Effects of hydroxyl, carboxyl, and amino functionalized carbon nanotubes on the functional diversity of microbial community in riverine sediment

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HIGHLIGHTS

- The functionalized MWCNTs negatively influenced the microbial metabolic function.
- The sediment microbial community preferred utilizing polymers and amino acids.
- MWCNT dosage had a greater impact on functional diversity than functionalization.

GRAPHICAL ABSTRACT

ABSTRACT

Nowadays, more and more attention is focused on the environmental harm brought by the wide production and use of carbon nanotubes. In this study, the metabolic function of sediment microbial community was investigated after unfunctionalized or functionalized multi-walled carbon nanotubes (MWCNTs) were incorporated. The surface functional groups on the studied functionalized MWCNTs in this work were hydroxyl, carboxyl, and amino, respectively. The metabolic functional diversity was determined by Biolog EcoPlates after one-month exposure to MWCNTs. Incorporating 0.5 wt% amino functionalized MWCNTs significantly decreased the microbial activity and diversity, and all types of MWCNTs caused great inhibition on the microbial metabolism at the dosage of 2.0 wt%. The sediment microbes preferred polymers and amino acids. Principal component and similarity analysis indicated that the microbial carbon metabolism was more affected by the MWCNT dosage compared with the functionalization, and 2.0 wt% amino functionalized MWCNTs made the greatest difference in metabolic function of sediment microbial community. These consequences may help to assess the environmental risks of MWCNTs from the aspect of ecological relevance of sediment microbial community.

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1. Introduction

As typical one-dimensional nanomaterials, carbon nanotubes (CNTs) have many unique properties and are extensively applied in numerous fields, such as polymer composites, medicine,

electronics, and energy (De Volder et al., 2013; Song et al., 2018; Zheng et al., 2018, 2019; Yang et al., 2019). The annual production amount of CNTs is as many as several thousand tons worldwide, and the CNT market is reported to be $3.43 billion in 2016 and projected to reach $8.70 billion by 2022 (Munk et al., 2017; De Marchi et al., 2018a). The increasing production and use of CNTs ineluctably cause the release of CNTs into the environment (Nowack et al., 2013; Chen et al., 2018; Yi et al., 2018). Water sediment is a major sink for CNTs, and CNTs may be released into the receiving water via multiple pathway, such as atmospheric deposition, surface runoff, open channel, sewage treatment plant, direct discharge, etc. In a one-year simulation study about the transport and distribution of released CNTs among various environmental compartments (atmosphere, water, soil, and sediment), it was found that 73.83% CNTs accumulated in the sediment (Liu and Cohen, 2014). This causes environmental concern on CNTs in sediment (Sun et al., 2015; Zindler et al., 2016).

Microbial community is a vital component of the aquatic sediment and is important for carbon and nitrogen cycling processes (Hunter et al., 2006; Madsen, 2011). Due to the unique nanostructure, CNTs may significantly affect the composition and function of microbial community. Chung et al. (2011) incorporated various concentrations of multi-walled carbon nanotubes (MWCNTs) into soil, and found that 5 mg g^{-1} of MWCNTs significantly reduced the microbial biomass (observed with an exposure time of 20 days) and inhibited the activity of extracellular enzymes (observed with an exposure time of 1, 4, and 11 days). Jin et al. (2014) reported that single-walled carbon nanotubes (SWCNTs) altered the soil microbial community composition after exposure for 25 days and the microbial biomass decreased with the SWCNT concentrations (0.03–1 mg g^{-1}). The experimental results of Shrestha et al. (2013) showed that 10 mg g^{-1} of MWCNTs caused a variation in the structure of soil microbial community after exposure for 90 days and increased the microbial populations that were more tolerant to polycyclic aromatic hydrocarbons. In a study about the effects of MWCNTs on a tomato plant and soil system, Khodakovskaya et al. (2013) watered the plant by using 50 mL of MWCNT suspensions (50 and 200 mg L^{-1}) once a week, and analyzed the soil microbial community after 9 weeks. Their results showed that the MWCNT exposure did not affect the diversity and richness of microbial community but altered the abundance of each bacterial group in the soil. In the above studies, chloroform fumigation-extraction, phospholipid fatty acid profiles, pyrosequencing, and denaturing gradient gel electrophoresis were applied for the analysis of microbial community. These methods are valuable for determining the composition and structure of microbial community, yet they are inadequate to describe the ecological correlation, as function and structure of microbial community do not always change in a consistent way (Tian et al., 2016; Ren et al., 2018). Studying the metabolic function of microbial community is helpful to understand the environmental significance of microbial community variations induced by nanomaterials. The Biolog EcoPlate is a useful tool for analyzing the functional diversity of microbial community and describing the microbial responses to environmental changes (Manjunath et al., 2018). Thus, the Biolog EcoPlate is applied to determine the differences in metabolic function of microbial communities.

Additionally, surface modification or functionalization is often required to enhance the performance of CNT composites during the applications of CNTs. The modified or functionalized CNTs may induce different toxic effects to organisms compared with unfunctionalized ones. For example, Zhang et al. (2015) studied different surface coatings on MWCNT toxicity towards green algae, and found that two synthetic surfactants (SDBS and TX100) increased the MWCNT toxicity, while humic acid alleviated the

toxicity. Zhou et al. (2017) reported that MWCNTs functionalized by hydroxyl and carboxyl were less cytotoxic but more genotoxic to human lung epithelial cells than unfunctionalized CNTs. In a recent study about the CNT toxicity to *Ruditapes philippinarum*, it was reported that carboxyl functionalized MWCNTs had greater toxic effects on the clams (De Marchi et al., 2018b). To our knowledge, few studies were performed to determine the relationship between the functionalization of CNTs and the CNT-induced variations in metabolic function of sediment microbial community. This work aims to investigate the functional diversity of microbial community in riverine sediment after unfunctionalized MWCNT (unf-MWCNT) and three MWCNTs functionalized with hydroxyl, carboxyl, and amino (MWCNT-OH, MWCNT-COOH, and MWCNT-NH$_2$) were incorporated. It is hoped that this work will be helpful for the risk assessment of CNTs.

2. Materials and methods

2.1. Sediment and MWCNTs

Sediment samples were taken from the Xiangjiang River, the largest river in Hunan Province, China. Basic properties of the sediment were determined according to previously reported methods (Song et al., 2017; Yan et al., 2017). Main mineral composition of the sediment was analyzed with XRD pattern. Four types of MWCNTs were used in this study, including unf-MWCNT, MWCNT-OH, MWCNT-COOH, and MWCNT-NH$_2$. These MWCNTs were obtained from Chengdu Organic Chemicals Co. Ltd., China. They all have a purity >95%, an outer diameter of 8–15 nm, an inner diameter of 3–5 nm, and a length of ~50 μm. The contents of functional group in MWCNT-OH, MWCNT-COOH, and MWCNT-NH$_2$ are 3.70 wt%, 2.56 wt%, and 0.45 wt%, respectively.

2.2. Experimental design

Nine treatments were conducted at an identical condition. A treatment without any MWCNTs was set as blank control (T1). In the experimental group, unf-MWCNT, MWCNT-OH, MWCNT-COOH, and MWCNT-NH$_2$ were respectively added to the sediment at the dosages of 0.5 wt% (T2, T4, T6, T8) or 2.0 wt% (T3, T5, T7, T9). According to previous studies and our pre-experiments (Fig. S1), MWCNTs significantly influenced the soil microbial community at concentrations more than 0.5 wt%, but the effects were not evident at low MWCNT concentrations (Chung et al., 2011; Shrestha et al., 2013; Kerfahi et al., 2015; Song et al., 2020). The used exposure level could bring about distinct changes in the microbial metabolic function, which helps to identify the differences resulting from different functionalization. Thus, these two MWCNT dosages were used in this study. After MWCNTs were added to the sediment, the mixtures were homogenized manually. During the experiment, all the sediment-MWCNT mixtures were kept at room temperature and the moisture was maintained at 50% (v/w). After one-month exposure, the microbial metabolic function was assessed.

2.3. Metabolic function assessment

The Biolog EcoPlate (Biolog Inc., USA) was used to determine the variations in microbial metabolic function. The microplate has 96 wells, including 3 blank control wells and 93 wells that contain 31 kinds of carbon sources in triplicate. These carbon sources were classified to six types: amines, amino acids, carbohydrates, carboxylic acids, phenolic compounds, and polymers (Table S1). They are selected carbon and energy sources for microbial growth and natural metabolic processes. When a carbon source is metabolized

by microbes, the coexisting dye (a colorless tetrazolium) would be simultaneously reduced and the color turns purple. The deeper purple indicates the higher utilization of corresponding carbon source (Stefanowicz, 2006). For analyzing the metabolism of microbial community, 10 g of sediment were added to 90 mL of 0.85% sterile sodium chloride solution, followed by a thorough shaking for half-hour. Then, the suspension was left to stand for another 30 min. After that, 150 μL of the supernatant were inoculated to each microplate well. All plates were incubated at 25 °C for seven days. The absorbance at each well was measured at 750 nm (turbidity) and 590 nm (color + turbidity) every 24 h.

2.4. Statistical analysis

Average well color development (AWCD) and Shannon-Wiener diversity index (H') were calculated based on carbon source utilization (Kumar et al., 2017; Liao et al., 2018). The calculation was performed with the following equations:

$$\text{AWCD} = \frac{1}{n}\sum(C_i - R) \tag{1}$$

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

where n is carbon source number, C_i (carbon source well) and R (control well) are calculated by using the absorbance value at 590 minus that at 750 nm, and $p_i = (C_i - R)/\sum(C_i - R)$. Result differences between treatments were tested with ANOVA. Heat map analysis was applied to visualize the relationship between different treatments and the metabolism of each carbon source in a two-dimensional chart. The heat map analysis was implemented with pheatmap package of R software (Wang et al., 2017). Principal component analysis (PCA) was conducted to identify the variation trend of microbial carbon metabolism in different treatments (Button et al., 2016). Similarity analysis of the carbon metabolism in different treatments was conducted based on Bray-Curtis similarity (Valentín-Vargas et al., 2018), and the paleontological statistic software package (PAST) was used for the analysis (Hammer et al., 2001). The microplate data at 168 h of incubation were used in heat map analysis, PCA, and similarity analysis (Song et al., 2019).

3. Results and discussion

3.1. Characterization of the sediment and MWCNTs

Microstructure of the MWCNTs was observed with scanning electron microscope (SEM). The SEM images showed a typical tubular shape, but the morphological difference between different types of MWCNTs was not distinct in the images (Fig. S2). The atomic percentages of MWCNTs were further determined by X-ray photoelectron spectroscopy to show their difference in elemental composition. The oxygen atomic percentages of unf-MWCNT, MWCNT-OH, MWCNT-COOH, and MWCNT-NH$_2$ are 3.32%, 4.83%, 6.96%, and 2.17%, respectively. The sediment has a texture of 49.0% clay, 27.6% silt, and 23.4% sand. The pH, cation exchange capacity, and organic carbon content of the sediment are 7.92, 10.8 cmol kg^{-1}, and 1.63% (w/w), respectively. Analysis of the X-ray diffraction (XRD) pattern of sediment suggests that the main mineral composition of sediment is quartz, gismondine, and muscovite (Fig. S2e).

3.2. Effect of functionalized MWCNTs on microbial functional diversity

3.2.1. Microbial activity and diversity index

The overall microbial activity was indicated by AWCD, and a higher AWCD value suggests a higher microbial metabolic activity. The changes of microbial activity in the presence of various types of MWCNTs are displayed in Fig. 1a. At the dosage of 0.5%, unf-MWCNT (T2) and MWCNT-OH (T4) showed no significant effect on the microbial activity compared with the treatment without any MWCNTs (T1), while exposure to MWCNT-COOH (T6) and MWCNT-NH$_2$ (T8) obviously decreased the AWCD from 0.79 to 0.66 and 0.59, respectively. At the dosage of 2.0%, all types of MWCNTs caused a decrease of the microbial activity compared with the blank control. Among these types of MWCNTs, MWCNT-COOH and MWCNT-NH$_2$ had a more obvious inhibition effect on the microbial activity, but no significant difference was observed between these two treatments. The higher toxicity of these functionalized MWCNTs to microbes in the sediment might be mainly attributed to the stronger interactions between them. It has been reported that CNTs modified by hydrophilic groups are easier to interact with the biomembrane of microbes via a lipid-asisted mechanism and have a better dispersity than unfunctionalized CNTs (Su et al., 2015). Additionally, the incorporation of these functionalized MWCNTs might change the sediment physicochemical properties such as pH and aggregate structure, indirectly affecting the sediment microbial community (Correia et al., 2015; Kerfahi et al., 2015).

The changes of Shannon-Wiener diversity index were similar to the variations of AWCD (Fig. 1b). The diversity index significantly decreased from 3.11 to 2.96 after the exposure to 0.5% MWCNT-NH$_2$. At the dosage of 2.0%, unf-MWCNT, MWCNT-OH, MWCNT-COOH, and MWCNT-NH$_2$ caused greater decreases of the microbial functional diversity, and the diversity index decreased to 2.85,
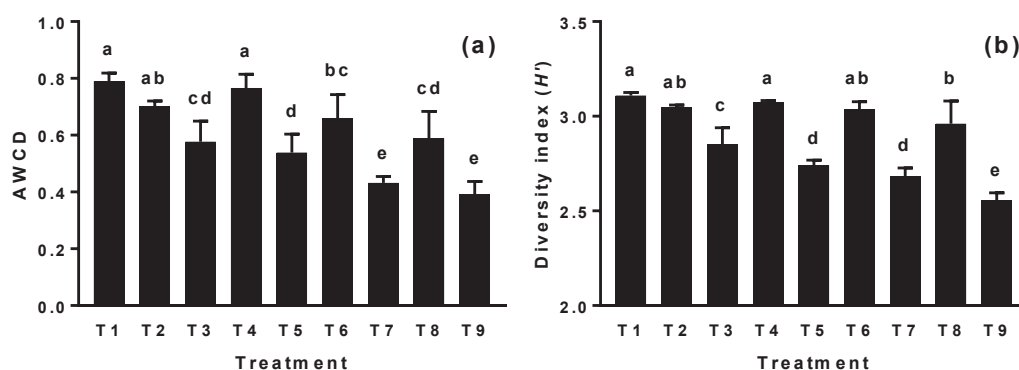
Fig. 1. Effects of different types of MWCNTs on the AWCD (a) and Shannon-Wiener diversity index (b) at 168 h of incubation. Different letters represent significant differences between treatments ($P < 0.05$).

2.74, 2.68, and 2.56, respectively. Similar changes in the diversity index and AWCD indicate the potential relevance between the decreased microbial diversity and the decreased carbon metabolic activity. Additionally, higher dosages of the MWCNTs resulted in a higher microbial toxicity. A large number of MWCNTs in the sediment increased the contact opportunity for microbes and MWCNTs (Wang et al., 2015). On the other hand, microbes might be adsorbed and enclosed by plenty of MWCNTs, and the isolation of microbes from external environment limited the nutrient availability thus prevented the microbial growth (Smith and Rodrigues, 2015; Song et al., 2018).

3.2.2. Heat map and principal component analysis

The relationship between microbial metabolism of each carbon source and different treatments is visualized by a heat map. As shown in Fig. 2, the rows are various carbon sources, and the columns refer to different treatments. The cell colors reflect the absorbance values according to the legend on the right, and suggest the carbon source utilization. It was found that D-xylose, 2-hydroxy benzoic acid, L-threonine, D-glucosaminic acid, α-keto butyric acid, and D, L-α-glycerol phosphate were not utilized by sediment microbial communities in all treatments. The maximum absorbance value for these carbon sources is only 0.06, which is less than the lowest level (0.15) that can indicate the carbon source utilization (Pardo et al., 2014; Feigl et al., 2017). L-asparagine, Tween 40, and Tween 80 were highly utilized carbon sources, and they are shown with a minimum absorbance value of 0.97 among all treatments. In the control group (T1), fifteen carbon sources have an absorbance

value more than one (shown with red, orange, and yellow cells in the heat map). Obvious inhibition of the carbon metabolism was observed at the MWCNT dosage of 2.0% (shown with increased blue cells in the heat map). The utilization characteristics of carbon sources in different treatments are varied. For example, at the dosage of 2.0%, the highest utilized carbon sources for the treatments with unf-MWCNT, MWCNT-OH, MWCNT-COOH, and MWCNT-NH$_2$ are L-asparagine, L-serine, γ-hydroxy butyric acid, and L-phenylalanine, respectively. This suggests the diversity and complexity of microbial metabolic changes in the presence of different functionalized MWCNTs. The microbial preference for carbon source types was further analyzed by heat map using different types of carbon sources as rows (Fig. S3). Overall, the sediment microbial communities had a preference of utilizing polymers and amino acids, with or without MWCNTs (shown with more red and orange cells in the rows of polymers and amino acids in the heat map). The addition of 2.0% MWCNT-COOH (T7) resulted in the greatest reduction in utilizing amino acids, while 2.0% MWCNT-NH$_2$ (T9) caused the greatest reduction in utilizing other five types of carbon sources.

PCA was conducted to visually distinguish the differences of microbial carbon metabolism between various treatments. The PCA results are showed with a biplot including loading plot and score plot (Fig. 3). A closer distance between treatments suggests a greater similarity in microbial carbon metabolism. The first principal component (PC1) and the second principal component (PC2) explain 36.43% and 23.61% of the original variables, respectively. Before conducting PCA, carbon sources that were not metabolized
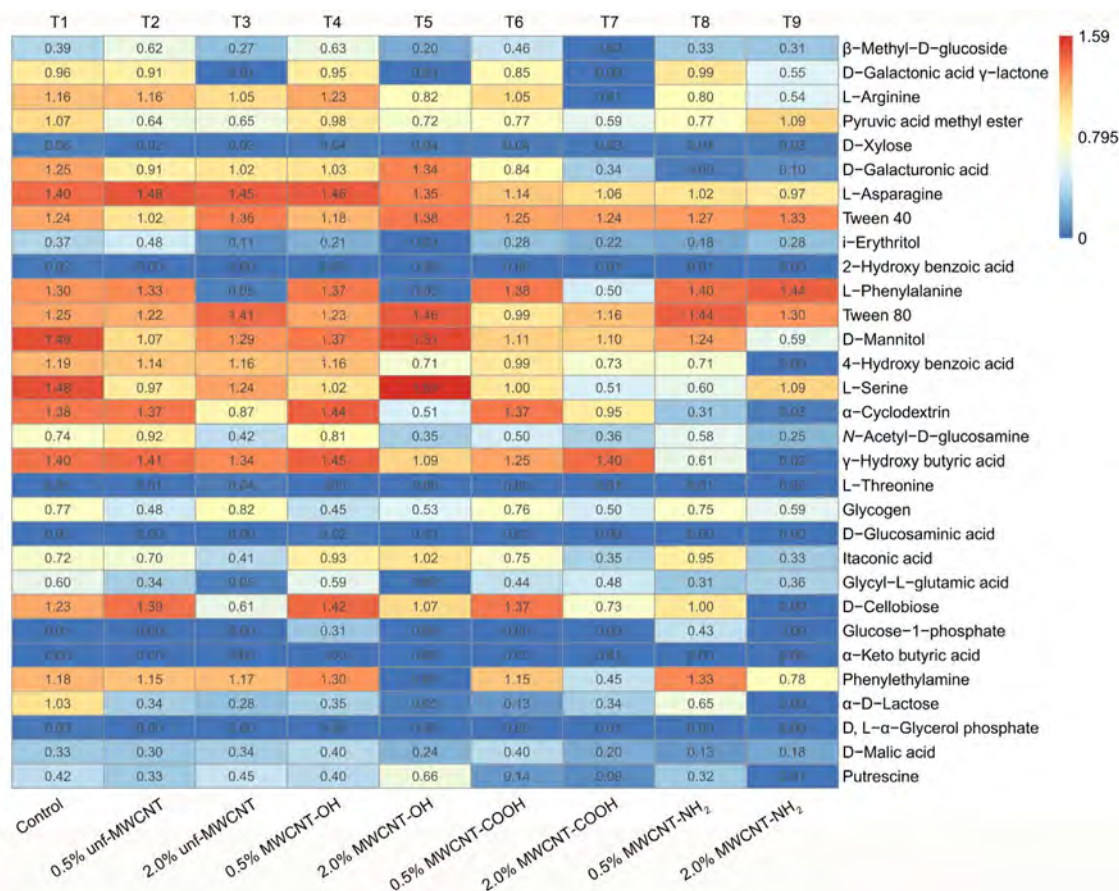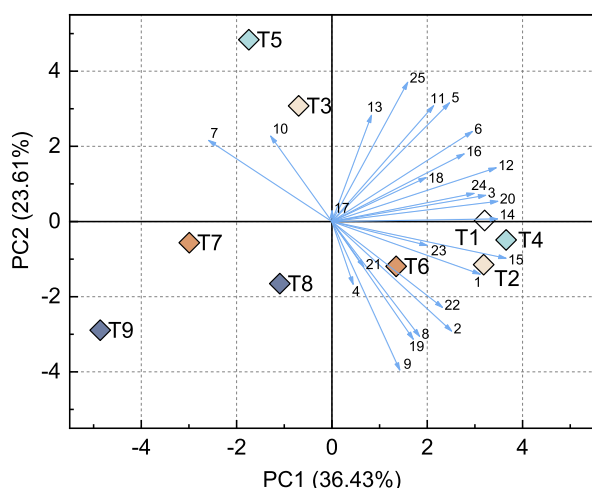

Fig. 2. Heat map analysis of microbial metabolism of 31 carbon sources in different treatments (T1–T9). Colors indicate the absorbance values according to the legend on the right, and a higher absorbance value suggests a higher degree of carbon source utilization. Numbers on the cells correspond to the specific absorbance values. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Fig. 3. Principal component analysis of the microbial metabolism of 25 utilized carbon sources. The results are displayed by the biplot including loading plot and score plot. Carbon sources that were not utilized by the sediment microbial communities were excluded according to the results of heat map analysis. 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-galacturonic acid; 6. L-asparagine; 7. Tween 40; 8. i-erythritol; 9. L-phenylalanine; 10. Tween 80; 11. D-mannitol; 12. 4-hydroxy benzoic acid; 13. L-serine; 14. α-cyclodextrin; 15. N-acetyl-D-glucosamine; 16. γ-hydroxy butyric acid; 17. glycogen; 18. itaconic acid; 19. glycyl-L-glutamic acid; 20. D-cellobiose; 21. glucose-1-phosphate; 22. phenylethylamine; 23. α-D-lactose; 24. D-malic acid; 25. putrescine.

Fig. 4. Similarity in microbial carbon metabolism between different treatments based on Bray-Curtis similarity.

by sediment microbes were excluded according to the results of heat map analysis. Excluding these carbon sources is helpful to improve the ability of the first two principal components in explaining the original variables (Fig. S4). As shown in Fig. 3, the sample point T3, T5, T7, T8, and T9 are distributed in the negative direction of most carbon source vectors, which suggests that the addition of 0.5% MWCNT-NH$_2$ or 2.0% all types of MWCNTs substantially reduced the microbial functional diversity in riverine sediment. The PC1 distinguishes the treatments with different dosages. Higher MWCNT dosage corresponds to a lower score on the PC1. Two carbon sources, Tween 40 and Tween 80, appear alone in the second quadrant. Combined with the above results, it indicates that the microbial metabolism of these two carbon sources were relatively stable under the interference with MWCNTs of different types and dosages. The distances between the control (T1) and the treatments with 0.5% MWCNTs (T2, T4, T6, and T8) are shorter than those between the control (T1) and the treatments with 2.0% MWCNTs (T3, T5, T7, and T9). This result suggests that the MWCNT dosage had a greater impact on microbial functional diversity than the functionalization.

3.2.3. Similarity analysis

Similarity analysis was further performed to quantitatively describe the carbon metabolic differences between various treatments. The Bray-Curtis similarity between different treatments is displayed in Fig. 4. The Bray-Curtis similarity is widely used to quantify the differences in species populations between two different sites in ecology, and it was used for analyzing the microbial differences based on the metabolic characteristic in this study. It can be found that the microbial metabolic differences between T1, T2, T4, and T6 were relatively small. These treatments showed a minimum similarity of 90.49%. At the dosage of 0.5%, MWCNT-NH$_2$ (T8) caused great changes in microbial metabolic function, and resulted in a dissimilarity of 19.31% between T8 and the other four treatments (T1, T2, T4, and T6). At the dosage of 2.0%,
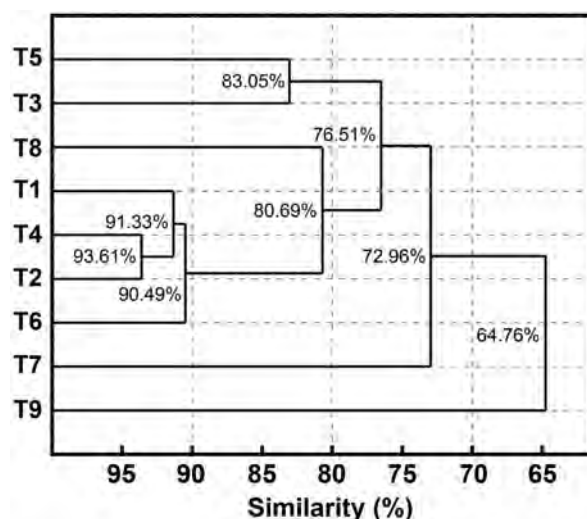
Table 1
Similarity percentage analysis (SIMPER) identifying the important carbon sources that contribute to the microbial metabolic dissimilarity between T1, T2, T4, and T6 versus T8.

Carbon source	Average dissimilarity	Percentage of contribution (%)	Cumulative contribution (%)
α-Cyclodextrin	2.658	13.76	13.76
D-Galacturonic acid	2.345	12.14	25.91
γ-Hydroxy butyric acid	1.878	9.727	35.63
L-Serine	1.250	6.475	42.11
4-Hydroxy benzoic acid	0.9886	5.120	47.23
α-D-Lactose	0.9296	4.814	52.04
Glucose-1-phosphate	0.8716	4.514	56.55
D-Cellobiose	0.8640	4.474	61.03
L-Arginine	0.8617	4.462	65.49
L-Asparagine	0.8478	4.390	69.88
Tween 80	0.6545	3.389	73.27
D-Malic acid	0.5614	2.907	76.18
N-Acetyl-D-glucosamine	0.4881	2.528	78.70
β-Methyl-D-glucoside	0.4767	2.469	81.17
Itaconic acid	0.4384	2.270	83.44
Glycyl-L-glutamic acid	0.4331	2.243	85.69
D-Mannitol	0.4176	2.162	87.85
i-Erythritol	0.3865	2.001	89.85
Pyruvic acid methyl ester	0.3832	1.985	91.83
Glycogen	0.3679	1.905	93.74
Phenylethylamine	0.3406	1.764	95.50
Putrescine	0.2323	1.203	96.71
Tween 40	0.2313	1.198	97.91
D-Galactonic acid γ-lactone	0.1890	0.9788	98.88
L-Phenylalanine	0.1232	0.6378	99.52
2-Hydroxy benzoic acid	0.02738	0.1418	99.66
D-Xylose	0.02289	0.1185	99.78
D-Glucosaminic acid	0.01831	0.09483	99.88
D, L-α-Glycerol phosphate	0.01829	0.09472	99.97
L-Threonine	0.004516	0.02338	99.99
α-Keto butyric acid	0.0009994	0.005175	100.0

all the treatments with MWCNTs (T3, T5, T7, and T9) showed a similarity less than 80% compared with the control. These results further demonstrate that the MWCNT dosage had a greater impact on microbial functional diversity than the functionalization. The addition of 2.0% MWCNT-NH$_2$ had a greatest impact on the sediment microbial metabolism, showing a similarity of only 64.76% between T9 and all other treatments.

Similarity percentage analysis (SIMPER) was further performed to identify important carbon sources that contributed to the microbial dissimilarity. The analysis result between T1, T2, T4, and T6 versus T8 is shown in Table 1. At the dosage of 0.5%, MWCNT-NH$_2$ (T8) caused significant changes in the microbial metabolism of α-cyclodextrin, D-galacturonic acid, γ-hydroxy butyric acid, L-serine, 4-hydroxy benzoic acid, and α-D-lactose (the first six carbon sources listed in Table 1), which contributed to over 50% dissimilarity between T8 and other four treatments (T1, T2, T4, and T6). Incorporating 2.0% unf-MWCNT (T3) and 2.0% MWCNT-OH (T5) into sediment had a great impact on the microbial metabolism of L-phenylalanine, D-galactonic acid γ-lactone, α-cyclodextrin, phenylethylamine, D-cellobiose, and glycyl-L-glutamic acid (Table S2), while adding 2.0% MWCNT-COOH (T7) made a considerable difference to the microbial metabolism of L-arginine, D-galactonic acid γ-lactone, L-phenylalanine, phenylethylamine, D-galacturonic acid, and D-cellobiose (Table S3).

3.2.4. Potential roles of MWCNT functionalization in microbial metabolism inhibition

The above results indicate that these functionalized MWCNTs could cause negative effects on the microbial metabolism, especially at high concentration. The microbial metabolism inhibition caused by MWCNT functionalization might mainly result from higher microbial toxicity of the functionalized MWCNTs. The MWCNT aqueous dispersions were observed by transmission electron microscope (TEM), and representative images were displayed in Fig. 5. The functionalization treatments significantly shortened the length of MWCNTs compared with the unfunctionalized MWCNTs, and shorter MWCNTs could cause higher microbial toxicity (Bussy et al., 2012). The presence of hydrophilic groups changed the affinity of MWCNTs with microbes. Su et al. (2015) reported that CNTs with hydrophilic groups on the surface were easier to interact with the biomembrane of microbes than unfunctionalized CNTs. In their experiments, 10 or 50 mg L^{-1} of hydroxyl-functionalized SWCNTs showed an inhibiting effect on the carbon source utilization and bacterial denitrification process, but no significant influence was observed with unfunctionalized SWCNTs at the same concentrations. Additionally, MWCNT-NH$_2$ showed relatively low dispersity and tended to aggregate compared with MWCNT-OH and MWCNT-COOH. This was also confirmed by the zeta potential distribution of the MWCNT
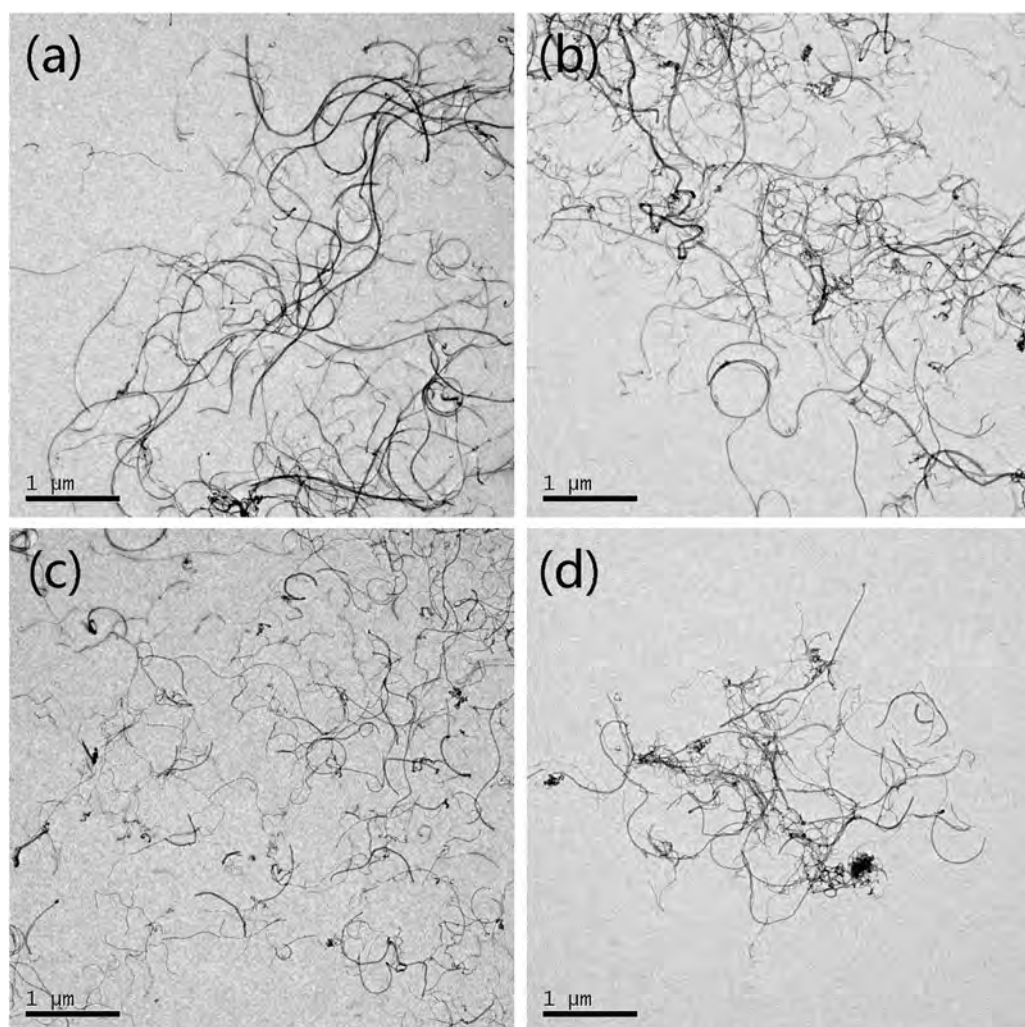

Fig. 5. TEM images of aqueous dispersion of the used unf-MWCNT (a), MWCNT-OH (b), MWCNT-COOH (c), and MWCNT-NH$_2$ (d).

aqueous dispersions (Fig. S5). The surface of MWCNT-NH$_2$ was more positively charged than MWCNT-OH and MWCNT-COOH, which might facilitate the aggregation of MWCNT-NH$_2$ with microbes and lead to higher toxicity (Bonventre et al., 2014). Wang et al. (2011) reported that carboxylated SWCNTs could be more toxic than unfunctionalized SWCNTs due to the formation of amorphous carbon fragments with higher toxicity during the carboxylation process. This might partly account for higher toxicity of MWCNT-COOH and MWCNT-NH$_2$ in this study. According to the MWCNT specification from the manufacturer, the used MWCNT-NH$_2$ was produced by using MWCNT-COOH as the starting material. Therefore, more amorphous carbon fragments formed in the MWCNT-NH$_2$ (Fig. 5d), and higher microbial toxicity was caused by the used MWCNT-NH$_2$.

4. Conclusions

In this study, the effects of hydroxyl, carboxyl, and amino functionalized MWCNTs on functional diversity of sediment microbial community were investigated. It was found that these functionalized MWCNTs could negatively influence the microbial carbon metabolism in sediment. At the dosage of 0.5%, MWCNT-NH$_2$ significantly decreased the microbial activity and diversity. At the dosage of 2.0%, all types of MWCNTs caused greater inhibition on the microbial metabolism. The heat map analysis showed that the sediment microbial communities had a preference of utilizing polymers and amino acids. PCA and similarity analysis suggested that the microbial carbon metabolism was more affected by the MWCNT dosage compared with the functionalization, and 2.0% MWCNT-NH$_2$ made the greatest difference in microbial metabolic function. These results indicated that the surface modification or functionalization should be considered when assessing the ecological risks of MWCNTs. Additionally, it is suggested to implement reliable strategies for MWCNT product evaluation and waste management to minimize the ecological risks of MWCNTs, especially for functionalized MWCNTs.

Credit author statement

Biao Song: Conceptualization, Methodology, Investigation, Writing - original draft. Zhuotong Zeng: Conceptualization, Methodology, Investigation, Writing - original draft. Guangming Zeng: Supervision, Project administration, Funding acquisition. Jilai Gong: Supervision, Project administration, Funding acquisition. Rong Xiao: Supervision, Project administration. Ming Chen: Software, Validation, Funding acquisition. Xiang Tang: Investigation, Visualization, Writing - review & editing. Shujing Ye: Data curation, Writing - review & editing. Maocai Shen: Writing - review & editing.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2020.128053.

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