Response of microbial community in the soil of halophyte after contamination with tetrabromobisphenol A

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Research Article

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Abstract

Coastal wetlands are subjected to increasing tetrabromobisphenol A (TBBPA) pollution, whereas knowledge on TBBPA degradation in marine environments is lacking. The changes of bacterial communities in TBBPA-polluted soil covered with halophytes were investigated. TBBPA could be degraded in the halophyte covered saline-alkali soil in microcosm experiment. Higher TBBPA removal occurred in the soil of *Kandelia obovata* compared with soils covered with *Suaeda australis* and *Phragmites australis* within 56 days of cultivation. The rhizosphere soils of *S. australis*, *P. australis*, and *K. obovata* mainly involved the classes of *Bacteroidia*, *Gammaproteobacteria*, *Alphaproteobacteria*, and *Anaerolineae*. Additionally, manganese oxidation, aerobic anoxygenic phototrophy, and fermentation funcitions were higher in the rhizosphere soil of *K. obovata* after TBBPA addition. This study indicated that the vegetation type is a vital factor influencing the biodegradation mechanism of TBBPA and other related organic pollutants in salt marsh ecosystem.

Introduction

Tetrabromobisphenol A (TBBPA; 4,4'-isopropylidenebis (2,6-dibromophenol)) is one of the most extensively used brominated flame retardants (BFRs), covering approximately 60% of the total worldwide usage [15]. It has been incorporated into numerous consumer products including plastics, textiles, and electronic circuit boards to protect materials against ignition [4]. TBBPA could act as hormones to interfere with the endocrine systems of organisms and humans due to its structural similarity to thyroxin [11]. However, it has been frequently detected in various environmental compartments and biota (ng/L-μg/L), and it is recognized as an important hazardous pollutant due to it may persist and bio-accumulate in the food chain [6, 10, 19, 35].

Physicochemical studies have shown the fate of TBBPA in the aquatic environment [2, 20] while biotransformation process is usually considered to play a major role in determining the fate of this compound in the natural environment. Several biotic studies focused mainly on reductive debromination of TBBPA to yield less brominated bisphenol A (BPA) under ananerobic conditions, and further removal of BPA was not occurred [29, 33]. However, TBBPA is probably long-term exposed to aerobic environment before partitioning into anaerobic conditions. So far, only a few published reports on aerobic biotransformation of TBBPA and mainly focused on freshwater sediment, river sediment, activated sludge, and soil [5, 16, 17]. Previous studies presented first evidence for aerobic TBBPA biodegradation in terrestrial settings, whereas there is limited study on degradation this compound and community-level changes in the coastal marine environment [7].

Coastal marine environments have received land-based source, river flow, stromwater runoff, sewage effluent and industrial wastewater [36]. Intertidal sediments are key transitional zones between terrestrial and aquatic environments, play an important role in biogeochemical cycles and ecosystem services [26]. It has been shown that TBBPA can be quite soluble under elevated pH [31]. On the other hand, a variety of industrial parks are located along the coastline in China and varying degree concentrations of BFRs have

been detected in sediments were significantly associated with the urbanization and industrialization of the coastal zone [18]. Thus, if disposed improperly, BFRs in the recycling sites that adjacent to soil and river water could be finally transferred to the marine, which can exert potentially devastating effects on ecosystem structure and function. Wang et al. [34] found that *Bacillus*, *Erythrobacter*, *Pseudomonas*, and *Rhodococcus* were the main genus in a mangrove sediment. Jiang et al. [13] demonstrated that the predominant genus in TBBPA polluted mangrove species sediments were *Anaerolineae*, *Geobacter*, *Pseudomonas*, *Flavobacterium*, and *Azoarcus*. Thus far, the degradation of this compound in coastal environments remains obscure.

We hypothesis that the intertidal sediments that undergo flooding with seawater and exposure to the atmosphere between high and low tides should have significant impacts on TBBPA elimination. The main study aims to compare the removal of TBBPA in the intertidal sediment under different type of vegetation coverages and then track changes in bacterial community by using 16S rRNA gene based Illumina sequencing approach. This study will expand the understanding of the extent of whether indigenous marine microbes in the coastal marine environment are capable of biodegrading TBBPA and provide information on the fate of this compound.

Material And Methods

Chemicals

TBBPA (98+% purity) for spiking was purchased from Tokyo Chemical Industry Co., Ltd (Tokyo, Japan). HPLC-grade methanol was obtained from Tedia Company Inc (USA). All other chemicals were ACS grade or better.

Characterization of Sediments

Species in a coastal wetland dominated by *Suaeda australis* (defined as “SUA” hereafter), *Phragmites australis* (PHR), *Kandelia obovata* (KAN), and bare mudflats (MUD) without plant coverage used in this study were collected. The plant-covered sediments were also collected at a depth of 0–20 cm using a soil auger from the coast of Zhoushan Island (29°32’-30°04’N, 121°30’-123°25’), in May 2022. These three plant species had high survival rate and strong adaptability in the study area. Moreover, the study site have no known history of TBBPA contamination. Sediments were placed on ice, transported to the laboratory immediately, and stored at 4°C until use. Large sand grains and plant debris in all freshly collected sediments were removed and thoroughly mixed to create a homogeneous sediment sample prior to use. Sediments were aired-dried at and sieved through 2 mm sieve before analysis. The characteristics of the sediments were determined, including pH, salinity, soil organic matter (SOM) [38].

Microcosm Set-up

For a comprehensive understanding of *in situ* biological remediation, microcosm were adopted due to its authenticity, flexibility, and security which could reflect the fate of the target compound [21, 32]. Two runs were carried out involving (i) sediment + plant only, (ii) sediment + plant amended with TBBPA. TBBPA

was spiked into the microcosms at a final concentration of 20 mg/kg, and the microcosms were stirred vigorously with seawater using a stainless spatula to make homogenized sediments. Then, SUA, PHR, and KAN were separately planted in the microcosm, while sediments without plantation were used as control. For comparison, mudflats was also polluted with TBBPA, while mudflats without TBBPA contamination was used as control.

TBBPA Analysis

Sediments were periodically collected after 0, 7, 14, 28, and 56 days and used to measure the residual TBBPA. Each sample was performed in triplicate. TBBPA concentrations were determined using UPLC I-Class Xevo TQ-S (Waters Corporation, USA). Chromatographic conditions: chromatographic column, ACQUITYTM UPLC BEH C18 (2.1 mm×100 mm, 1.7 μm); injection volume, 3 μL; sample temperature, 10°C; column temperature, 40°C; mobile phase was made up of ultrapure (A) and methanol (B), and eluted at 0.3 mL/min in a linear gradient (30%A, 0–1.0 min; 70 – 5%A, 1.0-1.1 min; 5%A, 1.1-4.0 min; 5–70%A, 4.0-4.3 min; 70%A, 4.3–5.5 min). The conditions for MS were as follows: ion source, electrospray ionization (ESI-); ionspray voltage, 2.3 kV; ionization temperature, 150°C; desolvation temperature, 600°C; cone gas flow, 150 L/h; desolvation gas flow, 800 L/h.

Soil DNA Extraction and High Throughput Sequencing

After 56 days of TBBPA contamination, the rhizospheric soils were collected separately, and the initial rhizospheric soils (i.e., without TBBPA contamination) were used as control. The total genomic DNA was extracted from the soils using the Soil DNA Kit (D5625, OMEGA) following the manufacturer's protocols. The concentration and purity of the DNA were quantified by Qubit®3.0 (Life Invitrogen). The integrity of the DNA was verified by running electrophoresis on 2% agarose gel. The V4-V5 regions of 16S rRNA genes of the samples were subjected to sequencing. The 16S rRNA genes were amplified using the primers 515F (5'- GTGCCAGCMGCCGCGG-3') and 907R (5'-CCGTCAATTCMTTTRAGTTT-3') by PCR (95°C for 5 min, followed by 27 ~ 30 cycles at 95°C for 30 s, 55°C for 30 s, and 72°C for 45 s and a final extension at 72°C for 5 min). PCR reactions were performed in 30 μL mixture containing 15 μL of 2 × Phanta Master Mix, 1 μL of each primer (10 μM), and 20 ng of template DNA. Amplicons were extracted from 2% agarose gels and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, U.S.) according to the manufacturer's instructions and quantified using Qubit®3.0 (Life Invitrogen).

The amplicon library was paired-end sequenced (2×250) on an Illumina Novaseq 6000 platform (Nanjing GenePioneer Co. Ltd) according to the standard protocols. The PCR chimeras were detected and filtered out using the Vsearch uchime-denovo algorithm. The PE reads were overlapped to assemble the final tag sequences with the minimum overlap length as 10 bp with PANDAseq [25]. Quality filtering of MiSeq reads was carried out by PRINSEQ according to specific filtering conditions to obtain the highquality clean tags. The sequences with ≥ 97% similarity were assigned to the same operational taxonomic units (OTUs) by Vsearch (Version 2.15.0). The phylogenetic affiliation of each 16S rRNA gene sequence was analyzed by uclust against the silva (SSU132)16S rRNA database using confidence threshold of 90%.

The rarefaction analysis based on QIIME (1.9.1) was conducted to reveal the diversity indices. Beta-diversity using both weighted and unweighted UniFrac was calculated by QIIME 1.9.1 [23]. The functional annotation of the bacterial taxa was performed using the Functional Annotation of Prokaryotic Taxa (FAPROTAX) database [22].

Results And Discussion

Soil Characterization

The physicochemical properties of SUA, PHR, KAN, and MUD soils are summarized in Table 1. The soil properties differed between the plant-covered soils and the barren soil (i.e., MUD). The soils were alkaline, and the soil pH of MUD was higher than that of three plant-covered soils. The soil salinity was highest in the MUD soil (22.8 g/kg) and dramatically declined in the soils with different plant coverage, indicating that desalinization was occurred in the plant-covered saline-alkali soils. The levels of SOM in all plant-covered soils were lower than that of MUD soil (28.0 g/kg) without plant coverage. The reduction of SOM was probably due to the continuous rainfall with only a small amount remained in the surface soil during the sampling period in May.

Table 1
Soil physicochemical parameters

Parameter	MUD	SUA	PHR	KAN
pH	8.20	8.53	8.49	8.65
Salinity (g/kg)	22.8	7.3	3.8	4.8
SOM (g/kg)	28.0	8.66	7.67	9.46

TBBPA Elimination in Microcosm

Time profile of TBBPA removal in different plant-covered soils is shown in Fig. 1. Results showed that TBBPA degradation occurred in three plant soils as compared with the MUD soil without plant. In plant-covered soils, KAN soil exhibited higher TBBPA removal, while TBBPA could be totally removed after 56 days of cultivation. Furthermore, the residual TBBPA concentrations were in the following order: SUA > PHR > KAN. Jiang et al. [13] demonstrated that TBBPA with initial concentration of 10 mg/kg was almost degraded in *Kandelia obovata* -planted sediment during the 93-day growth period, which is in line with the present study. It was found that TBBPA biodegradation in the mangrove sediments was enhanced with fungi enzymes under aerobic conditions [37].

Predominant Bacterial Community

Microbial communities in coastal sediments play vital roles in biogeochemical cycles [14]. Bacterial communities in the rhizosphere soils of different plant coverage at the class and phylum levels are

shown in Fig. 2 and Fig. 3, respectively. As shown in Fig. 2, more than 60% of total bacterial richness belonged to four classes: *Bacteroidia*, *Gammaproteobacteria*, *Alphaproteobacteria*, and *Anaerolineae*. Higher relative abundance of *Bacteroidia* was observed in MUD soil after contaminated with TBBPA at the end of the experiment. The relative abundance of *Bacteroidia* was higher in SUA and PHR-covered soil as compared to the initial status. As for KAN-covered soil, the higher relative abundance of *Bacteroidia* and *Actinobacteria* was found after 56-day cultivation with TBBPA. At phylum levels, the major phyla were *Bacteroidota*, *Proteobacteria*, *Gemmatimonadota*, *Chloroflexi*, and *Actinobacteriota* after 56-day cultivation. *Bacteroidota* had the highest abundance in MUD soil with TBBPA. Similarly, this species had also dominated in plant-covered soils.

Previous report showed that *Bacteroidota* might participate in the decomposition of organic matter [1]. The phylum *Proteobacteria* played vital roles in the nitrogen fixation and nutrient cycling [30]. *Gemmatimonadota* might related to plants and the rhizosphere [27]. *Chloroflexi* was directly associated with organic degradation [39]. *Actinobacteriota* played important roles in removing complex organic matter, nitrogen and phosphorus [40]. Yang et al. [37] demonstrated that the classes of *Gammaproteobacteria* dramatically decreased and *Alphaproteobacteria*, *Sphingobacteria*, and *Flavobacteria* increased after addition of TBBPA in mangrove sediments after 45 days of cultivation under aerobic conditions. The mainly phyla were *Proteobacteria*, *Chloroflexi*, *Firmcutes*, *Actinobacteria*, *Bacteroidetes*, *Acidobateria*, *Nitrospira*, and *Cyanobacteria* in the *A. marina* and *K. obovata* covered sediments after 93-day greenhouse pot experiment [13]. In our previous studies, *Pseudoalteromonas* sp. isolated from the TBBPA-polluted marine surface sediment played an important role in TBBPA biodegradation [7, 8]. These results suggested that different plant species with different capacity for enrichment of potential TBBPA-degrading bacteria in the soils.

Predictive Functional Profiling of KAN

To further reveal the relationship between bacterial community and function in KAN-covered soil, ecological functions were predicted by using FAPROTAX (Fig. 4). Results showed that fumarate, nitrogen, and sulphur metabolisms were predicted in KAN soil prior to TBBPA contamination. Previous study has demonstrated that the carbon, nitrogen, and sulphur cycles were widespread in coastal wetlands [3], which is in line with this study. At the end of the 56-day cultivation with TBBPA, manganese oxidation, aerobic anoxygenic phototrophy, xylanolysis, fermentation, cyanobacteria, oxygenic photoautotrophy functions were plentiful. It has been reported that Mn(III/IV) oxides were capable of oxidizing a wide range of compounds, including antibacterial agents, endocrine disruptors, and pesticides [28]. The aerobic anoxygenic phototrophy bacteria were widely distributed in marine environments, which played a unique role in bioremediation of pollution [12]. The fermentation function might be related to bacterial debromination of TBBPA [37].

Conclusions

This study revealed the responses in bacterial community structure and functional potential in the rhizosphere soils of saline-alkali tolerant plant with TBBPA contamination. SUA, PHR, and KAN- covered

soils played important roles in TBBPA elimination. Of which, the soil of KAN exhibited higher TBBPA degradation than that of SUA and PHR. More than 60% of total bacterial richness belonged to *Bacteroidia*, *Gammaproteobacteria*, *Alphaproteobacteria*, and *Anaerolineae* classes in the rhizosphere soils of the three plants as revealed by Illumina sequencing analyses. The predicted function profiling of manganese oxidation, aerobic anoxygenic phototrophy, and fermentation played essential roles in TBBPA biodegradation in the rhizosphere soil of KAN. This study could contribute to a better understanding of the rhizosphere microbiota in coastal wetland ecosystems.

Declarations

Author Contribution

CG and FZ: conceived and designed the study. QXS and RWG: conducted the literature search and performed the experiments. WKL: were involved in the analysis and interpretation of data. CG: drafted the manuscript. QS: The study was supervised and tutored. All authors read and approved the final manuscript.

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Ethics Approval

Not applicable.

Conflict of Interest

The authors declare no competing interests.

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Figures

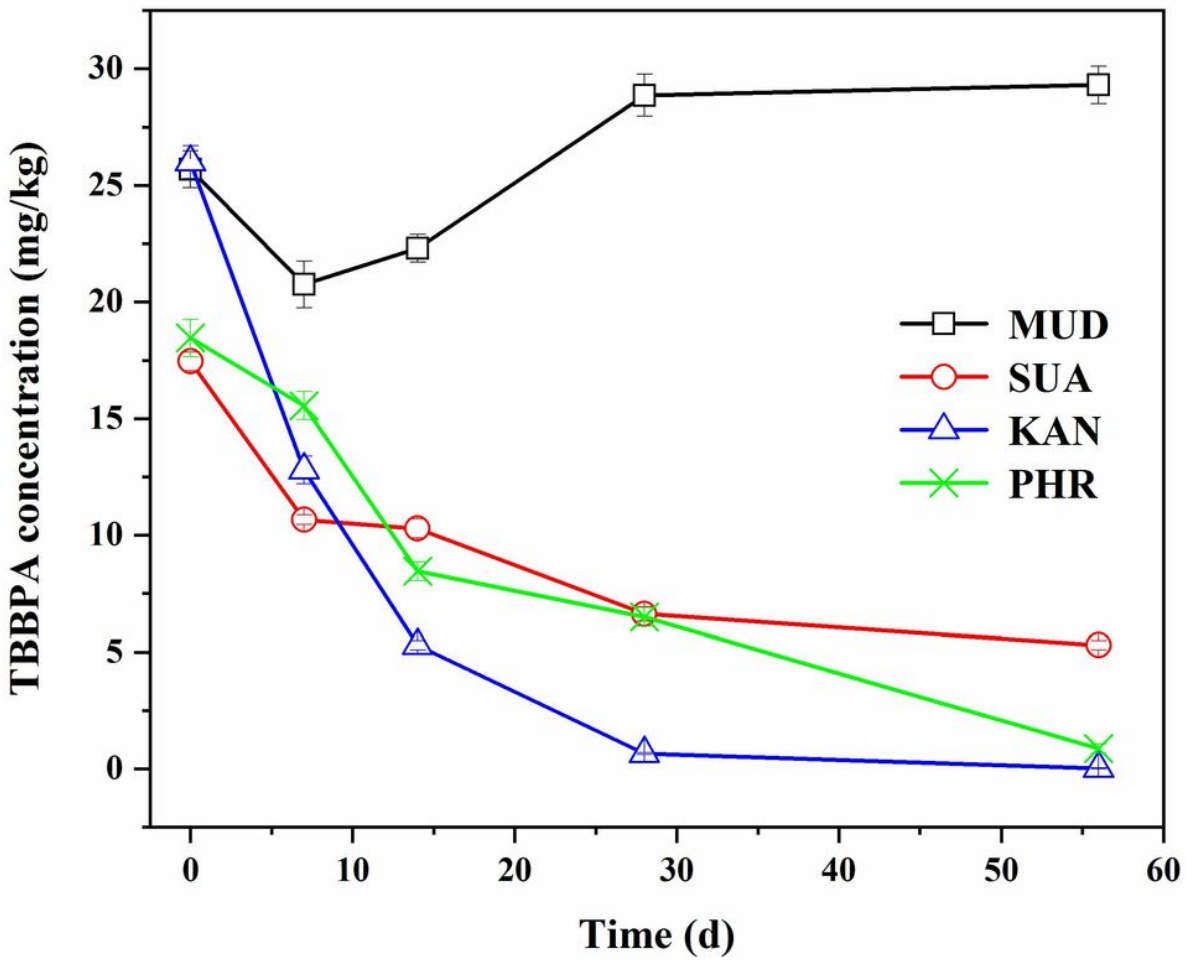

Figure 1

Time profile of TBBPA removal in different wetland soils.

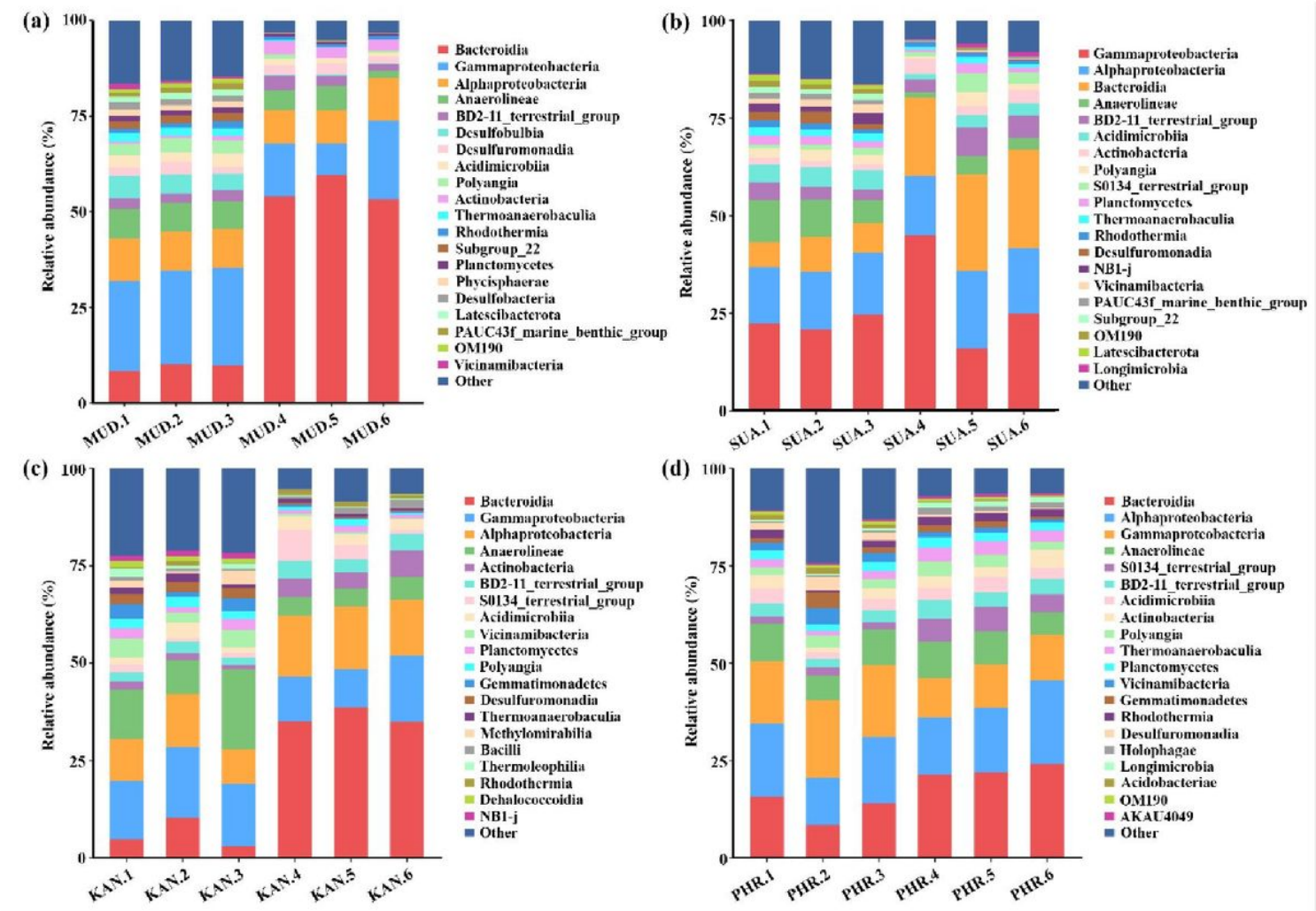

Figure 2

Bacterial community composition at class-level classification. Number 1-3 means the same experimental set (without TBBPA) performed in triplicate, while the number 4-6 means another experimental set (with TBBPA after 56 days).

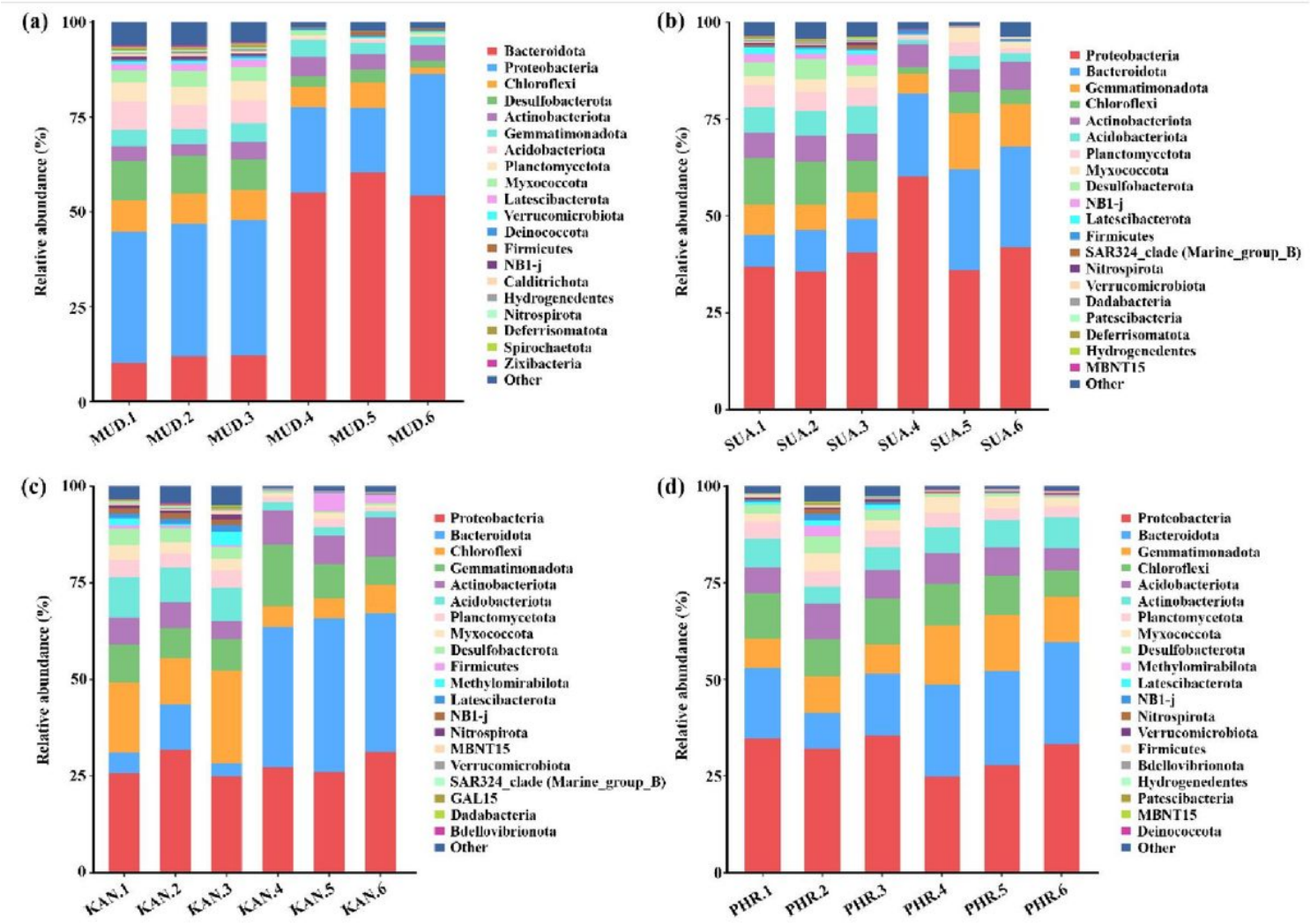

Figure 3

Bacterial community composition at phyla-level classification. Number 1-3 means the same experimental set (without TBBPA) performed in triplicate, while the number 4-6 means another experimental set (with TBBPA addition after 56 days).

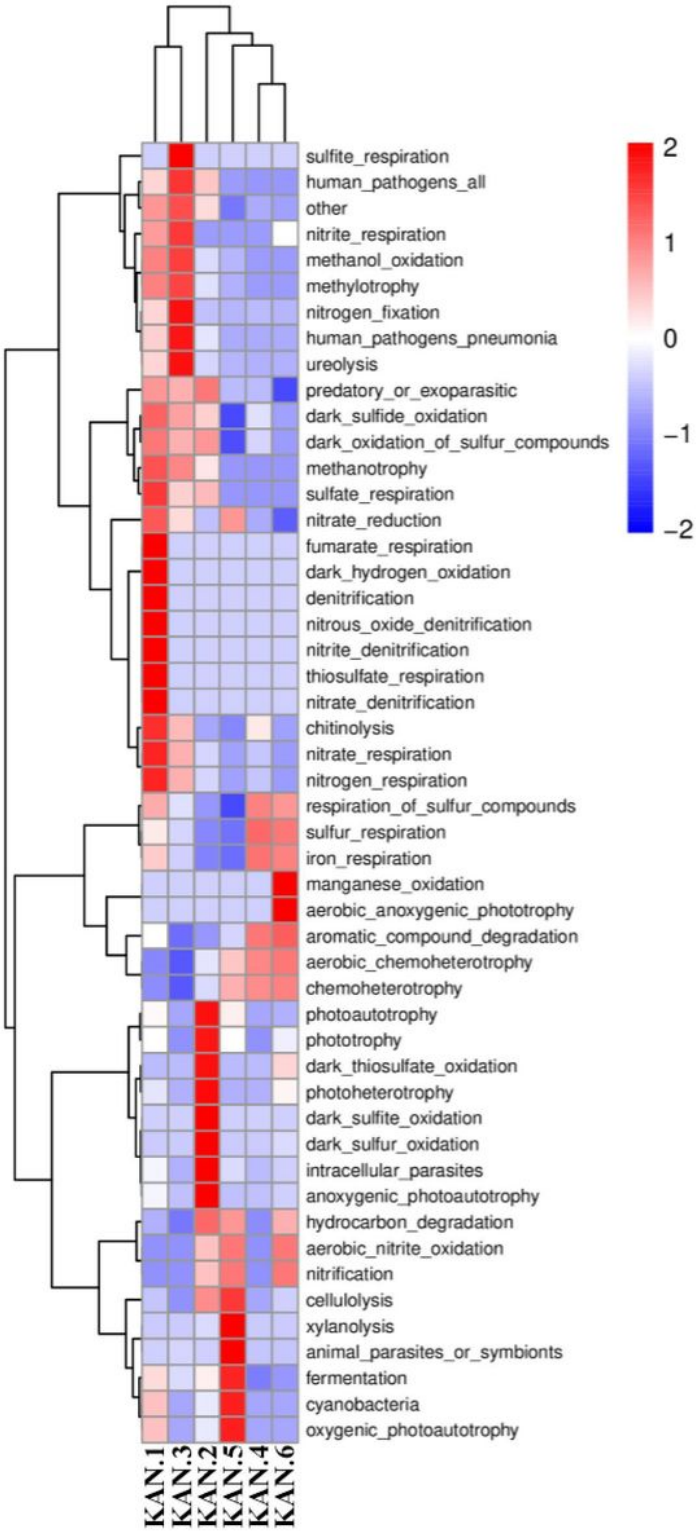

Figure 4

Functional predictions using FAPROTAX in the rhizosphere of KAN bacterial community.