Effects of multi-walled carbon nanotubes on metabolic function of the microbial community in riverine sediment contaminated with phenanthrene

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Abstract

The ecological risks of carbon nanotubes in the aquatic environment are of great concern. In this work, the effects of multi-walled carbon nanotubes (MWCNTs) on metabolic function of the microbial community in sediment contaminated with phenanthrene were investigated. The metabolic function was evaluated by Biolog ECO microplates a month later after MWCNTs of various dosages (0.5%, 1.0%, and 2.0%, w/w) were incorporated into the phenanthrene-contaminated sediment. The self-organizing map (SOM) algorithm and principal components analysis were used for data processing. The incorporation of 0.5% MWCNTs into the contaminated sediment significantly enhanced microbial activity (from 0.83 to 0.92, average well color development) and Shannon-Wiener diversity index (from 3.19 to 3.23) compared with the blank control. Clustering the microbial communities in different treatments on the trained SOM suggested that phenanthrene had a greater impact on the metabolic function of sediment microbial communities than MWCNTs in the experiments. The metabolic differences caused by MWCNTs were mainly reflected in the utilization of amino acids and polymers. The results of this study may contribute to evaluating the ecological risks of MWCNTs in the aquatic environment and developing the secure applications of MWCNTs.

1. Introduction

Carbon nanotubes (CNTs) are one-dimensional carbonaceous nanomaterials with a cylindrical graphite structure [1, 2]. Since they were first reported [3], continuous research has been conducted on the unique chemical, electrical, mechanical, optical, and thermal properties of CNTs. CNTs have found wide applications in biosensors, coatings and films, composite materials, energy storage, medical devices, microelectronics, and environment [1, 4-7]. The global market size of CNTs was $2.26 billion in 2015, and was estimated to reach $5.64 billion by 2020 at a compound annual growth rate of 20.1% [8]. As the production and application of CNTs increase, these nanomaterials will inevitably be released into the environment. Aquatic sediment is one of the main sinks of CNTs in the natural environment [9]. It is estimated that the concentration of CNTs in sediment has reached a level of μg/kg and will continue to increase [10, 11].

The potential ecological risks of CNTs in the aquatic environment are of great concern [12-14]. Due to the unique structural characteristics, CNTs may have toxic effects on aquatic organisms such as fish, crustaceans, algae, and bacteria [15, 16]. On the other hand, CNTs can interact with coexisting contaminants and alter their fates and environmental risks [17]. Our previous work has shown that incorporating CNTs of 0.5%, 1.0%, and 1.5% (w/w) into sediment significantly impeded the transport of sodium dodecyl benzene sulfonate through riverine sediment columns and increased the retardation factor from 5.10 to 42.7, 60.6, and 92.6, respectively [18]. Qian et al. [19] found that CNTs would change the specific surface area and zeta potential of

sediment, and the adsorption capacity of sediment for phosphorus increased from 0.664 to 0.996 mg/g with the increase of CNT content from 0% to 5% (w/w). Abbasian et al. [20] added CNTs of 0.1%, 0.5%, and 1.0% (w/w) to a fresh water sediment contaminated with crude oil, and found that the CNTs could increase the microbial abundance and the effects depended on both CNT dosage and oil concentration. Recent research by Myer et al. [21] showed that CNTs could reduce the toxicity of diphenhydramine to *Ceriodaphnia dubia* in sediment exposure, and their results suggested that the sediment containing carboxylated CNTs of 318 μg/g caused a 78.7%–90.1% decrease in 48-h mortality. Despite the progress that has been made in recent years, more work is needed to improve the knowledge of CNT ecological risks in the aquatic environment.

Sediment microbial community is sensitive to the variations of sediment environment and anthropogenic disturbance, which enables it to be an indicator for assessing the ecological risks of contaminants in the aquatic environment [22-24]. Most available studies about the effects of CNTs on sediment microbial communities focused on the microbial composition and structure diversity based on molecular methods such as pyrosequencing, terminal restriction fragment length polymorphism, and denaturing gradient gel electrophoresis [20, 25, 26]. These commonly used methods are helpful for analyzing microbial community structure, but are limited in reflecting the ecological relevance of community structure. The Biolog ECO microplate is a useful tool to study the microbial metabolism based on carbon utilization, and can provide valuable information about the ecological functions of

microbial community [27, 28]. Thus, the Biolog ECO microplate is used in this work. Polycyclic aromatic hydrocarbons (PAHs) are a group of persistent organic pollutants widely found in oil-contaminated sediments. Phenanthrene is a PAH with three benzene rings and commonly used as model PAH [29-31]. It is of great environmental concern because of its toxicity and persistence in the aquatic environment [32, 33]. In this study, multi-walled carbon nanotubes (MWCNTs) of various dosages (0.5%, 1.0%, and 2.0%, w/w) were incorporated into the sediment contaminated with phenanthrene. The primary objective of this work is to determine the effects of MWCNTs on the metabolic function of microbial community in the contaminated sediment. The results of this study will benefit the understanding of CNT-induced changes in sediment microbial community and provide valuable information for risk assessment of CNTs in the aquatic environment.

2. Materials and methods

2.1. Sediment and MWCNTs

Surface sediment samples (0–15 cm) were collected from five sites located in Changsha section of the Xiangjiang River, which is the largest river in Hunan Province, China. This river runs from south to north, and flows into the Dongting Lake belonging to the Yangtze River system. Three separate samples were taken from every site, and immediately transported to the laboratory after sampling. The collected samples were air-dried, crushed, and sieved through a sieve with a mesh aperture of one mm. Samples from different sites were manually homogenized prior to use. No

native PAHs were detected in the sediment. Phenanthrene was artificially spiked into the sediment (detailed spiking procedures are provided in the Supplementary Material). The final concentration of phenanthrene was detected at 2.03 mg/g in the prepared sediment. This level of phenanthrene concentration was chosen to match the total amount of residual PAHs commonly found in soil and sediment heavily contaminated with oil [34-37]. MWCNTs with a CNT content > 90%, a length of 5–20 μm, and an outer diameter of 10–20 nm were used in this study. They were purchased from Chengdu Organic Chemistry Co., Chinese Academy of Sciences, Chengdu, China.

2.2. Experimental design

Eight treatments were performed in the experiments. The details of the experimental design are displayed in Table 1. For the sediments contaminated with phenanthrene, MWCNTs were respectively added at weight ratios of 0% (T2), 0.5% (T4), 1.0% (T6), and 2.0% (T8), and the mixtures were manually homogenized. The same procedures were performed on uncontaminated sediments, and these treatments were used as controls (T1, T3, T5, and T7). Previous studies have shown that MWCNTs can significantly alter soil microbial activity and pollutant bioavailability at relatively high concentrations (> 0.5%, w/w), but have little effects on those at low concentrations [38-40]. Considering that our primary objective is to study the effects of MWCNTs on metabolic function of microbial community in the sediment, though the concentrations of MWCNTs used in this study (0.5%–2.0%, w/w) are relatively

high, they are suitable for purpose and may correspond to practical cases of accidental spills or CNT waste accumulation [39]. After incorporating CNTs into the sediment, ultrapure water was slowly added at a water/sediment ratio of 5:1 (v/w) to simulate the water-sediment system. The overlying water was removed at the end of one-month treatment and sediment samples were taken out for Biolog ECO microplate experiments.

Table 1

Experimental design for investigating the effects of MWCNTs on metabolic function of the microbial community in sediment contaminated with phenanthrene.

Treatment	Components		
Treatment 1 (T1)	Sediment	–	–
Treatment 2 (T2)	Sediment	Phenanthrene (0.2%, w/w)	–
Treatment 3 (T3)	Sediment		MWCNTs (0.5%, w/w)
Treatment 4 (T4)	Sediment	Phenanthrene (0.2%, w/w)	MWCNTs (0.5%, w/w)
Treatment 5 (T5)	Sediment	–	MWCNTs (1.0%, w/w)
Treatment 6 (T6)	Sediment	Phenanthrene (0.2%, w/w)	MWCNTs (1.0%, w/w)
Treatment 7 (T7)	Sediment	–	MWCNTs (2.0%, w/w)
Treatment 8 (T8)	Sediment	Phenanthrene (0.2%, w/w)	MWCNTs (2.0%, w/w)

2.3. Biolog ECO microplate experiments

The Biolog ECO microplate (Biolog Inc., California, USA) has 96 wells containing 31 kinds of carbon sources (2 amines, 6 amino acids, 10 carbohydrates, 7 carboxylic acids, 2 phenolic compounds, and 4 polymers) in triplicate and three wells without carbon sources (Table S1). Each well of the microplate also contains a colorless tetrazolium dye, which can be reduced to a purple formazan when the

carbon source is utilized by microorganisms [41]. The color shades of purple reflect the difference in utilization of carbon sources. For microbial community analysis, 10 g (dry weight) of sediments were added into 90 mL of sterile NaCl solution (0.85%, w/v), and the suspension was shaken at 200 rpm for 30 min. After standing for 30 min, 150 μL of supernatant were added to each well of the microplate. The inoculated microplates were incubated at 25 °C for 7 days. Absorbance was recorded at 590 nm (colour + turbidity) and 750 nm (turbidity) with a microplate spectrophotometer (Thermo Scientific Multiskan GO, USA) every 24 h [42].

2.4. Data processing

Average well color development (AWCD), which indicates the microbial activity was calculated with the following equation [27,42]:

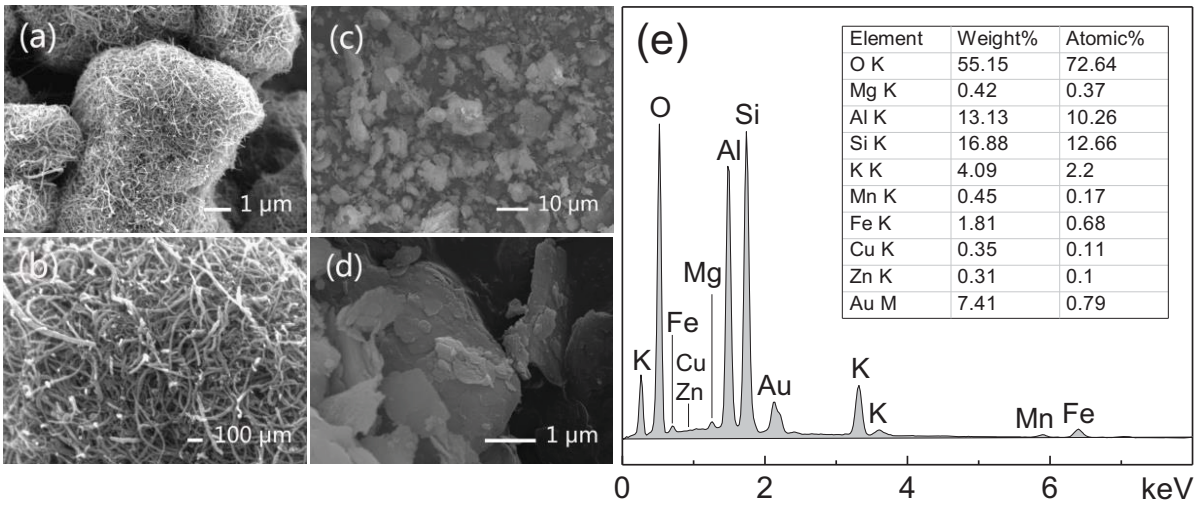
$$\mathrm{AWCD} = \frac{1}{31}\sum\left(C_i - R\right) \quad (3)$$

where C_i is the difference value of absorbance at 590 and 750 nm from the wells containing carbon sources, and R is the difference value of the blank well without carbon sources. The Shannon-Wiener diversity index (H') of the sediment microbial community was calculated according to the following equation [27]:

$$H' = -\sum\left(p_i \times \ln\ p_i\right) \quad (4)$$

where $p_i = (C_i - R) / \sum(C_i - R)$, C_i and R have the same meaning as those in Eq. (3). One-way analysis of the variance (ANOVA) followed by the least significant difference (LSD) test at a significance level of 0.05 was performed to compare the mean values. The self-organizing map (SOM) algorithm was used to classify the

165 microbial communities in different treatments based on the utilization of each carbon
166 source in the microplate. SOM is an artificial neural network widely used for
167 visualizing input data of high dimensionality in a two dimensional space through the
168 training of unsupervised learning [43]. The Matlab software and SOM toolbox were
169 used to implement the SOM algorithm according to previously reported instructions
170 [43-45]. The input data were normalized to the range between zero and one with a
171 linear transformation. The principal component analysis (PCA) was conducted to
172 determine how the microbial communities are different based on the microplate data.
173 The principal components that could explain over 5% of the total variance were
174 involved in the analysis. The PCA and SOM analysis were performed with microplate
175 data at 168 h of incubation [46].
176
177 **3. Results and discussion**
178 *3.1. Characterization of the sediment and MWCNTs*
179 The micromorphology of sediment and MWCNTs was characterized by scanning
180 electron microscope (SEM). The typical tubular structure of MWCNTs was observed
181 and the sediment particles varied in size and shape (Fig. 1). The measured pH value of
182 the sediment was 7.92 and it had an organic carbon content of 1.63% (w/w). Energy
183 disperse spectroscopy (EDS) analysis was performed to determine the elemental
184 composition of sediment, and the result showed that oxygen, silicon, aluminum, and
185 potassium were the main components (Fig. 1e).



Element	Weight%	Atomic%
O K	55.15	72.64
Mg K	0.42	0.37
Al K	13.13	10.26
Si K	16.88	12.66
K K	4.09	2.2
Mn K	0.45	0.17
Fe K	1.81	0.68
Cu K	0.35	0.11
Zn K	0.31	0.1
Au M	7.41	0.79

Fig. 1. SEM images of the used MWCNTs (a and b) and sediment (c and d), and EDS analysis of the sediment (e).

3.2. Effect of MWCNTs on microbial metabolic function

3.2.1. Microbial activity and diversity index

The overall microbial activity in different treatments is indicated by AWCD and shown in Fig. 2a. Compared with the blank control without MWCNTs and phenanthrene (T1), obvious increase of microbial activity was observed in the sediments with 0.5% MWCNTs (T3 and T4). For the phenanthrene-contaminated sediments, no significant differences of microbial activity were found between the sediment without MWCNTs (T2) and other groups except T4. Comparing the results of T3–T8, the sediments with 0.5% MWCNTs showed higher microbial activity than the sediments with higher content of MWCNTs. These results suggested that the addition of 0.5% MWCNTs could enhance the microbial activity in sediment. The reason might be that these MWCNTs acted as microenvironments for the attachment and growth of microbes and protected them from predation [20]. In the aquatic

environment, biofilms are the main form of microbial life, and the formation of biofilms can be promoted in the presence of CNTs due to the abundant sites on CNTs for microbial attachment [47]. Additionally, CNTs can adsorb external nutrients that are required for microbial growth, which improves the availability of nutrients [48]. When more MWCNTs (1.0% and 2.0%) were added, the increase of microbial activity might be inhibited due to the antibacterial effect of a large number of MWCNTs [38]. Bulk MWCNTs can envelop the adsorbed microorganisms and isolate them from the external environment, thus preventing their growth [4, 49].

Although the differences of overall microbial activity between certain treatment groups are not significant, the Shannon-Wiener diversity index (H') varies considerably (Fig. 2b). Shannon-Wiener diversity index is a widely used species diversity index that takes into account the species richness and evenness, and provides heterogeneity information for microbial community studies [50]. Compared with the blank control (T1), the addition of 0.5% MWCNTs significantly increased the diversity index of microbial community in phenanthrene-contaminated sediment (T4). The treatments with addition of only MWCNTs (T3, T5, and T7) showed lower diversity index than those with addition of both MWCNTs and phenanthrene (T4, T6, and T8). It has been reported that microbial diversity is positively correlated with environmental heterogeneity [51]. Thus, when multiple exogenous substances were incorporated, the microbial diversity increased with greater sediment heterogeneity. Additionally, no significant differences in the diversity index were found between the contaminated sediment without MWCNTs (T2) and those sediments incorporated

with MWCNTs (T4, T6, and T8). This result indicated that the addition of MWCNTs had little influence on microbial species of the sediment contaminated with phenanthrene in the experiments. Further analysis was performed with SOM algorithm and PCA to illustrate the microbial differences in utilization of each carbon source in the microplates.

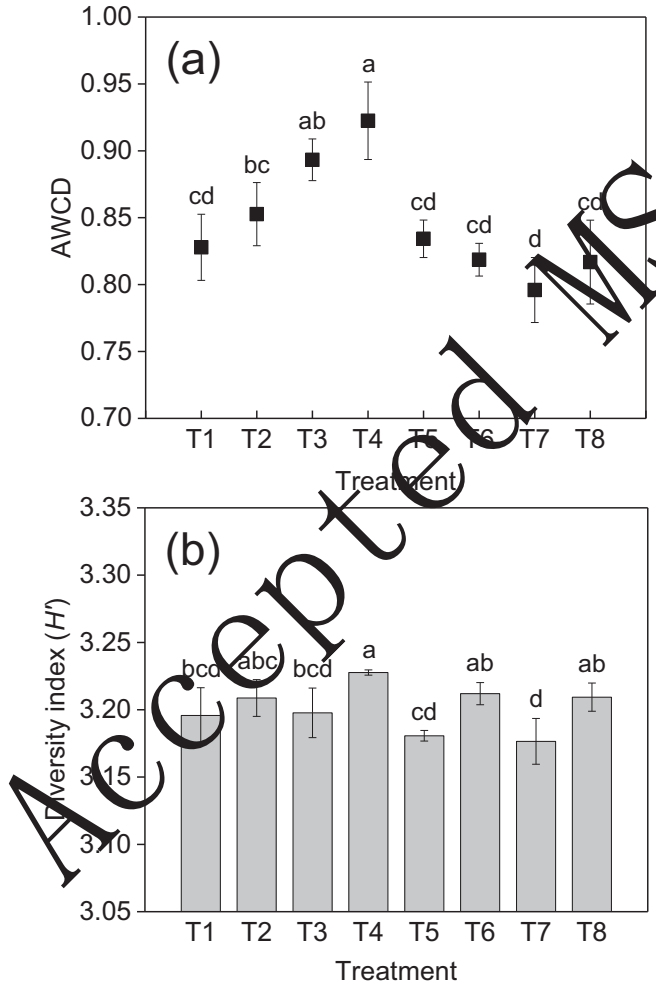


Fig. 2. AWCD of the Biolog ECO microplates (a) and Shannon-Wiener diversity index (b) from different treatment groups at 168 h. Different letters denote statistically significant differences ($P < 0.05$) between groups.

236 *3.2.2. SOM analysis*

237 The utilization of carbon sources in the microplate by microbial communities in
238 different treatments was analyzed with SOM algorithm. Through SOM analysis, the
239 relationship between the clusters of sediment microbial communities and the
240 utilization of each carbon source can be clearly identified. A 28-unit map (7 × 4) was
241 selected as the best compromise between a low quantization error and a number of
242 neurons close to the number of samples. The k-means algorithm was applied to cluster
243 the trained map and classify the microbial communities in different treatments into
244 four groups (Fig. 3, cluster I–IV). Microbial communities in the same cluster
245 exhibited more similar metabolic characteristics towards the 31 carbon sources. It was
246 found that all the control treatments (T1, T3, T5, and T7) were in the cluster I. The
247 main characteristic of this cluster was that the sediments in these treatments did not
248 contain phenanthrene, thus it suggested that phenanthrene had a greater impact on the
249 metabolic function of sediment microbial communities than MWCNTs in the
250 experiments. This could be because phenanthrene is easier to be bioaccumulated and
251 involved in the microbial metabolism than MWCNTs [14, 52]. The treatment T2
252 appeared in the cluster II and III, but not the cluster IV. This result indicated that, for
253 the phenanthrene-contaminated sediment, the addition of 2.0% MWCNTs (T8) caused
254 more significant differences in the microbial metabolic function than other treatments
255 (T4 and T6). Freixa et al. [15] have reported that the dose-effect relationships between
256 CNT exposure and biological response are not linear. Aquatic organisms may have
257 adaptive mechanisms to the exposure of CNTs at relatively low concentrations, but

suffer distinct negative impacts when the concentration of CNTs is extremely high. On the other hand, the incorporation of 2.0% MWCNTs caused higher retention of phenanthrene in sediment, which was shown with lower phenanthrene concentration in the overlying water (Fig. S1). Co-exposure to these MWCNTs and phenanthrene resulted in more significant differences in the metabolic function.

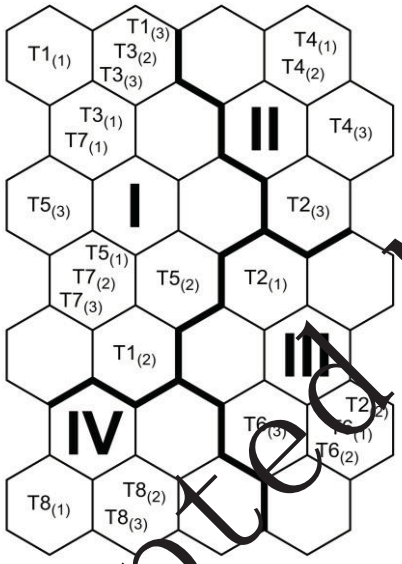


Fig. 3. Distribution and clustering of microbial communities in different treatments (T1–T8) on the SOM based on the utilization of carbon sources. Clusters (I–IV) were derived from k-means algorithm applied to the trained SOM. Numbers in the brackets indicate the repetitions.

The component planes that show the absorbance for each carbon source on the trained SOM were displayed in Fig. 4. At the top left corner of Fig. 4, a unified distance matrix (U-matrix) visualizes the relative distances between adjacent neurons. The matrix can help to identify clusters of microbial communities in different treatments on the SOM [53]. On the whole, L-asparagine, tween 80, D-mannitol, and

274 *N*-acetyl-D-glucosamine were highly utilized by the sediment microbes (shown with a
275 minimum absorbance value more than 1.20). D-xylose, 2-hydroxy benzoic acid,
276 phenylethylamine, and D, L-α-glycerol phosphate were not utilized by the sediment
277 microbes (shown with a maximum absorbance value less than 0.15). Most of the
278 maximum absorbance values appeared in the neurons at the top left, top right, and
279 bottom left corner, which respectively correspond to T1 (or T3), T4, and T8 by
280 reference to the microbial community distribution in Fig. 3. The microbial
281 communities in T1 and T3 showed the highest utilization for L-arginine, L-asparagine,
282 i-erythritol, itaconic acid, and D-malic acid, while D-galactonic acid γ-lactone,
283 glycogen, D-glucosaminic acid, and putrescine were most effectively utilized by the
284 sediment microbes in T8. The component planes of 15 carbon sources (including
285 β-methyl-D-glucoside, D-galacturonic acid, tween 80, D-mannitol, 4-hydroxy benzoic
286 acid, L-serine, α-Cyclodextrin, *N*-acetyl-D-glucosamine, γ-hydroxy butyric acid,
287 L-threonine, glycyl-L-glutamic acid, D-cellobiose, glucose-1-phosphate, α-keto
288 butyric acid, and α-D-lactose) displayed the maximum absorbance values in the
289 neurons corresponding to T4. The high utilization of these carbon sources by the
290 sediment microbes in T4 could account for the higher activity and diversity index of
291 microbial community in the contaminated sediment with 0.5% MWCNTs.

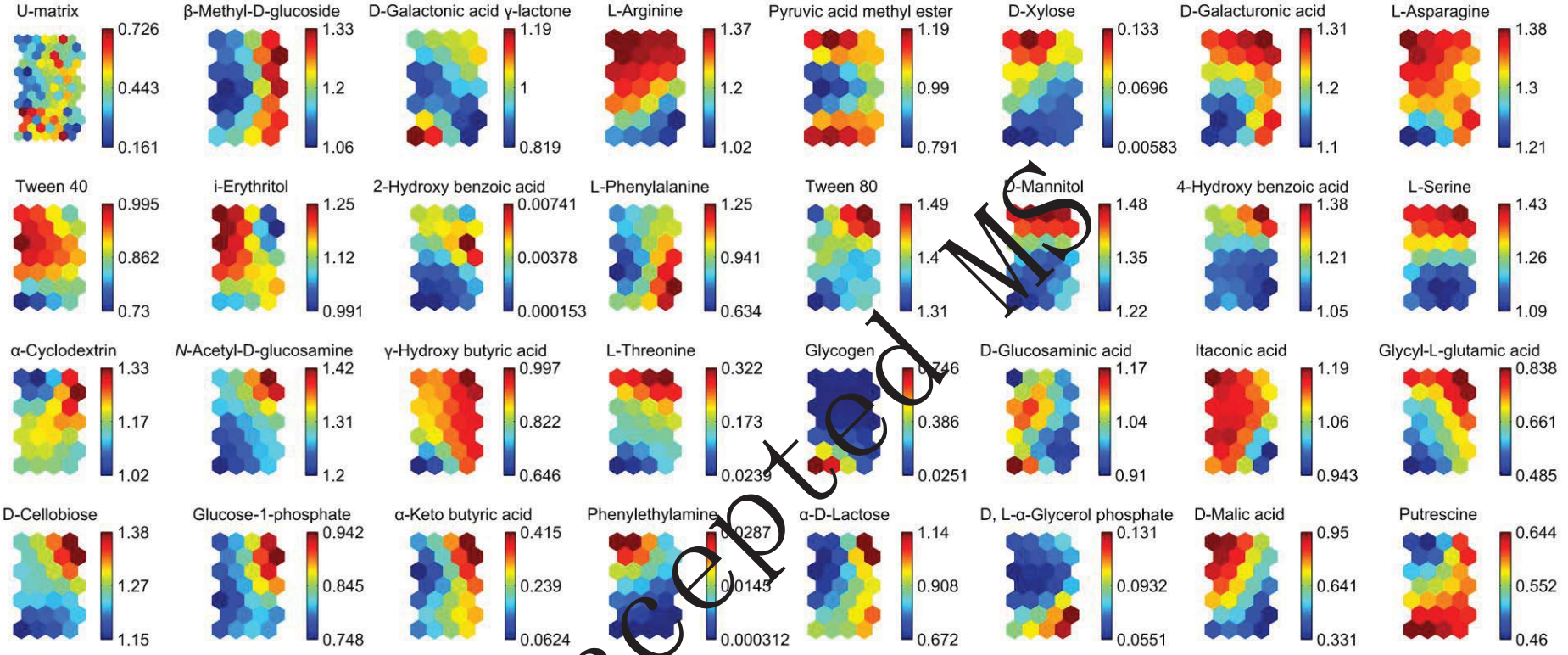


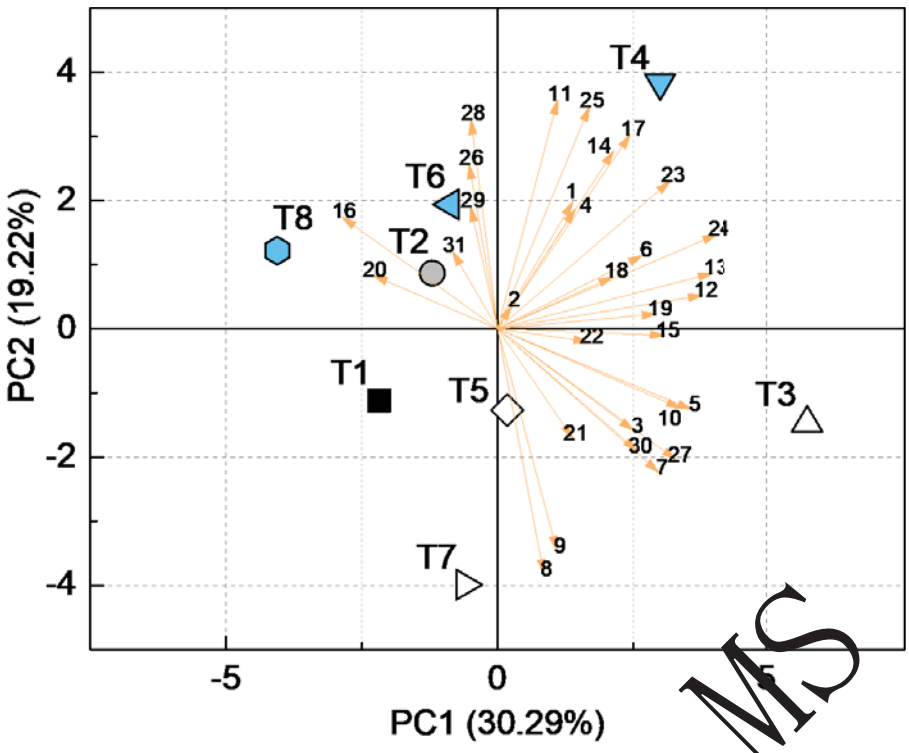
Fig. 4. Analysis of the absorbance for each carbon source on the trained SOM. Colors indicate the value of the absorbance of each unit in the map, according to the colorbars on the right. Numbers beside the colorbars correspond to the absorbance values. A higher absorbance value indicates a higher degree of carbon source utilization. Each map corresponding to one carbon source should be compared to the map representing the distribution of microbial communities in different treatments presented in Fig. 3. In the U-matrix, additional hexagons exist and visualize the distances between all pairs of adjacent map units.

298 *3.2.3. Principal component analysis*

299 PCA was further performed to distinguish the microbial communities in different
300 treatments based on the utilization of 31 carbon sources. PCA is a statistical procedure
301 that orthogonally transforms a set of original variables into linearly uncorrelated
302 variables which are called principal components [54]. The results of PCA analysis are
303 displayed by the biplot method with the first two principal components (Fig. 5). The
304 first principal component (PC1) and the second principal component (PC2) explained
305 30.29% and 19.22% of the original variables, respectively. Vectors in the figure
306 indicate the direction in which the utilization of carbon source increases. Most of the
307 vectors are in the first and the fourth quadrants where T4 and T3 locate in. These
308 vectors indicate the specific carbon sources that contributed to the enhanced metabolic
309 function of microbial communities in the sediments incorporated with 0.5%
310 MWCNTs.

311 In the principal component space, the distinctions of different treatments can be
312 related to the differences in carbon source utilization by examining the correlation of
313 carbon source variables to the principal components [55]. Important carbon sources
314 for distinguishing the microbial communities of different treatments are displayed in
315 Table 2. At least half of these carbon source variables are explained by PC1 or PC2.
316 According to the results of Table 2 and the distribution of T1–T8 in Fig. 5, the PC1
317 distinguishes the treatments with different MWCNT addition. The metabolic
318 differences caused by MWCNTs are mainly reflected in utilizing five out of six amino
319 acids, three out of ten carbohydrates, two out of seven carboxylic acids, and three out

of four polymers. Microbial communities in the sediments with 0.5% MWCNTs (T3 and T4) showed a higher response in utilizing five amino acids (L-arginine, L-asparagine, L-serine, L-threonine, and glycyl-L-glutamic acid), three carbohydrates (D-mannitol, *N*-acetyl-D-glucosamine, and D-cellobiose), two carboxylic acids (D-galacturonic acid and D-malic acid), and a polymer (tween 80), but a lower response in utilizing other two polymers (α-cyclodextrin and glycogen) than those in other treatments. The PC2 distinguishes the treatments with and without phenanthrene. It is positively correlated to L-phenylalanine, *N*-acetyl-D-glucosamine, glucose-1-phosphate, α-D-lactose, α-keto butyric acid, and 4-hydroxy benzoic acid, but negatively correlated to i-erythritol and tween 40. Comparing the results of PCA and SOM analysis, the high (or low) responses to these important carbon sources correspond to high (or low) absorbance values on the SOM. For example, PC2 is negatively correlated to i-erythritol with a correlation coefficient of −0.730, and the scores of treatments without phenanthrene (T1, T3, T5, and T7) on the PC2 are negative. Correspondingly, the neurons representing T1, T3, T5, and T7 on the SOM of i-erythritol showed higher absorbance values with red color. These results can help to understand the effects of MWCNTs on metabolic function of the microbial community in sediment contaminated with phenanthrene.



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339 **Fig. 5.** Principal component analysis of the microbial metabolism of 31 carbon sources in

340 different treatments (T1–T8). The results are displayed by the biplot method using the

341 variable eigenvectors scores. Vectors indicate the direction in which the utilization of carbon

342 source increases. 1. β-methyl-D-glucoside; 2. D-galactonic acid γ-lactone; 3. L-arginine; 4.

343 pyruvic acid methyl ester; 5. D-xylose; 6. D-galacturonic acid; 7. L-asparagine; 8. tween 40; 9.

344 i-erythritol; 10. 2-hydroxy benzoic acid; 11. L-phenylalanine; 12. tween 80; 13. D-mannitol;

345 14. 4-hydroxy benzoic acid; 15. L-serine; 16. α-cyclodextrin; 17. *N*-acetyl-D-glucosamine; 18.

346 γ-hydroxy butyric acid; 19. L-threonine; 20. glycogen; 21. D-glucosaminic acid; 22. itaconic

347 acid; 23. glycyl-L-glutamic acid; 24. D-cellobiose; 25. glucose-1-phosphate; 26. α-keto

348 butyric acid; 27. phenylethylamine; 28. α-D-lactose; 29. D, L-α-glycerol phosphate; 30.

349 D-malic acid; 31. putrescine.

350

351

352 **Table 2**

353 Correlation coefficients (*r*) of important carbon source variables to the first two principal

354 components.

PC1		PC2	
Carbon source	*r*	Carbon source	*r*
Amino acids		***Amino acids***	
L-Arginine	0.568	L-Phenylalanine	0.753
L-Asparagine	0.671		
L-Serine	0.697	***Carbohydrates***	
L-Threonine	0.664	i-Erythritol	−0.730
Glycyl-L-glutamic acid	0.719	*N*-Acetyl-D-glucosamine	0.639
		Glucose-1-phosphate	0.731
Carbohydrates		α-D-Lactose	0.688
D-Mannitol	0.898		
N-Acetyl-D-glucosamine	0.553	***Carboxylic acids***	
D-Cellobiose	0.915	α-Keto butyric acid	0.542
Carboxylic acids		***Phenolic compounds***	
D-Galacturonic acid	0.606	4-Hydroxy benzoic acid	0.585
D-Malic acid	0.582		
		Polymers	
Polymers		Tween 40	−0.806
Tween 80	0.855		
α-Cyclodextrin	−0.648		
Glycogen	−0.521		

355

356

357 **4. Conclusions**

358 Overall, the experimental results suggested that MWCNTs of high concentrations

359 (0.5%–2.0%, w/w) could bring about significant changes in the metabolic function of

360 sediment microbial communities. The phenanthrene-contaminated sediment with

361 0.5% MWCNTs showed the highest microbial activity and Shannon-Wiener diversity

362 index. The metabolic differences caused by MWCNTs mainly reflect in the utilization

363 of 13 carbon sources (including five amino acids, three carbohydrates, two carboxylic

364 acids, and three polymers) on the Biolog ECO microplate. Clustering the microbial

365 communities in different treatments on the trained SOM suggested that phenanthrene

had a greater impact on the metabolic function of sediment microbial communities than MWCNTs in the experiments. The variations of microbial metabolic function provide valuable information for evaluating the ecological risks of CNTs in the aquatic environment. This study is a community level physiological profiling. Further research is needed to develop the knowledge with other methods of molecular biology and ecology.

Acknowledgements

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