b were set as 0.72 and 0.49 and used according to DeSutter et al. (1998) and $\log K_{ow}$ was set as -3.49 according to Wang et al. (2011).

The acute risk for aquatic organisms was assessed according to the TU (Rasmussen et al., 2015). TU was calculated to understand the toxicity of glyphosate, according to Eq. (6):
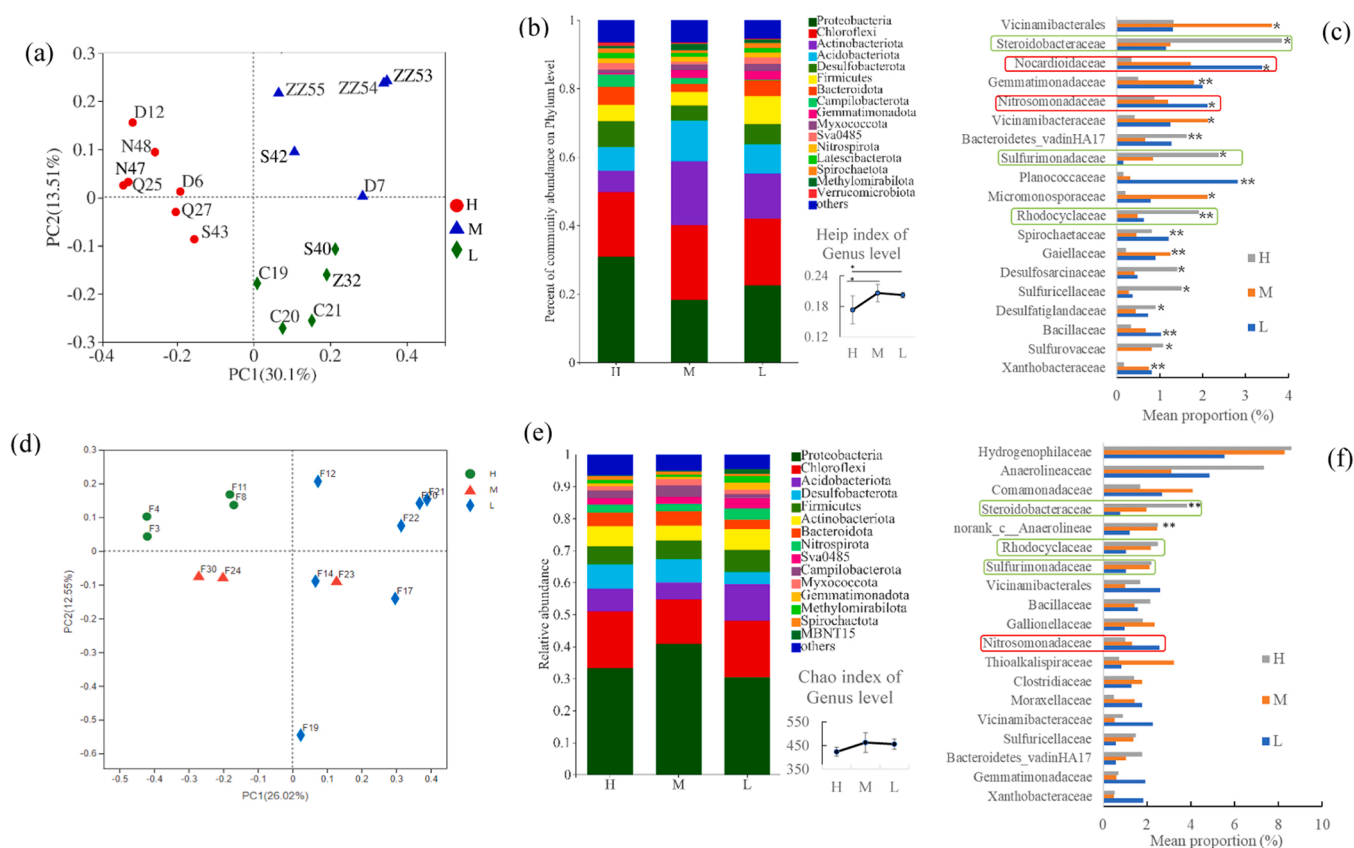
$$TU = \frac{C_i}{LC_{50}} \qquad (6)$$

Fig. 3. Sediments with high (group H: sites D6, D12, Q25, Q27, S43, N47, N48, n = 7), medium (group M: sites D7, S42, ZZ53, ZZ54, ZZ55, n = 5), and low (group L: sites C19, C20, C21, Z32, S40, n = 5) glyphosate concentration in 10/1/2020 and sediments with high (group H: sites F3, F4, F8, F11, n = 4), medium (group M: sites F23, F24, F30, n = 3), and low (group L: sites F12, F14, F17, F19, F20, F21, F22, n = 7) glyphosate concentration in 11/26/2021 from the Baiyangdian Lake showed significant difference in bacterial community (p < 0.05). (a) and (d): PCoA analysis of sediment bacterial community from different sampling sites on OTU level (R^2 =0.3823, p = 0.001; R^2 = 0.2785, p = 0.005); (b) and (e): bacterial community composition in H, M and L group on phylum level; (c) and (f): difference in bacterial abundance at family level among H, M and L group using Kruskal Wallis H test (green box line indicated higher abundance in glyphosate-highly enriched sediments; red box line indicated higher abundance in glyphosate-lowly enriched sediments). Asterisk* and double asterisks** indicated significant difference (p < 0.05) and extremely significant difference (p < 0.01), respectively. Heip index and Chao index of diversity for bacteria in H, M, L group were placed at the side of (b) and (e), respectively, to indicate the difference in α-diversity of bacterial communities among three groups.

where C_i is the concentration of glyphosate in the sample i, and LC_{50} is the concentration of glyphosate causing a 50% effect in the benchmark organism. According to Tsui and Chu (2004), LC_{50} was set as 1000 mg/L. TU > 1 indicates an ecological risk, whereas TU < 1 indicates no ecological risk.

2.4. Bacterial community in sediment

In order to discover the possible effect of glyphosate on microbial communities in the Baiyangdian lake sediment, after the glyphosate concentration was determined, seven, five, five sediment samples with glyphosate highly (H: 2.99 ± 3.00 mg/kg), moderately (M: 0.32 ± 0.30 mg/kg) and lowly (L: undetectable, < 0.001 mg/kg) enriched respectively from the October samples of 2020 (Fig. 3a), and four, three, seven sediment samples from the November samples of 2021(Fig. 3d) were selected to analyze the bacterial community soon after the sample collection.

DNA extraction and sequencing: DNA was extracted from 0.32 to 0.41 g of wet sediment using EZNA ® Bacterial DNA Kit following the manufacturer's instructions (Omega Biotech, USA). DNA of equal amount extracted from triplicate sediment samples were mixed. DNA concentration and quality was determined with NanoDrop 2000 Spectrophotometer (Thermo Fisher, USA) and examined by gel electrophoresis. All extracted DNA was stored at − 80 °C before use. The V3–V4 region of the bacterial 16 S rRNA gene was amplified by PCR using universal primers 338 F (5′-ACTCCTACGGGAGGCAGCAG-3′) and barcoded primer 806 R (5′-GGACTACHVGGGTWTCTAAT-3′). Sediment DNA was amplified using polymerase chain reaction (PCR) in triplicate with a 20 μl mixture, which 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 FastPfu DNA Polymerase, and 10 ng of template DNA. The PCR amplification of 16 S rRNA gene was performed as follows: initial denaturation at 95 °C for 3 min, followed by 27 cycles of the following procedures: denaturing at 95 °C for 30 s, annealing at 55 °C for 30 s, and elongation at 72 °C for 45 s; the final extension was at 72 °C for 10 min and then holding at 10 °C. The PCR product was extracted from 2% agarose gel and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) following manufacturer's instructions. The concentrations of the purified PCR products were measured using QuantiFluor™-ST (Promega, USA), pooled in equimolar concentrations, and then sequenced on the Illumina MiSeq PE300 platform at the Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China).

Processing of sequencing data: The raw 16 S rRNA gene sequencing reads were demultiplexed, quality-filtered by Trimmomatic and merged by FLASH. Briefly, a threshold of average quality scores > 50 over 20 bp window size was used to filter the unqualified reads, and only overlapping sequences longer than 10 bp and < 0.2 mismatches were assembled. The jointed sequences with ambiguous characters and lengths < 200 bp were discarded. Operational taxonomic units (OTUs) with 97% similarity for bacteria were clustered using UPARSE (version 7.1), and chimeric sequences were identified and removed using UCHIME. The taxonomy of each OTU representative sequence was analyzed by RDP Classifier (http://rdp.cme.msu.edu/) against the 16 S rRNA database (Silva SSU132) using confidence threshold of 0.7. A total of 687825 and 459606 high-quality 16 S rRNA sequences were generated for 17 October and 14 November samples. The rarefaction curves showed clear asymptotes (data not shown), which indicated a near-complete sampling of the community. Sequencing numbers of each sample were rarified to the sample with the minimum number of reads (34784 reads for October sample and 21044 reads for November sample) to correct sampling effects on diversity. Beta (β) diversity was examined to verify variation in bacterial community structure among samples (Figs. 3a and 3d) after subsampling.

2.5. Statistical analyses

GIS-based interpolation (Inverse Distance Weighting (IDW) Interpolation Algorithm) was used to illustrate the spatial distribution of glyphosate in sediments (Fig. 2c ~ 2f). Bacterial community function prediction (Fig. 4) was carried out using FAPROTAX (functional annotation of prokaryotic taxa) module (https://cloud.majorbio.com/). FAPROTAX is a database that maps bacterial or archaeal taxa to metabolic or ecologically relevant functions (i.e., nitrogen fixation, sulfate respiration or hydrocarbon degradation) using literature on culture representatives. The FAPROTAX provides a python script to convert OTUs tables into functional tables based on taxa identified in a sample and functional phenotype of each taxon in the FAPROTAX database (Sansupa et al., 2021). Exploratory analysis was conducted by Microsoft Office Excel (Microsoft Corporation Inc., Redmond, USA). Statistical comparisons by ANOVA and Spearman's rank correlation analysis were implemented with SPSS 18.0 (SPSS Inc., Chicago, USA).

3. Results

3.1. Glyphosate distribution in sediment of Baiyangdian Lake and its risk assessment

Fig. 2a showed that highest glyphosate concentration(8.63 mg/kg) but lowest detection frequency (13%) occurred in August sediment samples; second highest glyphosate concentration(4.38 mg/kg) but highest detection frequency (35%) occurred in April sediment samples; mean value of the detected glyphosate concentration in August (3.94 ± 3.18 mg/kg) was the significantly highest among the four seasons ($p < 0.05$);however, there was no significant difference among the other three seasons (October:0.91 ± 1.32 mg/kg; April: 0.48 ± 0.97 mg/kg; November: 0.68 ± 1.15 mg/kg).

Comparison between April and August (Fig. 2b) suggested that the highest sediment glyphosate concentration happened in submergent macrophytes community located at the large clear water area of reed marshes away from the lake village and emergent macrophytes (reed) community located at the lakeside reed border nearby the Anxin county in April and August, respectively.

Samples collected near or at village (e.g., 33, 34), cropland and farm ditch/pond (e.g., 48, 56) showed relatively higher values of glyphosate in comparison with samples collected in other sites in October. According to Fig. 2, the higher glyphosate enriched sites generally located close to villages, farmlands, towns, and service industrial regions. While the lower glyphosate enriched sites generally located open water far away from human residential area under less artificial disturbances. Meanwhile, the water body's apparent feelings on some samples with high glyphosate enrichment were turbid and smelly, only a few water bodies were clear occasionally; but most water bodies with low glyphosate enrichment were clean and no sign of turbidity, and blankets of aquatic vegetation such as reed (*Phragmites australis*), lotus (*Nelumbo nucifera*), cattail (*Typha angustifolia*), Pondweed (*Potamogeton pectinatus*), hornwort (*Ceratophyllum demersum*), Brasenia (*Brasenia schreberi*) or Nymphaea (*Nymphoides peltatum*) densely cover the open water surface (Table S2).

Glyphosate accumulation hotspots harbored total 18 samples exhibiting RQ > 0.1 (≈ 10%) and total 5 samples exhibiting RQ > 1 (≈ 3%), indicating the medium to high chronic risk to sediment-dwelling organisms due to the glyphosate exposure in some glyphosate enriched sites in the Baiyangdian Lake (Table 1). The potential acute toxicity in aquatic and benthic organisms at site 47-r3 (TU= 2.09, exceeded the acute risk threshold 1) in Nanliuzhuang village and farmland drainage ditch where near the Anxin county downtown suggested the probable sources of glyphosate from agriculture, household, municipal sewage, tourism, and recreational activities involving pollution and disturbance to the ecological toxicity for aquatic organisms, especially in August.
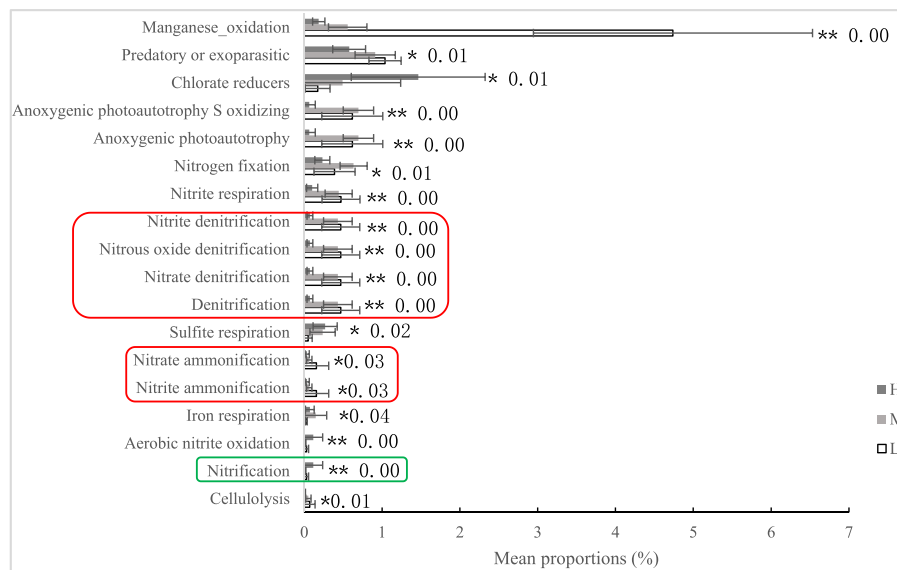
Fig. 4. Predicted bacterial metabolic functions with significant differences among H, M and L groups (red box line indicated higher predicted function abundance in glyphosate-lowly enriched sediments; green box line indicated higher abundance in glyphosate-highly enriched sediments)(a) and linear regression between the values of abundance of certain bacterial metabolic function predicted by FAPROTAX over glyphosate concentration in sediments from the Baiyangdian Lake (n = 17, all October samples of H, M and L groups) (b and c). Numbers beside error bars in plot (a) were p values of significance. Rising and declining trends were presented separately in plots (b) and (c) and different functions were illustrated by different colors. The r value (regression coefficient) which was marked by the function abbreviation beside the linear trend was showed to indicate the degree to which a linear trend was. Asterisk* and double asterisks** indicated significance ($p < 0.05$) and extreme significance ($p < 0.01$), respectively.
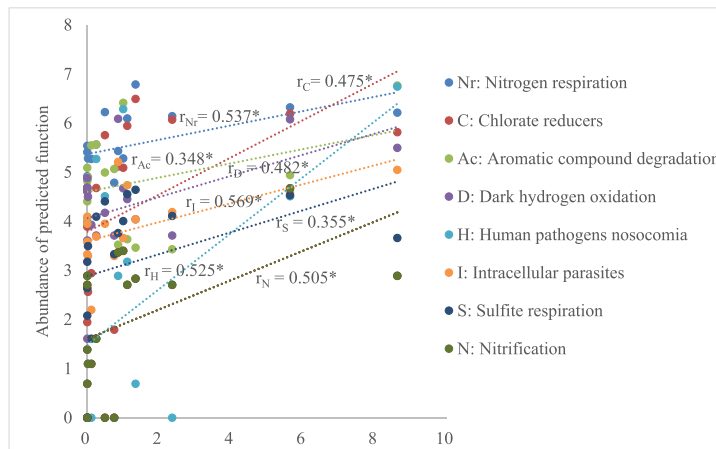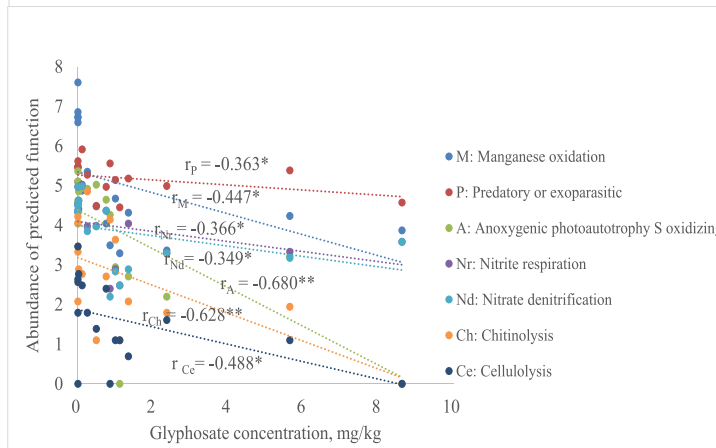
3.2. Effect of glyphosate accumulation on bacterial community composition

In order to detect the possible effect of glyphosate accumulation on sediment biogeochemistry, seven (sites 6, 12, 25, 27, 43, 47, 48 with glyphosate concentration of 2.99 ± 3.00 mg/kg representing the highly (H) glyphosate-enriched scenario), five (sites 7, 42, 53, 54, 55 with glyphosate concentration of 0.32 ± 0.30 mg/kg representing the moderately (M) glyphosate-enriched scenario) and five (sites 19, 20, 21, 32, 40 with non-detected glyphosate representing the lowly (L)

glyphosate-enriched scenario) typical samples were selected to check the differences in bacterial community composition (Fig. 3a ∼ 3c), respectively, to further explore the ecological effects of glyphosate accumulation in the Baiyangdian lake. To avoid confusion, the names of the 17 typical October samples were displayed by adding the first letter of the full Chinese name of the sampling site before the sample ID number in Fig. 3a. To determine the general rule of the effect of glyphosate on bacterial community, four (sites F3, F4, F8, F11), three (sites F23, F24, F30) and seven (sites F12, F14, F17, F19, F20, F21, F22) typical samples from November sampling sites were selected to

Table 1

Chronic risk, as risk quotient (RQ) and acute risk, as toxic unit (TU) of glyphosate to sediment-dwelling organisms and aquatic biota for typical sampling site in the Baiyangdian Lake.

RQ

Site ID	10/1/2020	Site ID	10/1/2020	Site ID	4/18/2021	8/18/2021	Site ID	4/18/2021	8/18/2021	Site ID	11/26/2021
1	0.00	27	0.00	3-r1	0.00	0.00	41-r3	0.01	0.00		0.84
3	0.00	30	0.00	3-r3	0.00	0.00	41-s3	0.16	0.00		0.05
4	0.00	32	0.00	3-s1	0.02	0.00	45-r2	0.35	0.00		0.00
5	0.00	34	1.45	3-s2	0.30	0.00	45-s1	0.00	0.00		0.03
7	0.00	35	0.08	19-r1	0.00	0.65	45-s2	0.00	0.00		0.00
8	0.00	36	0.00	19-r2	0.02	0.00	45-s3	0.09	0.00		0.03
9	0.00	37	0.04	19-r3	0.00	0.05	47-r1	0.00	0.63		0.00
10	0.05	39	0.00	19-s1	0.03	0.00	47-r3	0.02	3.00		0.00
11	0.00	40	0.00	19-s2	1.52	0.00	47-s1	0.01	0.00		0.00
12	0.00	41	0.00	19-s3	0.08	0.00	47-s2	0.00	1.92		0.00
13	0.00	42	0.00	25-r1	0.00	1.96	47-s3	0.11	0.00		0.00
15	0.00	46	0.00	25-r3	0.03	0.00	55-s1	0.26	0.00		0.00
16	0.00	48	0.47	25-s1	0.13	0.00	55-s3	0.00	0.00		0.00
25	0.39	55	0.17	41-r1	0.00	0.00					0.00
26	0.02	56	0.82	41-r2	0.00	0.00					0.00

TU

Site ID	10/1/2020	Site ID	10/1/2020	Site ID	4/18/2021	8/18/2021	Site ID	4/18/2021	8/18/2021	Site ID	11/26/2021
1	0.00	27	0.00	3-r1	0.00	0.00	41-r3	0.00	0.00		0.30
3	0.00	30	0.00	3-r3	0.00	0.00	41-s3	0.05	0.00		0.01
4	0.00	32	0.00	3-s1	0.01	0.00	45-r2	0.05	0.00		0.00
5	0.00	34	0.99	3-s2	0.18	0.00	45-s1	0.00	0.00		0.02
7	0.00	35	0.05	19-r1	0.00	0.13	45-s2	0.00	0.00		0.00
8	0.00	36	0.00	19-r2	0.01	0.00	45-s3	0.33	0.00		0.01
9	0.00	37	0.02	19-r3	0.00	0.02	47-r1	0.00	0.12		0.00
10	0.01	39	0.00	19-s1	0.01	0.00	47-r3	0.00	2.09		0.00
11	0.00	40	0.00	19-s2	0.46	0.00	47-s1	0.00	0.00		0.00
12	0.00	41	0.00	19-s3	0.02	0.00	47-s2	0.00	0.58		0.00
13	0.00	42	0.00	25-r1	0.00	0.54	47-s3	0.03	0.00		0.00
15	0.00	46	0.00	25-r3	0.00	0.00	55-s1	0.04	0.00		0.00
16	0.00	48	0.15	25-s1	0.02	0.00	55-s3	0.00	0.00		0.00
25	0.04	55	0.02	41-r1	0.00	0.00					0.00
26	0.01	56	0.18	41-r2	0.00	0.00					0.00

Note: Considering the RQ approach, RQ < 0.01 indicates negligible chronic risk; 0.01 < RQ < 0.1 indicates low chronic risk (indicated in green); 0.1 < RQ < 1 indicates medium chronic risk (indicated in yellow); and RQ > 1 indicates high chronic risk (indicated in red). Considering the TU approach, TU < 1 indicates no acute risk (green); TU > 1 indicates acute risk (red) (Pérez et al., 2021).

represent H, M, L glyphosate-enriched scenario to recheck the impact of glyphosate accumulation on bacterial community composition (Fig. 3d ~ 3 f).

Sediments containing high-level glyphosate harbored distinct bacterial communities from those containing medium- and low-level glyphosate, as demonstrated by phylum-level community composition (Figs. 3b and 3e), PCoA based on the Bray-Curtis distance at OTU level (Figs. 3a and 3d) and difference analyses (Figs. 3c and 3f). The glyphosate-H group had significantly higher abundances of Proteobacteria and Campilobacterota but significantly lower abundances of

Actinobacteriota and Acidobacteriota than glyphosate -L groups (p < 0.05). In October case (Fig. 3b), M group seemed closer to L group and both distinct from H group; in November case (Fig. 3e), M group seemed closer to H group and both distinct from L group; the sample distribution in Figs. 3a and 3d also proved this phenomenon. Generally, along with the glyphosate concentration increased from lower glyphosate group to higher glyphosate group (L and M to H in Fig. 3b and L to M and H in Fig. 3e), the bacterial community composition shifted in a similar manner: Proteobacteria and Campilobacterota significantly increased but Actinobacteriota and Acidobacteriota significantly decreased.

The family-level differences in bacterial community composition among H, M, L groups strongly proved by Kruskal-Wallis H test bar plot (Figs. 3c and 3f). Despite the change magnitude among groups in November did not reach the significant level as much as those among groups in October, higher abundance of Proteobacteria families Steroidobacteraceae and Rhodocyclaceae and Campilobacterota family Sulfurimonadaceae, while lower abundance of Proteobacteria family Nitrosomonadaceae were found in sediments from H group (Figs. 3c and 3f) in both October and November. Besides, significantly lower Nocardioidaceae and Micromonosporaceae of Actinobacteriota, Vicinamibacteraceae of Acidobacteriota, and Planococcaceae of Firmicutes in H group were found among October groups (Fig. 3c). Further, Hydrogenophilaceae and Gallionellaceae of Proteobacteria were higher while Vicinamibacterales of Acidobacteriota was lower in H group with varying degrees among November groups (Fig. 3f).

Additionally, as shown in two mini plots beside Figs. 3b and 3e, α-diversity indices (Heip index, Chao index) at genus level were calculated to indicate the significantly lower microbial community diversity in H group than M and L groups in October sediment samples (p < 0.05). Chao index for the H group in November sediments was lower but not significantly relative to M and L group. Significantly negative correlation (r = −0.425, p < 0.05, n = 24) between α Diversity index (Coverage) of bacterial community and glyphosate concentration in October sediment samples (Table S3) confirmed the lower bacterial diversity in higher glyphosate accumulation scenario. Besides, correlation heatmap confirmed stimulatory effect of glyphosate on Steroidobacteraceae (Fig. S1b and S1d). These results suggested that glyphosate accumulation affected bacterial community composition/diversity at least partly. And the most sensitive taxon was Steroidobacteraceae of Proteobacteria which was significantly stimulated by glyphosate accumulation (Figs. 3c and 3f, FigS1b and S1d).

3.3. Functional responses of bacterial community to the sediment glyphosate accumulation

Sediment attached bacteria consisted of a wide array of different functional potentials in C, N, P, and S biogeochemical cycling (Table 2). The disproportionation of responses for different bacteria to glyphosate (Fig. 3; Fig.S1; Table S3) led to disproportionation of predicted bacterial metabolic functions (predicted by FAPROTAX) in different glyphosate concentration level (Fig. 4). Figs. 4a and 4b showed bacterial metabolic functions such as "Aromatic compound degradation", "Sulfite respiration" and "Nitrification" were stimulated with increasing glyphosate concentration. However, bacterial metabolic functions of "Nitrate denitrification", "Anoxygenic photoautotrophy S oxidizing", "Cellulolysis" and "Manganese oxidation" were promoted in sediments with lower glyphosate concentration (Figs. 4a and 4c). Microbial predicted functions by FAPROTAX (Fig. 4) corroborated that many microbial groups were involved in the biodegradation of toxic organic compounds, oxidation of sulfur compounds / iron sulfide minerals / pyrite, and reduction of nitrate (Table 2) in the anaerobic freshwater lake sediments of this study, which included groups from Steroidobacteraceae, Rhodocyclaceae, *Thiobacillus*, Gallionellaceae and Sulfurimonadaceae due to their positive responses to glyphosate accumulation (Fig. 3).

Table 2
Potential function of the typical responding bacteria to glyphosate.

Taxonation	Potential function	Reference
Probably stimulated by Gly		
Proteobacteria Steroidobacteraceae	The Steroidobacteraceae family is related to the **degradation of sulfadiazine** ($C_{10}H_{10}N_4O_2S$, antibiotic). Iron–sulfur metabolic potential; Implicated in **iron reduction.** Suppressed by lindane and endosulfan (organochlorine pesticide).	Zhang et al. (2021) M. Wang et al. (2019); Tsuji et al. (2020) S.C. Wu et al. (2021)
Rhodocyclaceae	Polycyclic Aromatic Hydrocarbons (PAHs) degraders.	Singleton et al. (2015)
Hydrogenophilaceae	Genus *Thiobacillus*: **Sulfur-oxidizing bacteria (SOB)** containing genes encoding sulfite dehydrogenase, APS reductase, ATP sulfurylase, rhodanese, etc. Sulfur-based autotrophic **denitrifiers for nitrate removal**. Leaching bacteria grow on **ferrous** sulphate or **copper** sulphide for **bioleaching**.	Luo et al. (2018) Beller et al. (2006) Yang et al. (2018) Jensen and Webb (1995) Donati et al. (1997)
Gallionellaceae	Denitrification coupled to Fe (II) oxidation and carbon fixation; "nitrate-dependent Fe (II) oxidation".	Huang et al., 2021; Tominski et al. (2018)
Campilobacterota Sulfurimonadaceae	**Sulfur-oxidizing**, degrading toxic organic compounds, tolerate and detoxify heavy metals. Oxidation of sulfur compounds coupling to the **reduction of nitrate or molecular oxygen**. **CO_2 fixation stability** depending on pH and ionic strength conditions.	Li et al. (2019) Luo et al. (2018) Inagaki et al. (2004) Kwon et al. (2018)
Probably inhibited by GLY		
Proteobacteria Nitrosomonadaceae	**Ammonia oxidizing bacteria (AOB)**, obligate autotrophs and obtain C only from CO_2 fixation via the Calvin cycle; inhibited by variety of compounds such as dicyandiamide (DCD).	Clark et al. (2021)
Actinobacteriota Nocardioidaceae MB-A2–108 Microtrichales	Plant bacterial endophytes may transmit vertically from shoot into the root−soil interface to increase potential **phytoremediation** efficiency; Sulfamethoxazole (antibiotic)-degrading bacteria. Rhizosphere actinobacteria associated with rusty root disease. Bacteriosis control by secreting antibiotics corresponding functions associated with **glycolysis** and/or gluconeogenesis, porphyrin and chlorophyll metabolism, **photosynthetic** proteins, and transcription factors.	Luo et al. (2019) Ouyang et al. (2019) Q. Wang et al. (2019) Deng et al. (2020) Beckmann et al. (2017)
Acidobacteriota Vicinamibacteraceae	Polyphosphate-accumulating organisms (PAOs) as intracellular storage was observed for the key compounds polyphosphate and glycogen.	Kristensen et al. (2021)
Firmicutes *Paenisporosarcina*	**Denitrification** heterotrophically. **Nitrogen removal**. Carbon and nitrogen cycling enhancement.	Zheng et al. (2018)

Note: Gly was short for glyphosate.

4. Discussion

4.1. Current status of glyphosate accumulation in sediments of the Baiyangdian Lake

According to the Soil Environment Quality Standard of China, there were bunch of samples were far beyond the threshold value for

glyphosate (0.50 mg/kg), including 3 out of 56 October samples, 4 out of 54 April samples, 5 out of 48 August samples and 1 of 29 November samples. The low over-limit ratio seemed to indicate that glyphosate pollution in Baiyangdian sediments was not as serious as it was supposed. However, when compared with other usually-concerned glyphosate pollution regions in a global scale, the glyphosate concentration in this study was at a medium to high level (Table S4). It was higher than those reported in Europe and the United States, but lower than the high values of the Argentinian pampas, where the typical glyphosate polluted farming area had maximum glyphosate concentrations among glyphosate hotspots all around the world (Primost et al., 2017). Because of being applied with the herbicides directly, the agricultural soil generally contained higher glyphosate concentration than sediment. It was clear that sediment compartment was an important sink of the herbicide, and the ubiquitous occurrence of herbicide with even low concentrations in sediments (in mg/kg scale) would largely reflect the intensive use of glyphosate for weed control in the Baiyangdian Lake district. Even though only single herbicide glyphosate was adopted for risk assessment, RQ of sites 19, 25, 34, 47 exceeded the risk threshold 1 due to their high glyphosate concentration (4.38, 5.64, 4.19, 8.63 mg/kg) beyond the PNEC (1.25 mg/kg). There were 10% and 3% of the sampling sediments exhibiting medium and high chronic risk to sediment-dwelling organisms and the high TU value (2.09) of site 47 suggested the potential acute risk of glyphosate exposure to aquatic and benthic organisms in some cases. Besides, Guo et al. (2014) pointed out that organochlorine pesticides in core sediments of Baiyangdian Lake exhibited potential environmental risk because of 5.9% of the p,p′-DDT concentrations and 41.2% of the ΣDDT concentrations observed in the surface sediment were higher than the ERL(effect range low) values (the usage of DDT was officially banned in China since 1983). According to Pérez et al. (2021), the risk level would only be higher when glyphosate, legacy pesticides (e.g., DDT) and other current use chemicals (e.g., imidacloprid, atrazine, acetochlor) were incorporated, therefore, the combined risks of various chemicals should be concerned in the complex freshwater ecosystems under anthropogenic impacts.

Since the International Agency on Research on Cancer found glyphosate to be probably carcinogenic in 2015, several countries have banned or restricted glyphosate use. Austria became the first EU country to ban glyphosate in July 2019. Italy, Canada, Spain, Netherlands, and Portugal already reduced or banned glyphosate, and France and Germany are processing glyphosate ban (Brovini et al., 2021). According to the European Commission, glyphosate is currently approved for use in the European Union until Dec. 15, 2022. Other countries that have some sort of legislation around glyphosate include: Sri Lanka, Thailand, Vietnam, etc. Under such situation the tasks to prevent ecological risk and protect aquatic ecosystem and human health should be scheduled onto the restrictions regarding the use of glyphosate in China. Thus, it is recommended that the current Chinese agricultural chemicals regulations should be modified.

Glyphosate degradation is performed co-metabolically and decomposition rate should depend on the environmental factors, especially the activity of soil microorganisms (Muskus et al., 2020). Biodegradation products of glyphosate can be sarcosine – as the effect of direct activity of C–P lyase or aminomethylphosphonic acid formed as the result of the activity of glyphosate oxidoreductase and glyoxylic acid (Klimek-Ochab et al., 2004). Tejada (2009) believed that high levels of glyoxylic acid in the soil, consuming high levels of oxygen to their degradation, could be "subtract" to soil microorganisms. This aspect would reduce the soil MBC and consequently their intracellular activities (dehydrogenase), and their extracellular activities (urease, ß-glucosidase, phosphatase and arylsulphatase). Our results that inhibitory effects of glyphosate on MBC, ß-glucosidase and bacterial community diversity but stimulatory effects on phosphatase activity (Table S3 and Fig. 3) seem to support that less tolerate species were inhibited meanwhile some resistant and degrading species could use glyphosate as phosphorus, carbon and nitrogen source in this study (Zhan et al., 2018). This suggested the

present glyphosate concentration in Baiyangdian lake sediments was sufficient to affect the bacteria biomass, diversity, activity and community composition, agreeing with Chávez-Ortiz et al. (2022) who found glyphosate reduced C mineralization but increased phosphatase as well as changes in microbial community diversity and structure.

However, physicochemical and biological characteristics of the sediment (e.g., SOC, pH, MBC, microbial activity, NO_3-N, NH_4-N, clay fraction, etc.) influenced the environmental fate and behavior of glyphosate in the lake (Table S3 and Fig.S2; Carretta et al., 2021; De Gerónimo and Aparicio, 2022). Significantly lower SOC but higher NH_4-N (though not significant) in H group (Fig.S2(b, q, r, s)) suggested the reduced condition with less oxygen and more pollutants in the glyphosate-accumulated sites promoted the NH_4-N accumulation while the lower SOC in relative to L group suggested amount of plant residue input caused high SOC accumulation in L group. Palomeque et al. (2019) found ammonium-N increased in response to glyphosate. According to Zablotowicz et al. (2008), the co-application of glufosinate with nitrogen fertilizers may alter atrazine cometabolism, thereby reasonable inferring on probably effects of co-application of N fertilizer (ammonium sulfate, ammonium nitrate, etc.), and herbicides (glyphosate, glufosinate, atrazine, etc.) on glyphosate degradation. Several studies revealed glyphosate promoted nitrification and inhibited denitrification (Das et al., 2022) thus alter the NH_4-N and NO_3-N contents, in turn, the N source availability would be a key factor influencing to glyphosate biodegradation (Zhan et al., 2018). Glyphosate caused oxidative stress and exhibited negative effects on photosynthesis (Radwan and Fayez, 2016), which would be the reasons of significantly higher peroxidase and catalase (Fig.S2(i,j)), and lower (though not significant) DOC_{ow} and DOC_{sw} (Fig.S2(d,e)) in H group. Liu et al. (2019) highlighted that the interaction between glyphosate and low microplastics content negatively affected DOC dynamics, leading to the loss of bioavailable C loss. There was a consensus that glyphosate adsorption decreased with increasing pH since the negative charge would challenge the formation of strong adsorbent–cation–P(glyphosate) bonds by ligand exchange between the glyphosate phosphonate group and singly coordinated Al-OH and Fe-OH groups (De Gerónimo and Aparicio, 2022). Significantly negative correlation between glyphosate concentration and pH in this study agreed that (Table S3). However, due to the differences in other influencing factors such as clay content and iron oxides besides pH (De Gerónimo et al., 2018), pH in H, M and L groups did not vary consistently with the glyphosate concentration (Fig.S2(m)). According to Gimsing et al. (2007), the clays appeared to have two well-defined glyphosate adsorption sites, a first adsorption site being on the external surface and a second one on the interlayer space, and due to the differences in charge and molecular size, competition between glyphosate and phosphate for adsorption sites made glyphosate affinity to sediment was changeable. Those maybe the reasons why glyphosate was significantly positive correlated with large water-stable aggregate but significantly negative correlated with middle large and small aggregate shown in Table S3. Based on the discuss above, we can assume that the glyphosate accumulation, fate and environmental behavior in the lake sediment mainly involved in the interaction with the sediment physicochemical and biological properties. Regulation of NH_4-N, NO_3-N, pH, sediment textures would be important to glyphosate fate in the lake ecosystem, and glyphosate occurrence and concertation plus appearance of community level biosensor such as biomass, diversity, activity were direct field evidence to evaluate glyphosate ecological risk level in glyphosate application and surrounding area.

4.2. Sediment bacterial community structure and function in response to glyphosate accumulation

Zabaloy et al. (2012) said glyphosate addition to the microcosms had minimal effects on both structural and functional measures of the microbial community. Newman et al. (2016) reported Proteobacteria (particularly gammaproteobacteria) increased in relative abundance

while relative abundance of Acidobacteria decreased in soil rhizosphere-associated bacterial communities following long-term glyphosate application on glyphosate tolerant corn and soybean in the greenhouse. Yang et al. (2021) found glyphosate effect on the composition of the soybean rhizosphere bacterial community but no impact on the structure of the rhizosphere microbial community during a single planting season. Our results highlighted the stimulatory effect of glyphosate on Proteobacteria and Campilobacterota while inhibitory effect on Actinobacteria and Acidobacteria. Although the shift of bacterial community could not be completely attributed to glyphosate since other factors besides glyphosate also contributed the changes in sediment bacterial community composition and structure (Fig.S1), the significantly positive correlations between glyphosate concentration and abundance of dominant families Steroidobacteraceae, Rhizobiales incertae sedis, Pseudomonadaceae within Phylum Proteobacteria; families Desulfatiglandaceae, Desulfosarcinaceae, Desulfobaccaceae within Phylum Desulfobacterota; family Anaerolineaceae within Chloroflexi; and family Flavobacteriaceae within Phylum Bacteroidota (Table S3) would partly support the impacts of glyphosate on the bacterial communities. According to Zhang et al. (2022), who successfully isolated a novel glyphosate-degrading bacterial strain, *Chryseobacterium* sp. Y16C (phylum: Bacteroidota; order: Flavobacteriales; famliy: Weeksellaceae), which efficiently degraded glyphosate and induced a slight alteration in the diversity and composition of soil microbial community, the positive responding bacteria taxa in our results may be informative for isolation of glyphosate-degrading species for bioremediation of glyphosate contaminated soil.

Lorch et al. (2021) elucidated glyphosate reduced the abundance and altered the community structure of soil culturable pseudomonads in a temperate grassland, and the negative correlation between glyphosate concentration and Pseudomonadaceae abundance was also found in this study despite it was not significant (Table S3). They deduced the glyphosate induced bacterial community alterations were most likely associated with the crucial ecosystem functions given that some members of the stimulated or inhibited bacteria were involved in biogeochemical processes, an increase or decrease in their abundance could lead to significant changes in nutrient status (Newman et al., 2016).

According to previous studies, Steroidobacteraceae family was related to the degradation of sulfadiazine ($C_{10}H_{10}N_4O_2S$, antibiotic) (Zhang et al., 2021) and had potential iron–sulfur metabolic function (Tsuji et al., 2020). Besides, under N fertilization, the abundance of Steroidobacteraceae was significantly and positively correlated with soil NO_3-N content (A.L. Wu et al., 2021). Liang et al. (2020) found abundant sulfur-oxidizing bacteria, such as *Sulfurifustis*, *Sulfuriferula* and *Thiobacillus* in a pilot pyrrhotite constructed wetland for the glyphosate and nutrients removal from simulated agricultural runoff. They confirmed *Thiobacillus* and *Sulfurimonas* used pyrrhotite (FeS) as the electron donor to reduce NO_3-N and higher glyphosate was adsorbed on the produced goethite (FeO(OH)) in the biofilter. Huang et al. (2021) also found *Sulfurimonas* and *Sulfurovorum* (Campylobacterota) reduce NO_3-N by oxidizing sulfur compounds in water system. According to Santos et al. (2019), glyphosate adsorption was favored at pH 4–10, thus the pH_{sed} ranged of 7 ~ 8 would favor the glyphosate adsorption; De Gerónimo and Aparicio (2022) pointed out that the greatest glyphosate adsorption occurred at a certain pH value and then adsorption decreased with increasing or decreasing pH, it seemed the favorable pH in H group (Fig. S2) would promoted the glyphosate adsorption onto the organic-rich sediment particles with high iron oxide minerals content in the Baiyangdian Lake (Cheng et al., 2017). Glyphosate was widely observed binding to minerals even abiotic degradation of glyphosate could happen largely following the glycine pathway rather than the AMPA (aminomethylphosphonic acid) pathway due to the cleavage of the C–N bond being disproportionately affected by the interaction of glyphosate with Mn-oxide (Li et al., 2018). Particularly, pH and aerobic/anaerobic conditions were key factors sensitively affected by the degradation of organic matter in sediment through alteration of the forms of Fe and S

(Wang et al., 2020). Overall, the significant responses of SOB in high glyphosate scenario (Table 2) rather than in glyphosate-no detected scenario and their related functions such as nitrate denitrification, Fe/Cu/Mn bioleaching, heavy metal and toxic organic compound detoxification, and antibiotic and aromatic hydrocarbons biodegradation indicated the effect of glyphosate accumulation on nitrogen, sulfur, iron, and other elements' cycling in a shallow eutrophic lake (Fig. 4).

Zabaloy et al. (2012) indicated glyphosate increased respiration in the reference grassland soil, potentially due to a stress response of glyphosate sensitive species, while it depressed respiration in the chronically exposed soil, probably as a result of selection for organisms acclimated for rapid assimilation of substrates from the cometabolic decay of the molecule. These phenomena also seemed to occur in this study (nitrogen and sulfite respiration increased but nitrite respiration decreased with glyphosate concentration in Fig. 4). Several studies have examined the effect of glyphosate on nitrification and denitrification, but the results were highly variable. Carlisle and Trevors (1986) described that denitrification in soil was either unaffected or stimulated while nitrification was inhibited by glyphosate partially due to some NO_3-N reducing microorganisms capable of using glyphosate as a nutrient source, probably P; while Zabaloy et al. (2017) reported that non-target effects of glyphosate when combined with N fertilizer on nitrifying microbes. Therefore, it was reasonable that we could conclude that the combined effects of stimulation of sulfur-based denitrifiers (such as *Thiobacillus* and *Sulfurovorum*) for nitrate removal or reduction and the inhibition of denitrification, TN/NO_3-N removal related bacteria such as *Paenisporosarcina* by glyphosate plus the highest NH_4-N contents in H group (Fig.S2(q ~ s)) resulted in the significantly increasing trend of predicted nitrification function abundance (Figs. 4a and 4b) as well as the significantly decreasing trend of predicted denitrification function abundance (Figs. 4a and 4c) with the increasing glyphosate concentration.

Interestingly, glyphosate effects on nitrification and denitrification based on some specific species such as denitrifers' stimulation and nitrifers' inhibition (Figs. 3c and 3f; Table 2) to high glyphosate concentration seemed different from those based on bacterial community's function prediction using FAPROTAX (nitrification increased but denitrification decreased with increasing glyphosate concentration, Fig. 4). The inconsistence reflected the concerned scale (e.g., individual, group or community) of functional subjects affected the stimulatory or inhibitory results of glyphosate effect. Besides, microbial functional assemblages predicted by the FAPROTAX analysis and actual performance were both impacted by physicochemical properties. Hence, the field real glyphosate distribution, concentration and actual bacterial community composition shifts in the lake sediments and the trying to detect their correlations to reveal the possible glyphosate effect on the functional bacteria at the prototype scale in-situ became the main novelty point of this work as compared to previous existing works. More information needs to be discovered at the C, N and S cycling functional gene level. And the control experiments also need to be conducted to reveal the mechanisms behind the stimulatory effect of glyphosate on Steroidobacteraceae and inhibitory effect on denitrification. Meanwhile, the dose threshold of the glyphosate effect also needs to be further studied.

5. Conclusions

This study raised attention to glyphosate effect on freshwater lake sediment-attached organisms: (1) through assessing the potential chronic risk to sediment-dwelling organisms by RQ and acute toxicity to aquatic organisms by TU and found the relevant moderate to high chronic risk and low toxicity in the Baiyangdian lake; (2) through comparing the bacterial community composition, diversity, abundance and predicted function to highlight the positively corresponding Steroidobacteraceae and inhibited denitrification in relatively high glyphosate concentration scenario. pH, NH_4-N, SOC, ß-glucosidase were the predominant factors influencing glyphosate effect. From the current

status, glyphosate seems not the predominant factor influencing bacterial community as crucial as pH, NH$_4$-N, SOC, ß-glucosidase, and the differences in bacterial community composition, diversity, activity and function among three glyphosate concentration levels may not be strictly regulated only by the difference in glyphosate concentration. However, this study was also worthwhile, supporting field evidence to verify former experimental claims such as the inhibitory effect of glyphosate on Nitrosomonadaceae species and glyphosate-induced oxidative stress. Considering that glyphosate was one node in the bacteria–environment interaction network and various factors were coevolutionary across time and space in the lake ecosystem, the observed alterations were most likely associated with the human activities including the usage of glyphosate. For the first time, we reported the probable consequences of the glyphosate enrichment in lake sediments which would induce diverse bacterial responses that contributed to crucial ecosystem functions in a subtropic natural lake under the threats of climate change and anthropogenic disturbances. But the magnitude of the effects of glyphosate in natural systems relative to that reported under controlled experimental conditions and the mechanisms of the impacts of physiochemical and biochemical alterations in ambient water qualities and nutrient concentrations need to be further studied.

In all, glyphosate may affect ecosystem functions including nutrient cycle and carbon balance through microbially mediated processes, such as nitrification/denitrification by functional microbes (e.g., Steroidobacteraceae, Sulfurimonadaceae and Desulfosarcinaceae). Quantities of glyphosate and glyphosate-based herbicides in the environment need to be further monitored for the accurate assessment of glyphosate impact on the related microbial community composition and function with the aggravation of human disturbance, laying the groundwork for improvement of agronomic management and development of bioremediation strategies for glyphosate contamination toward field success.

CRediT authorship contribution statement

Rong Xiao: Writing – original draft, Data analysis, Visualization. **Bo Kuang:** Data acquisition. **Yanping Hu:** Data acquisition and analysis. **Yaping Wang:** Investigation. **Junhong Bai:** Conceptualization, Funding acquisition. **Ling Zhang:** Data acquisition and curation. **Chen Wang:** Data curation. **Zhuoqun Wei:** Visualization. **Kegang Zhang:** Project administration, Data acquisition. **Milko A. Jorquera:** Conceptualization, Methodology. **Marco Campos:** Methodology, Project administration. **Jacquelinne J. Acuñad:** Review and editing. **Wenbin Pan:** Methodology and review.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2022.114445.

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