Carbon 144 (2019) 1–7

Contents lists available at ScienceDirect

Carbon

journal homepage: www.elsevier.com/locate/carbon

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

Biao Song [a, b, 1], Ming Chen [a, b, 1], Shujing Ye [a, b, 1], Piao Xu [a, b, 1], Guangming Zeng [a, b, *], Jilai Gong [a, b, **], Juan Li [a, b], Peng Zhang [a, b], Weicheng Cao [a, b]

[a] College of Environmental Science and Engineering, Hunan University, Changsha 410082, PR China
[b] Key Laboratory of Environmental Biology and Pollution Control (Hunan University), Ministry of Education, Changsha 410082, PR China

ARTICLE INFO

Article history:
Received 16 October 2018
Received in revised form
2 December 2018
Accepted 3 December 2018
Available online 4 December 2018

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

* Corresponding author. College of Environmental Science and Engineering, Hunan University, Changsha 410082, PR China.
** Corresponding author. College of Environmental Science and Engineering, Hunan University, Changsha 410082, PR China.
E-mail addresses: zgming@hnu.edu.cn (G. Zeng), jilaigong@hnu.edu.cn (J. Gong).
[1] These authors contributed equally to this article.

https://doi.org/10.1016/j.carbon.2018.12.016
0008-6223/

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.

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 (color + 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]:

$$\mathrm{AWCD} = \frac{1}{31}\sum(C_i - R) \tag{1}$$

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]:

$$H' = -\sum(p_i \times \ln p_i) \tag{2}$$

where $p_i = (C_i - R)/\sum(C_i - R)$, C_i and R have the same meaning as

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)

those in Eq. (1). 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 microbial communities in different treatments based on the utilization of each carbon source in the microplate. SOM is an artificial neural network widely used for visualizing input data of high dimensionality in a two dimensional space through the training of unsupervised learning [43]. The Matlab software and SOM toolbox were used to implement the SOM algorithm according to previously reported instructions [43–45]. The input data were normalized to the range between zero and one with a linear transformation. The principal component analysis (PCA) was conducted to determine how the microbial communities are different based on the microplate data. The principal components that could explain over 5% of the total variance were involved in the analysis. The PCA and SOM analysis were performed with microplate data at 168 h of incubation [46].

3. Results and discussion

3.1. Characterization of the sediment and MWCNTs

The micromorphology of sediment and MWCNTs was characterized by scanning electron microscope (SEM). The typical tubular structure of MWCNTs was observed and the sediment particles varied in size and shape (Fig. 1). The measured pH value of the sediment was 7.92 and it had an organic carbon content of 1.63% (w/w). Energy disperse spectroscopy (EDS) analysis was performed to determine the elemental composition of sediment, and the result showed that oxygen, silicon, aluminum, and potassium were the main components (Fig. 1e).

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].

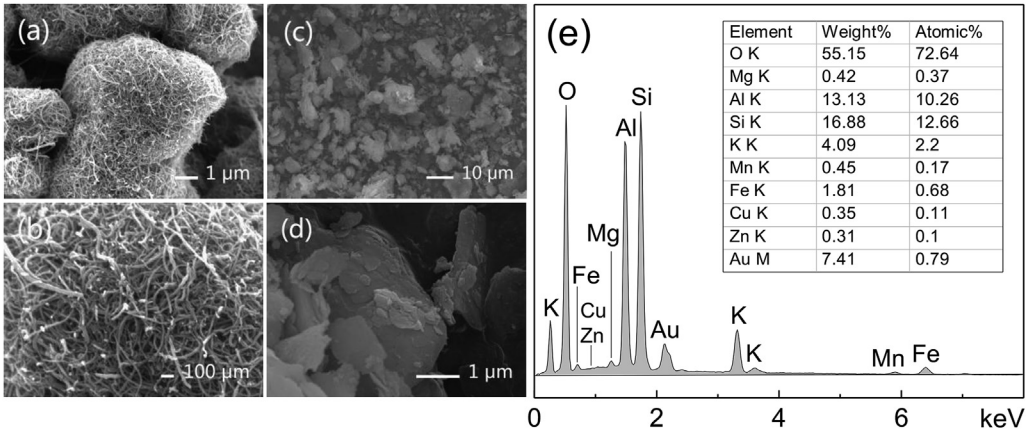

Fig. 1. SEM images of the used MWCNTs (a and b) and sediment (c and d), and EDS analysis of the sediment (e).

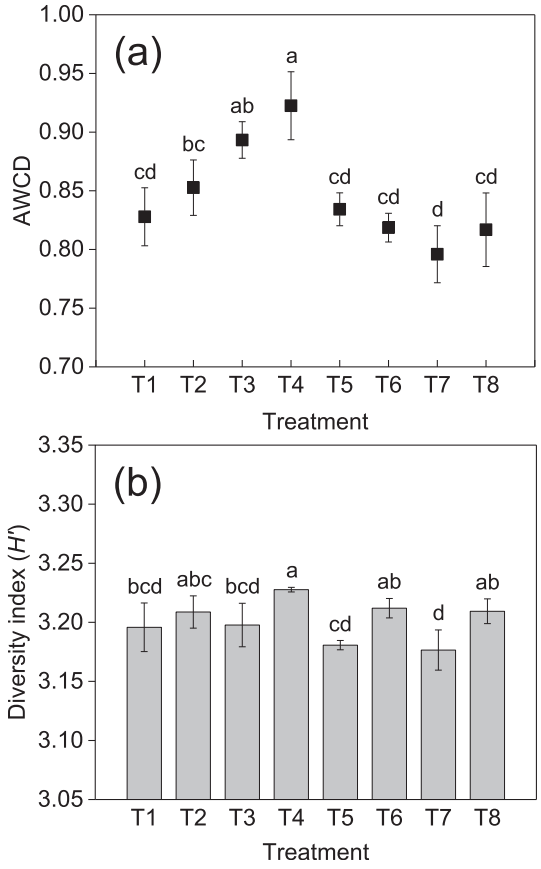

Fig. 2. AWCD of the Biolog ECO microplates (a) and Shannon-Wiener diversity index (b) from different treatment groups at 168 h. Different letters denote statistically significant differences ($P < 0.05$) between groups.

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.

3.2.2. SOM analysis

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

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 *N*-acetyl-D-glucosamine were highly utilized by the sediment microbes (shown with a minimum absorbance value more than 1.20). D-xylose, 2-hydroxy benzoic acid, phenylethylamine, and D, L-α-glycerol phosphate were not utilized by the sediment microbes (shown with a maximum absorbance value less than 0.15). Most of the maximum absorbance values appeared in the neurons at the top left, top right, and bottom left corner, which respectively correspond to T1 (or T3), T4, and T8 by reference to the

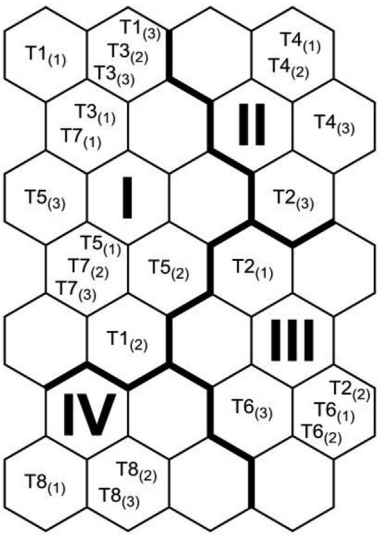

Fig. 3. Distribution and clustering of microbial communities in different treatments (T1–T8) on the SOM based on the utilization of carbon sources. Clusters (I–IV) were derived from k-means algorithm applied to the trained SOM. Numbers in the brackets indicate the repetitions.

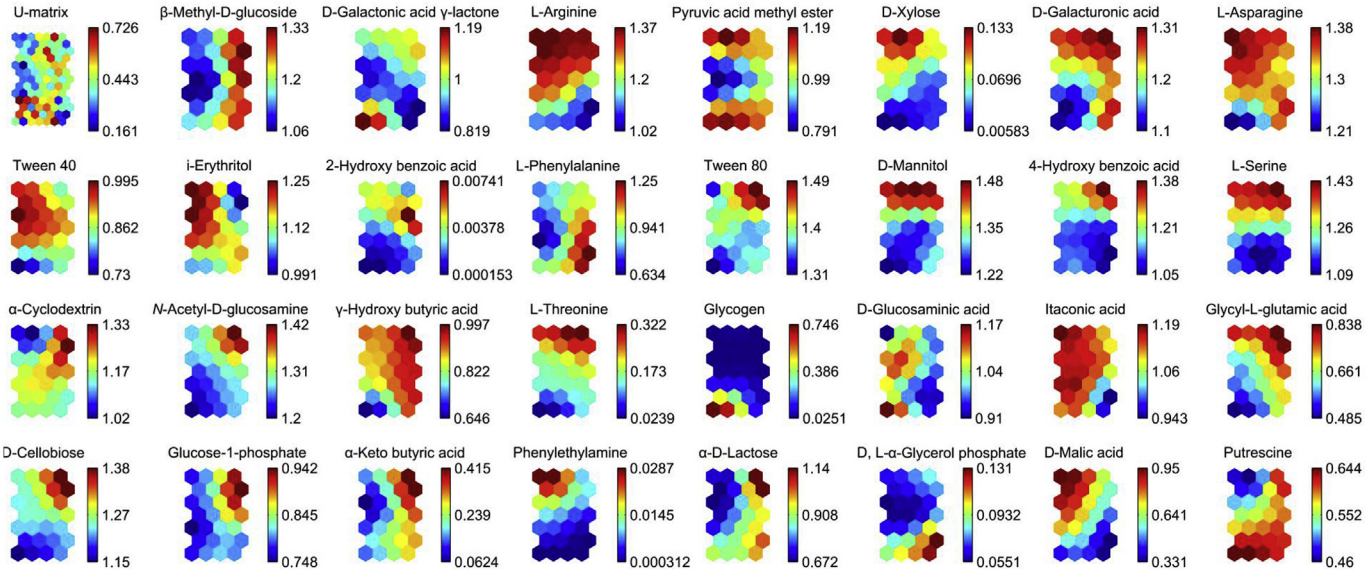

Fig. 4. Analysis of the absorbance for each carbon source on the trained SOM. Colors indicate the value of the absorbance of each unit in the map, according to the colorbars on the right. Numbers beside the colorbars correspond to the absorbance values. A higher absorbance value indicates a higher degree 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. (A colour version of this figure can be viewed online.)

microbial community distribution in Fig. 3. The microbial communities in T1 and T3 showed the highest utilization for L-arginine, L-asparagine, i-erythritol, itaconic acid, and D-malic acid, while D-galactonic acid γ-lactone, glycogen, D-glucosaminic acid, and putrescine were most effectively utilized by the sediment microbes in T8. The component planes of 15 carbon sources (including β-methyl-D-glucoside, D-galacturonic acid, tween 80, D-mannitol, 4-hydroxy benzoic acid, L-serine, α-Cyclodextrin, *N*-acetyl-D-glucosamine, γ-hydroxy butyric acid, L-threonine, glycyl-L-glutamic acid, D-cellobiose, glucose-1-phosphate, α-keto butyric acid, and α-D-lactose) displayed the maximum absorbance values in the neurons corresponding to T4. The high utilization of these carbon sources by the sediment microbes in T4 could account for the higher activity and diversity index of microbial community in the contaminated sediment with 0.5% MWCNTs.

3.2.3. *Principal component analysis*

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

In the principal component space, the distinctions of different treatments can be related to the differences in carbon source utilization by examining the correlation of carbon source variables to the principal components [55]. Important carbon sources for distinguishing the microbial communities of different treatments are displayed in Table 2. At least half of these carbon source variables

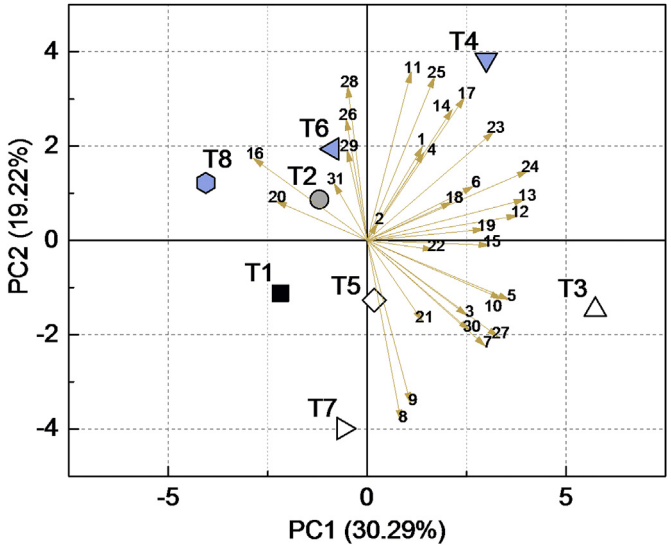

Fig. 5. Principal component analysis of the microbial metabolism of 31 carbon sources in different treatments (T1–T8). The results are displayed by the biplot method using the variable eigenvectors scores. Vectors indicate the direction in which the utilization of carbon source increases. 1. β-methyl-D-glucoside; 2. D-galactonic acid γ-lactone; 3. L-arginine; 4. pyruvic acid methyl ester; 5. D-xylose; 6. D-galacturonic acid; 7. L-asparagine; 8. tween 40; 9. i-erythritol; 10. 2-hydroxy benzoic acid; 11. L-phenylalanine; 12. tween 80; 13. D-mannitol; 14. 4-hydroxy benzoic acid; 15. L-serine; 16. α-cyclodextrin; 17. *N*-acetyl-D-glucosamine; 18. γ-hydroxy butyric acid; 19. L-threonine; 20. glycogen; 21. D-glucosaminic acid; 22. itaconic acid; 23. glycyl-L-glutamic acid; 24. D-cellobiose; 25. glucose-1-phosphate; 26. α-keto butyric acid; 27. phenylethylamine; 28. α-D-lactose; 29. D, L-α-glycerol phosphate; 30. D-malic acid; 31. putrescine. (A colour version of this figure can be viewed online.)

are explained by PC1 or PC2. According to the results of Table 2 and the distribution of T1–T8 in Fig. 5, the PC1 distinguishes the treatments with different MWCNT addition. The metabolic differences caused by MWCNTs are mainly reflected in utilizing five out of six amino acids, three out of ten carbohydrates, two out of seven carboxylic acids, and three out of four polymers. Microbial

Table 2
Correlation coefficients (r) of important carbon source variables to the first two principal components.

PC1	r	PC2	r
Carbon source		Carbon source	
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		

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.

4. Conclusions

Overall, the experimental results suggested that MWCNTs of high concentrations (0.5%–2.0%, w/w) could bring about significant changes in the metabolic function of sediment microbial communities. The phenanthrene-contaminated sediment with 0.5% MWCNTs showed the highest microbial activity and Shannon-Wiener diversity index. The metabolic differences caused by MWCNTs mainly reflect in the utilization of 13 carbon sources (including five amino acids, three carbohydrates, two carboxylic acids, and three polymers) on the Biolog ECO microplate. 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 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

This work was supported by National Natural Science Foundation of China (51378190, 51508177, 51521006, 51579095, 51709101), the Program for Changjiang Scholars and Innovative Research Team in University (IRT-13R17).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.carbon.2018.12.016.

References

[1] M.F.L. De Volder, S.H. Tawfick, R.H. Baughman, A.J. Hart, Carbon nanotubes: present and future commercial applications, Science 339 (2013) 535–539.
[2] M. Zhang, J. Li, Carbon nanotube in different shapes, Mater. Today 12 (2009) 12–18.
[3] S. Iijima, Helical microtubules of graphitic carbon, Nature 354 (1991) 56–58.
[4] B. Song, P. Xu, G. Zeng, J. Gong, P. Zhang, H. Feng, et al., Carbon nanotube-based environmental technologies: the adopted properties, primary mechanisms, and challenges, Rev. Environ. Sci. Biotechnol. 17 (2018) 571–590.
[5] J.L. Gong, B. Wang, G.M. Zeng, C.P. Yang, C.G. Niu, Q.Y. Niu, et al., Removal of cationic dyes from aqueous solution using magnetic multi-wall carbon nanotube nanocomposite as adsorbent, J. Hazard Mater. 164 (2009) 1517–1522.
[6] W. Xiong, Z. Zeng, X. Li, G. Zeng, R. Xiao, Z. Yang, et al., Multi-walled carbon nanotube/amino-functionalized MIL-53(Fe) composites: remarkable adsorptive removal of antibiotics from aqueous solutions, Chemosphere 210 (2018) 1061–1069.
[7] H. Yi, D. Huang, L. Qin, G. Zeng, C. Lai, M. Cheng, et al., Selective prepared carbon nanomaterials for advanced photocatalytic application in environmental pollutant treatment and hydrogen production, Appl. Catal. B Environ. 239 (2018) 408–424.
[8] W.G. Kim, S.D. Yong, S.J. Yook, J.H. Ji, K.H. Kim, G.N. Bae, et al., Comparison of black carbon concentration and particle mass concentration with elemental carbon concentration for multi-walled carbon nanotube emission assessment purpose, Carbon 122 (2017) 228–236.
[9] H.H. Liu, Y. Cohen, Multimedia environmental distribution of engineered nanomaterials, Environ. Sci. Technol. 48 (2014) 3281–3292.
[10] T.Y. Sun, N.A. Bornhöft, K. Hungerbühler, B. Nowack, Dynamic probabilistic modeling of environmental emissions of engineered nanomaterials, Environ. Sci. Technol. 50 (2016) 4701–4711.
[11] A.A. Koelmans, B. Nowack, M.R. Wiesner, Comparison of manufactured and black carbon nanoparticle concentrations in aquatic sediments, Environ. Pollut. 157 (2009) 1110–1116.
[12] E.J. Petersen, L. Zhang, N.T. Mattison, D.M. O'Carroll, A.J. Whelton, N. Uddin, et al., Potential release pathways, environmental fate, and ecological risks of carbon nanotubes, Environ. Sci. Technol. 45 (2011) 9837–9856.
[13] X. Ren, G. Zeng, L. Tang, J. Wang, J. Wan, H. Feng, et al., Effect of exogenous carbonaceous materials on the bioavailability of organic pollutants and their ecological risks, Soil Biol. Biochem. 116 (2018) 70–81.
[14] A. Mottier, F. Mouchet, É. Pinelli, L. Gauthier, E. Flahaut, Environmental impact of engineered carbon nanoparticles: from releases to effects on the aquatic biota, Curr. Opin. Biotechnol. 46 (2017) 1–6.
[15] A. Freixa, V. Acuña, J. Sanchís, M. Farré, D. Barceló, S. Sabater, Ecotoxicological effects of carbon based nanomaterials in aquatic organisms, Sci. Total Environ. 619–620 (2018) 328–337.
[16] S. Boncel, J. Kyzioł-Komosińska, I. Krzyżewska, J. Czupioł, Interactions of carbon nanotubes with aqueous/aquatic media containing organic/inorganic contaminants and selected organisms of aquatic ecosystems – a review, Chemosphere 136 (2015) 211–221.
[17] X. Ren, G. Zeng, L. Tang, J. Wang, J. Wan, Y. Liu, et al., Sorption, transport and biodegradation – an insight into bioavailability of persistent organic pollutants in soil, Sci. Total Environ. 610–611 (2018) 1154–1163.
[18] B. Song, P. Xu, G. Zeng, J. Gong, X. Wang, J. Yan, et al., Modeling the transport of sodium dodecyl benzene sulfonate in riverine sediment in the presence of multi-walled carbon nanotubes, Water Res. 129 (2018) 20–28.
[19] J. Qian, K. Li, P. Wang, C. Wang, M. Shen, J. Liu, et al., Effects of carbon nanotubes on phosphorus adsorption behaviors on aquatic sediments, Ecotoxicol. Environ. Saf. 142 (2017) 230–236.
[20] F. Abbasian, R. Lockington, T. Palanisami, M. Megharaj, R. Naidu, Multiwall carbon nanotubes increase the microbial community in crude oil contaminated fresh water sediments, Sci. Total Environ. 539 (2016) 370–380.
[21] M.H. Myer, M.C. Black, Multi-walled carbon nanotubes reduce toxicity of

diphenhydramine to Ceriodaphnia dubia in water and sediment exposures, Bull. Environ. Contam. Toxicol. 99 (2017) 321–327.
[22] S. Xu, M.L. Silveira, K.S. Inglett, L.E. Sollenberger, S. Gerber, Soil microbial community responses to long-term land use intensification in subtropical grazing lands, Geoderma 293 (2017) 73–81.
[23] A. Chroňáková, V. Radl, J. Čuhel, M. Šimek, D. Elhottová, M. Engel, et al., Overwintering management on upland pasture causes shifts in an abundance of denitrifying microbial communities, their activity and N_2O-reducing ability, Soil Biol. Biochem. 41 (2009) 1132–1138.
[24] B. Song, G. Zeng, J. Gong, J. Liang, P. Xu, Z. Liu, et al., Evaluation methods for assessing effectiveness of in situ remediation of soil and sediment contaminated with organic pollutants and heavy metals, Environ. Int. 105 (2017) 43–55.
[25] Y. Ge, J.H. Priester, M. Mortimer, C.H. Chang, Z. Ji, J.P. Schimel, et al., Long-Term effects of multiwalled carbon nanotubes and graphene on microbial communities in dry soil, Environ. Sci. Technol. 50 (2016) 3965–3974.
[26] J.R. Lawrence, M.J. Waiser, G.D.W. Swerhone, J. Roy, V. Tumber, A. Paule, et al., Effects of fullerene (C60), multi-wall carbon nanotubes (MWCNT), single wall carbon nanotubes (SWCNT) and hydroxyl and carboxyl modified single wall carbon nanotubes on riverine microbial communities, Environ. Sci. Pollut. Res. 23 (2016) 10090–10102.
[27] V. Feigl, É. Ujaczki, E. Vaszita, M. Molnár, Influence of red mud on soil microbial communities: application and comprehensive evaluation of the Biolog EcoPlate approach as a tool in soil microbiological studies, Sci. Total Environ. 595 (2017) 903–911.
[28] M. Rutgers, M. Wouterse, S.M. Drost, A.M. Breure, C. Mulder, D. Stone, et al., Monitoring soil bacteria with community-level physiological profiles using Biolog™ ECO-plates in The Netherlands and Europe, Appl. Soil Ecol. 97 (2016) 23–35.
[29] M.G. Waigi, F. Kang, C. Goikavi, W. Ling, Y. Gao, Phenanthrene biodegradation by sphingomonads and its application in the contaminated soils and sediments: a review, Int. Biodeterior. Biodegrad. 104 (2015) 333–349.
[30] D.W. Lee, H. Lee, A.H. Lee, B.O. Kwon, J.S. Khim, U.H. Yim, et al., Microbial community composition and PAHs removal potential of indigenous bacteria in oil contaminated sediment of Taean coast, Korea, Environ. Pollut. 234 (2018) 503–512.
[31] S. Ye, G. Zeng, H. Wu, C. Zhang, J. Liang, J. Dai, et al., Co-occurrence and interactions of pollutants, and their impacts on soil remediation—a review, Crit. Rev. Environ. Sci. Technol. 47 (2017) 1528–1553.
[32] J.Y. Wu, Z.G. Yan, Z.T. Liu, J.D. Liu, F. Liang, X.N. Wang, et al., Development of water quality criteria for phenanthrene and comparison of the sensitivity between native and non-native species, Environ. Pollut. 196 (2015) 141–146.
[33] L. Sun, Z. Zuo, M. Chen, Y. Chen, C. Wang, Reproductive and transgenerational toxicities of phenanthrene on female marine medaka (*Oryzias melastigma*), Aquat. Toxicol. 162 (2015) 109–116.
[34] J. Duan, W. Liu, X. Zhao, Y. Han, S.E. O'Reilly, D. Zhao, Study of residual oil in Bay Jimmy sediment 5 years after the Deepwater Horizon oil spill: persistence of sediment retained oil hydrocarbons and effect of dispersants on desorption, Sci. Total Environ. 618 (2018) 1244–1253.
[35] L. Yuan, L. Han, W. Bo, H. Chen, W. Gao, B. Chen, Simulated oil release from oil-contaminated marine sediment in the Bohai Sea, China, Mar. Pollut. Bull. 118 (2017) 79–84.
[36] Š. Petrová, J. Rezek, P. Soudek, T. Vaněk, Preliminary study of phytoremediation of brownfield soil contaminated by PAHs, Sci. Total Environ. 599–600 (2017) 572–580.
[37] S. Kuang, Z. Wu, L. Zhao, Accumulation and risk assessment of polycyclic aromatic hydrocarbons (PAHs) in soils around oil sludge in Zhongyuan oil field, China, Environ. Earth Sci. 64 (2011) 1353–1362.
[38] H. Chung, Y. Son, T.K. Yoon, S. Kim, W. Kim, The effect of multi-walled carbon nanotubes on soil microbial activity, Ecotoxicol. Environ. Saf. 74 (2011) 569–575.
[39] B. Shrestha, V. Acosta-Martinez, S.B. Cox, M.J. Green, S. Li, J.E. Cañas-Carrell, An evaluation of the impact of multiwalled carbon nanotubes on soil microbial community structure and functioning, J. Hazard Mater. 261 (2013) 188–197.
[40] E.J. Petersen, R.A. Pinto, P.F. Landrum, J.W.J. Weber, Influence of carbon nanotubes on pyrene bioaccumulation from contaminated soils by earthworms, Environ. Sci. Technol. 43 (2009) 4181–4187.
[41] A. Stefanowicz, The biolog plates technique as a tool in ecological studies of microbial communities, Pol. J. Environ. Stud. 15 (2006) 669–676.
[42] Z. Jiang, P. Li, Y. Wang, B. Li, Y. Wang, Effects of roxarsone on the functional diversity of soil microbial community, Int. Biodeterior. Biodegrad. 76 (2013) 32–35.
[43] J. Leflaive, R. Céréghino, M. Danger, G. Lacroix, L. Ten-Hage, Assessment of self-organizing maps to analyze sole-carbon source utilization profiles, J. Microbiol. Methods 62 (2005) 89–102.
[44] J. Vesanto, J. Himberg, E. Alhoniemi, J. Parhankangas. Self-Organizing Map in matlab : the SOM toolbox. Proceedings of the Matlab DSP Conference. Comsol Oy, Espoo, Finland; p. 35-40.
[45] W.P. Tsai, S.P. Huang, S.T. Cheng, K.T. Shao, F.J. Chang, A data-mining framework for exploring the multi-relation between fish species and water quality through self-organizing map, Sci. Total Environ. 579 (2017) 474–483.
[46] J.H. Li, Y.J. Yang, B.W. Li, W.J. Li, G. Wang, J.M.H. Knops, Effects of nitrogen and phosphorus fertilization on soil carbon fractions in alpine meadows on the qinghai-Tibetan plateau, PloS One 9 (2014) e103266.
[47] V.K.K. Upadhyayula, V. Gadhamshetty, Appreciating the role of carbon nanotube composites in preventing biofouling and promoting biofilms on material surfaces in environmental engineering: a review, Biotechnol. Adv. 28 (2010) 802–816.
[48] A. Patel, S. Tiwari, P. Parihar, R. Singh, S.M. Prasad, Chapter 2 - carbon nanotubes as plant growth regulators: impacts on growth, reproductive system, and soil microbial community, in: D.K. Tripathi, P. Ahmad, S. Sharma, D.K. Chauhan, N.K. Dubey (Eds.), Nanomaterials in Plants, Algae and Microorganisms, Academic Press, 2019, pp. 23–42.
[49] S.C. Smith, D.F. Rodrigues, Carbon-based nanomaterials for removal of chemical and biological contaminants from water: a review of mechanisms and applications, Carbon 91 (2015) 122–143.
[50] C. Pesciaroli, C. Viseras, C. Aguzzi, B. Rodelas, J. González-López, Study of bacterial community structure and diversity during the maturation process of a therapeutic peloid, Appl. Clay Sci. 132–133 (2016) 59–67.
[51] E.E. Curd, J.B.H. Martiny, H. Li, T.B. Smith, Bacterial diversity is positively correlated with soil heterogeneity, Ecosphere 9 (2018) e02079.
[52] E.J. Petersen, Q. Huang, J.W.J. Weber, Bioaccumulation of radio-labeled carbon nanotubes by *Eisenia foetida*, Environ. Sci. Technol. 42 (2008) 3090–3095.
[53] R. Olawoyin, A. Nieto, R.L. Grayson, F. Hardisty, S. Oyewole, Application of artificial neural network (ANN)–self-organizing map (SOM) for the categorization of water, soil and sediment quality in petrochemical regions, Expert Syst. Appl. 40 (2013) 3634–3648.
[54] H. Abdi, L.J. Williams, Principal component analysis, Wiley Interdiscip. Rev. Comput. Stat. 2 (2010) 433–459.
[55] J.L. Garland, A.L. Mills, Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source utilization, Appl. Environ. Microbiol. 57 (1991) 2351–2359.