Effects of typical engineered nanomaterials on 4-nonylphenol degradation in river sediment: basing on bacterial community and function analysis

Piao Xu [a, b, 1], Ming Chen [a, b, 1], Cui Lai [a, b, 1], Guangming Zeng [a, b, *], Danlian Huang [a, b, *], Han Wang [a, b], Xiaomin Gong [a, b], Lei Qin [a, b], Yuanyuan Liu [c], Dan Mo [a, b], Xiaofeng Wen [a, b], Chengyun Zhou [a, b], Rongzhong Wang [a, b]

[*] Corresponding authors. Fax: +86-731-88823701; Tel: +86-73- 88823701.

E-mail: zgming@hnu.edu.cn (G.M. Zeng); huangdanlian@hnu.edu.cn (D.L. Huang).

[a] College of Environmental Science and Engineering, Hunan University, Changsha 410082, P.R. China.

[b] Key Laboratory of Environmental Biology and Pollution Control, Ministry of Education, Hunan University, Changsha 410082, P.R. China.

[c] College of Chemistry and Chemical Engineering, Changsha University of Science and Technology, Changsha 410076, P.R. China.

1 These authors contribute equally to this article.

ABSTRACT

In this study, we presented a detailed investigation on the effects of typical engineered nanomaterials (ENMs) (including Fe_2O_3 nanoparticles, Fe_3O_4 nanoparticles and multi-wall carbon nanotubes (MWCNTs) on 4-nonylphenol (4NP) degradation, diversity and function of bacterial communities in sediments. Results demonstrated that iron oxides promoted 4NP degradation and enzyme activities in sediments, while MWCNTs inhibited those activities. LEfSe analysis suggested that iron oxides incorporation discriminative enriched iron-reducing bacteria, including *Rhizobiales*, *Shewanella* and *Shewanellaceae* contributing to iron reduction and organic degradation. PICRUSt analysis demonstrated that 4NP contamination promoted the expression of biodegradation related genes, including amino acid metabolism, carbohydrate metabolism, energy metabolism and xenobiotic biodegradation and metabolism. Interesting, compare to MWCNTs, we further found that iron oxides incorporation brought about an enhanced expression of iron regulated proteins, including ferric uptake regulator (Fur), diphtheria toxin regulator (DtxR), ferrous iron transport (FeoB) and iron complex transport systems. These results indicate that iron oxides endow a better advantage in 4NP degradation, in contrast, pragmatic prospection of MWCNTs is necessary since the fact of extending persistence of 4NP in sediments. The study may be favored to evaluate the secure applications of ENMs in the aquatic environment basing on full understanding of their environmental fate.

Keywords: Engineered nanomaterials, 4-nonylphenol, bacterial response, bacterial functions, iron regulated proteins

1. Introduction

The increasing global production and application of engineered nanomaterials (ENMs) resulted in the increasing release into the environment. A recent study estimated that approximately 20,000 t of ENMs are expected to end up in municipal incineration facilities worldwide on an annual basis.[1] Among numerous ENMs, iron based nanomaterials and carbon based nanomaterials are widely applied and discharged. CNTs have been proposed for large-scale applications, such as hydrogen storage devices, quantum computers, agricultural smart delivery systems, chemical sensors, optical devices, catalyst supports, and also environmental applications.[2] Numerous studies also demonstrated the wide application of iron oxide nanomaterials (e.g., Fe_2O_3, Fe_3O_4 and FeOOH) in ceramics, leather, catalysts and also wastewater treatment.[3, 4] According to a market research report, production capacity for carbon nanotubes (CNTs) products was about 4,065 tons in 2010, and exceeded 12,300 tons in 2015.[5] The significant expansion in the application of these ENMs in large-scale commercial applications led to their eventual accumulation in environment. Once released into the environment, ENMs may accumulate in soils and/or sediments.

As the ultimate sink of various pollutants, the aquatic sediments tend to be the primary storage for ENMs as well. There is a growing concern about the fate of ENMs and their interactions with co-existed contaminants, for their potential effects on transformation and bioavailability.[6, 7] Additionally, once be accumulated into the soil/sediment, ENMs may contact soil particles, contaminants, as well as microorganisms. Microorganisms as a vital component in the ecosystem play an

important role in the fate of contaminants and ENMs. Published data for the fate of ENMs in environment primarily focused on their effect on the transportation and bioavailability of contaminants, usually in simulative porous medium or in soil. For example, Hofmann and Kammer[8] investigated the synergetic transport between carbon nanomaterials and hydrophobic organic pollutants (HOCs) in porous media, and proved that carbon nanomaterials can act as pollutant carriers to influence the transport of pollutants.

Specifically, 4-nonylphenol (4NP) has drawn increasing concern due to its estrogenic effects and ubiquity in environment.[9, 10] For example, the production and consumption loads of 4NP in the United States were reported to be 194,000 tones and 163,000 tons in 2006, respectively.[11] Meanwhile, 4NP is a breakdown product of nonylphenol ethoxylates (NPEs) widely used in detergent, textile and pesticides,[12] but more persistent, lipophilic and toxic than the parent NPEs.[13] Approximately 60% of NP was released into the aquatic environment via discharge of wastewater treatment plant (WWTP) effluents mainly as the intermediate products of NPEs.[14] Commonly, 4NP could be potentially removed by the naturally occurring microorganisms harbored in sediments and bacterial strains with bioremediation potentials have also been identified. Several bacterial strains isolated from soils, sediments or activated sludge were found to participate in the direct degradation of 4NP, such as *Pseudomonas* sp.,[15] *Sphingomonas* sp.[16, 17] and *Stenotrophomonas* sp..[18] Drastic shifts in microbial activity and bacterial community members largely driven by the organic contaminants are widely reported in pure soil or river sediment.[19, 20] Previous studies have demonstrated

that such contaminations stimulated the enrichment of organic-degrading microbial community.[21, 22] Exploring microbial communities can provide invaluable information in biological understanding of biodegradation. However, few studies concentrated on investigating the microbial response to the co-existence of ENMs and organic contaminants, which is beneficial to control risks of ENMs basing on the understandings of their interactions. Especially, scarce studies have been done in natural sediments, because of their uncertainty and complexity of dynamic characteristics.

Addressing the lack of information about the interactions between NPEs and ENMs, especially that how bacterial community and functions are affected by and responded to the stress associated with the co-existence of organic pollutants and ENMs, this study aims to explore the interactions among ENMs, environmental contaminants, bacterial communities and their implications to the microbial functions in sediments. Accordingly, the actual environmental risks posed by ENMs are mainly determined by the species and bioavailability of ENMs in environment. Numerous studies demonstrated the application of iron oxide nanomaterials (e.g., Fe_2O_3, Fe_3O_4 and FeOOH) and multi-wall carbon nanotubes (MWCNTs) to remove contaminants or lower bioavailability in wastewater.[2-4] Hence, we chose Fe_3O_4 nanoparticles, Fe_2O_3 nanoparticles and MWCNTs, which are widely applied and discharged,[23-25] as representative ENMs. Typically, our study combines distinctive bacterial function analysis basing on PICRUSt to provide a more complete investigation of sediment microbiological state than is typically reported. In this study, we aim to (1) evaluate the

effect of typical ENMs on 4NP transformation and degradation in water-sediment interface, (2) determine bacterial community response in sediments driven by 4NP contamination and ENMs incorporation, and (3) explore the variation of bacterial metabolism functions in those perturbated sediments in order to elucidate the impacting mechanisms associated with each ENMs.

2. Materials and methods

2.1. Materials

Sediment samples were collected from the 5–15 cm layer from the surface in Xiangjiang River along Xiaoxiang road near Hunan university in Changsha, China. After air drying, sediments were grinded and passed through a 2 mm sieve prior to the experiments. The sediments had a neutral pH (6.76) and an organic carbon content of 24.9 g kg^{-1}. Fe_2O_3 nanoparticles and Fe_3O_4 nanoparticles (>99.5% purity) were purchased from Jingkang new material technology Co. Ltd (Changsha). MWCNTs (>98% purity) were purchased from Chengdu Organic Chemicals Co. Ltd. of the Chinese Academy of Sciences. Detail information including shape, size and surface areas of MWCNTs and iron oxide nanoparticles were provided in Table S1 (Supporting information, SI). Ultrapure water was used throughout all experiments.

2.2. Experiment setup

Natural sediment with ultrapure water was set as blank control (group A). Natural sediment with 4NP supernatant was also used as control setup (group B). ENMs incorporated sediment was prepared by adding Fe_3O_4 nanoparticles (group C), Fe_2O_3 nanoparticles (group D) and MWCNTs (group E) at a mass proportion of 0.5%. The

prepared ENMs-sediment mixtures were then homogenized via agitating at 30 rpm for 72 h. Then, 20 g of as-prepared sediment was weighed and transferred into a serum bottle and added with 200 mL of 4NP solution (10 mg L^{-1}, dissolved in 10% methanol, pH 7.0). The samples were placed at 25 ℃ for 45 days, and the supernatant and sediment were collected at each time interval. Furthermore, the sediments with 30 mg L^{-1} 4NP (at the solid-to-liquid ratio of 1:10) were placed at 5, 15, 25 and 35 ℃ to investigate 4NP sedimentation and degradation, simulating the seasonal temperature variation in Changsha.

2.3. 4NP extraction and analysis

4NP concentrations were extracted by ultrasonic extraction with the addition of acetone and n-hexane (1:1, v/v) solution.[26] 2.0 g sediments were extracted by adding 15 mL extracting solution and ultrasonic extracted for 30 min. The supernatant was collected by centrifugation at 4 ℃. The ultrasonic extraction process was repeated for three times, and the supernatant was mixed. The supernatant was removed by rotary evaporation and then 1 mL of methyl alcohol was added to dilute the extracted 4NP. 4NP concentration was determined by HPLC equipped with fluorescent detector. Meanwhile, Fe(II) extraction and detection were carried out following Li et al..[27]

2.4. Sediment enzyme activity analysis

Urease (EC 3.5.1.5) activity was detected using the modified method of Kandeler and Gerber.[28] For urease activity detection, 2.0 g of sediments were mixed with 1 mL methylbenzene for 15 min, then 2 mL urea solution (100 g L^{-1}) and 4 mL potassium citrate buffer were added and incubated at 38 ℃ for 24 h, before finally adding ultrapure

water to a final volume of 25 mL. The supernatant was filtered, and the ammonium concentration of the filtered extracts was determined by measuring the absorbance at 578 nm via UV-Vis spectrophotometer (UV 2550, Shimadzu). Dehydrogenase activity (EC 1.1.1.1; DHA) was assayed according to Tu et al..[29] Polyphenol oxidase (EC1.10.3.2, PPO) activity was determined via evaluating the oxidation of catechol in the presence of phosphate buffer,[30] and expressed as μg of catechol oxidized h^{-1} g^{-1} of sediments (basing on dry weight).

2.5. Fe(II) extraction and analysis

Microbial available Fe(II) in sediments was extracted via 0.5 M HCl at the solid-to-liquid ratio of 1:10.[31] After immediate mixing, the mixture was vibrated at 120 rpm at 30 ℃ in dark for 24 h. Thereafter, the extraction solution was centrifugated (4000 rpm, 10 min) and filtrated for Fe(II) analysis. The concentrations of the Fe(II) were determined by the Fe(II)-selective reagent ferrozine.[32]

2.6. DNA extraction and 16S rRNA high-throughput sequencing analysis

The total genomic DNA was extracted from 0.5 g of wet sediments (sediments with 10 mg L^{-1} 4NP at 30 ℃, at day 2, 5, 15 and 30) using the E.Z.N.A.TM Soil DNA Kit (Omega Biotek, USA) according to the manufacturer's instructions. PCR amplification was carried out on a MyCycler thermal cycler (Bio-Rad, Hercules, CA, USA) using the forward primer 341F and reverse primer was 806R, targeting the V3-V4 hypervariable regions. The purified PCR amplicons were sequenced using the Illumina Miseq (300-bp paired-end reads) platform at Mega genomics Technology Co., Ltd. (Beijing, China). The sequences were aligned against the SILVA

(http://www.arbsilva.de/) database, and clustered into operational taxonomic units (OTUs) at 97% similarity. Alpha and beta diversities were then determined using the Quantitative Insights Into Microbial Ecology (QIIME, version 1.6) pipeline.[33] Phylogenetic investigations of communities by reconstruction of unobserved states (PICRUSt) was applied to evaluate the metabolic characteristics of bacterial communities in the sediments.[34]

2.7. Statistical analysis

Weighted and Unweighted UniFrac[35] distances were calculated from the normalized OTU tables for each experiment. α-diversity measures were calculated by the function 'diversity' using the Shannon method in the R package Vegan.[36] Additionally, principal coordinates analysis (PCoA) was used to visualize the variation in the microbial community composition among samples and potential clustering. A metagenomic biomarker discovery approach was employed with LEfSe linear discriminant analysis (LDA) coupled with effect size measurement which performed a nonparametric Wilcoxon sum-rank test followed by LDA analysis.

3. Results and discussion

3.1. Biodegradation rates of 4NP

In our study, quick adsorption and sink of 4NP onto sediments were detected in this study. For example, at day 5, residual 4NP concentration in the aqueous solution was detected to be below 0.05 mg L^{-1} (Fig. 1a). The fast sedimentation of 4NP was perhaps due to its high octanol−water partition coefficient (average log K_{ow} 4.48) and organic carbon partition coefficient (log K_{oc} 5.22 ± 0.38),[9] causing the rapid

sedimentation of 4NP. This suggested that sediments tended to be the primary environmental sink of 4NP in surface aquatic systems. Results were consistent with previous study that owing to its high hydrophobicity and octanol-water partition coefficient,[9] 4NP tends to be easily adsorbed to soil/sediments, which in turn become their storage in the environment.

Hence, evaluating degradation of 4NP in sediments is important in the understanding of the fate of organic contaminants in the aquatic environment. Fig. 1b shows the dynamic 4NP levels residual in sediments. Almost complete removal was observed at day 45 in sediments incorporated with iron oxides (below 1.0 mg kg^{-1}), whereas 3.97 and 15.26 mg kg^{-1} was observed in sediments without ENMs and with MWCNTs, respectively. For example, Yuan et al.[9] reported that the half-lives of 4NP in river sediments varied from 13 to 99 d under aerobic conditions, and 16 bacterial strains capable of aerobically degrading 4NP and NP1EO as carbon sources were isolated. Meanwhile, as shown in Fig. S1, the residual 4NP concentration in sediment at 5 ℃ was higher than that at 15, 25 and 35 ℃, suggesting that higher temperature favored 4NP degradation. At day 8, the residual 4NP levels at 35 ℃ were 66.95, 20.31, 30.18 and 115.28 mg kg^{-1}, accompanied with the degradation efficiencies of 77.68%, 93.23%, 89.94% and 61.57%, respectively.

It was apparent that Fe_3O_4 and Fe_2O_3 nanoparticles incorporation significantly stimulated 4NP degradation. Accordingly, application of iron oxides to strengthen organic pollution remediation has been widely recognized, taking advantages of their higher chemical activity and good biocompatibility, avoiding environmental and health

risks caused by the addition of iron compounds. Hansel et al.[37] found that the addition of iron oxides can promote the morphological transformation of valence elements in the environment, thus, favoring degradation of organic matter. Bonneville et al.[38] also observed that nano α-Fe_2O_3, amorphous α-Fe_2O_3 and lepidocrocite could be dissolved and transformed by *Shewanella putrefaciens*. Typically, nano α-Fe_2O_3 was first attached to the surface of the bacteria, and then reduced to Fe(II) catalyzed by Fe(III) reductase, showing higher iron reduction rate.

In contrary, MWCNTs incorporation limited the 4NP degradation in sediments with relatively higher residual 4NP level. Accordingly, significant inhibition of 4NP degradation might ascribed to two possible reasons: (i) MWCNTs can act as carries of organic contaminants, which may prolong their existence in environment in the case of their lower accessibility to microbes. For instance, Li et al.[39] demonstrated that CNTs restrained the polyaromatic hydrocarbons by reducing their bio-accessibility in soils, which was quite consistent with our observations. (ii) Potential cytotoxicity and microbial inactivation of MWCNTs might suppress the microbial degradation of 4NP.[40] Microbial inactivation was a theoretical pre-testing indicator of CNTs environmental impact and toxicity in soils and sediments. Our previous studies also confirmed the inactivation of microbial activity and exacerbated microbial inactivation occurred with MWCNTs even along with the reduced Cd bioavailability.[41] Accordingly, we inferred that the inhibition of MWCNTs on 4NP degradation could be associated to their adsorption ability resulting in the lower bio-accessibility and their potential toxicity to microbes in the sediments.

3.2. Temporal course of enzyme activity

Commonly, enzymes partially exist in solid or liquid phase of soils at the combined or free state, which involved in the breakdown or oxidation of organic matter.[42, 43] In our study, activities of polyphenol oxidase (PPO), dehydrogenase (DHA) and urease were investigated. As shown in Fig. 2a, PPO activities in the 4NP contaminated samples were significantly higher than the controls, indicating that PPO activity is stimulated response to 4NP. Highest PPO activity was observed at day 9, and then gradually decreased accompanied by the gradual 4NP degradation. In addition, PPO activity was significantly promoted in Fe_2O_3 and Fe_3O_4 incorporated samples compared to those with MWCNTs. PPO is an important oxidoreductase ubiquitous in soils and sediments contributing to the decomposition and transformation of aromatic compounds.[44] It is also implicated in catalyzing several different phenols to produce *o*-quinones.[29] Thus, increased PPO activity was found, which could be responsible for the demand-driven degradation of 4NP.

Comparatively, unlike PPO, activities of DHA and urease decreased immediately after 4NP contamination. Generally, activities of urease and DHA reflect the contamination level.[42] As shown in Fig. 2(b, c), initial 4NP contamination singularly inhibited DHA and urease activities due to potential toxicity of 4NP to microorganisms. Previous studies also reported the rapid decline in DHA activity in the heavy metal and organic pollutants contaminated soils.[45] Additionally, the inhibition of DHA and urease was also remitted with the addition of Fe_3O_4 and Fe_2O_3. MWCNTs incorporation

represented the most serious and long-duration enzyme inactivation, which was possible depending on the higher residual 4NP level and potential toxicity of MWCNTs.

Considering the potential microbial toxicity of ENMs, direct comparison of impacts on enzyme activity (PPO and urease) was conducted with iron oxides and MWCNTs incorporation respectively. As shown in Fig. 2d, MWCNTs inhibited PPO activity, with the activity changing in response below 0.5, while addition of Fe_3O_4 and Fe_2O_3 nanoparticles elevated PPO activity in sediments. Similar results were found in urease activity that MWCNTs were more toxic than iron oxides. Results were consistent with previous studies showing that MWCNTs exposure inhibited microbial biomass, respiration and enzyme activities in soils and sediments.[46] In contrary, iron oxide nanoparticles were much more environmental friendly with good biocompatibility.[3] Accordingly, we speculated that the variation in microbial enzyme activities in our study was mainly affected in two ways, one by influencing the 4NP degradation pathway and the other, via the intrinsic fate of iron oxide nanoparticles and MWCNTs.

3.3. Bacterial community composition and individual taxon abundance

For in-depth understanding of microbial variation in the reactors, pyrosequencing was used with 16s rRNA-specific oligonucleotide primers. Mean of 69235 clean tags was obtained. The total numbers of OTUs in the five different groups were 12793, 17517, 17434, 17645 and 16446 for groups A, B, C, D and E, respectively (Fig. S2). There were noteworthy overlaps in the differentially abundant OTUs. The similar OTUs among the five groups were about 6926, and the unique OTUs in group A, B, C, D and E were 799, 886, 752, 1023 and 685, respectively. Further analysis of the OTUs

271 in the tested samples showed that the core OTUs shared in all 20 samples were 832 (Fig.
272 3a), with the increasing observed species response to 4NP contamination. Samples at
273 day 2 and 5 exhibited the most abundant unique OTUs in the 4NP contaminated samples.
274 Furthermore, the decrease in the total and unique OTUs in the MWCNTs incorporated
275 samples suggested possible microbial inhibitory role of MWCNTs.

276 The PCoA plot based on Weighted Unifrac distances suggested that the bacterial
277 community structure varied significantly among 4NP contaminated samples and the
278 blank control (Fig. 3b). Additionally, within-sample diversity (α-diversity) revealed
279 variation in the five groups (Fig. 3c). The lowest number of observed species was
280 observed in the blank control, while 4NP contamination elevated the observed species
281 greatly. Chao1 and Shannon index analysis further demonstrated that 4NP
282 contamination contributed to significant promotion of the bacterial biodiversity. Results
283 suggested that 4NP contamination stimulated bacterial growth, resulting in a richer
284 bacterial biodiversity. No significant differences were found in the 4NP contaminated
285 sediment incorporated with Fe_2O_3, Fe_3O_4 and MWCNTs.

286 Phylogenetic analysis assigned the 16S rRNA gene sequences to different taxa,
287 allowing us to further explore the dynamics of the bacterial community (Fig. 3d). The
288 taxonomic distributions of each bacterial sample were determined at the phylum and
289 genus level. Apparently, phyla distributions were markedly different among 20 tested
290 samples. The samples could be simply classified into four subjects. In detail, subject A
291 included samples with 4NP contamination at day 15 (also including MWCNTs
292 incorporated sample at day 30). Subject B involved in the samples at initial day 2 and

293 5 with relatively high 4NP levels. Subject C was classified as the samples without 4NP
294 contamination (blank controls), while subject D contained samples with 4NP
295 contamination at day 30 (except for samples with MWCNTs). Results demonstrated
296 that 4NP contamination significantly altered the bacterial communities.

297 Apparently, *Proteobacteria* were dominant phylum and did not vary significantly
298 in all samples, which is indicative of their admirable tolerance to the stress relative to
299 4NP. In subject C, *Proteobacteria* and *Firmicutes* dominated the community,
300 accounting for over 70% of all the sequences generated. *Firmicutes* were the most
301 abundant phylum in natural sediments with a relatively stable proportion of 36.86%–
302 56.81%. Interestingly, initial 4NP exposure (day 2, 5 and 15) led to a significant
303 decrease in the proportion of *Firmicutes* to below 17%. Meanwhile, we also observed
304 the recovery of *Firmicutes* at day 30 with 4NP degradation (except for samples with
305 MWCNTs with high levels of 4NP). In subjects A and B with high levels of 4NP,
306 *Proteobacteria* (22.72%–35.19%), *Actinobacteria* (15.46%–27.76%) and
307 *Acidobacteria* (9.78%–19.22%) were the most abundant groups. Furthermore,
308 *Actinobacteria*, *Acidobacteria*, *Chloroflexi* and *Gemmatimonadetes* increased
309 significantly response to 4NP contamination. Results indicated the vital roles of these
310 bacteria in organic tolerance and degradation in sediments. Notably, *Acidobacteria*,
311 which have been reported to be abundant in soils and sediments, were characterized by
312 their ability to withstand metal-contaminated, organic-contaminated, acidic, and other
313 extreme environments.[47] Previous studies also reported the roles of *Acidobacteria* in
314 microbial degradation of lignocellulosic plant biomass.[48, 49] Moreover, recent studies

315 have reported that members of *Chloroflexi* were ubiquitous in the environment, and
316 some of them played important roles in organic matter degradation.[50] The large
317 proportion and demand driven of the these phyla are indicatives of their contribution to
318 biodegradation.[51]

319 Further comparison of the bacterial communities was conducted at the class level
320 to further investigate the impact of 4NP contamination and ENMs incorporation (SI,
321 Fig. S3). Comparison between groups A and B revealed that 4NP contamination led to
322 the sharp decrease in *Bacilli*, *Clostridia*, *Spartobacteria*, *Betaproteobacteria*,
323 *Bacteroidia* and *Sphingobacteriia*, suggesting their sensibility to 4NP contamination
324 owing to the potential toxicity. In group B, *Gammaproteobacteria* and *Flavobacteria*
325 were observed to be in highest abundance. Meanwhile, comparison among three groups
326 with ENMs revealed that *Negativicutes*, *Acidobacteria*, *Caldilineae*, *Themomicrobia* and
327 *Ktedonobacteria* were the dominant classes in groups C and D with iron oxide
328 nanomaterials, whereas the most represented bacterial classes in group E were *Soil*
329 *Crenarchaeotic Group* (SCG), *α-proteobacteria*, *Deinococci* and *Phycisphaerae*.
330 Although the dominant classes varied among the treatments, most bacterial classes were
331 shared by all sediments. For example, *Nitrospira*, *Chloroflexia*, *Acidimicrobiia*,
332 *Acidobacteria*, *Gemmatimonadetes* and *Thermoleophilia* significantly increased in
333 response to 4NP contamination, but did not vary significantly with various ENMs
334 incorporation. These bacteria were also widely reported involved in the degradation of
335 organic contaminants and with potential ability to withstand other numerous

contaminants,[52-54] which may reflect the ecological coherence of the contaminated sediments.

3.4. Discriminative bacterial community analysis

To identify the taxa that differed significantly among the 4NP contaminated samples, linear discriminant analysis effect size (LEfSe) was employed. As shown in Fig. 4, 16 bacterial clades presented statistically significant differences with an LDA threshold of 2.0. Most bacteria were significantly enriched in group E with MWCNTs, while only 1 and 2 clades showed favored abundances in group C and D, respectively. For example, higher taxonomic levels of *Thermales* (including *Deinococcus*, *Deinococci*, *Thermales*, *Thermaceae* and *Thermus*), *Phycisphaerae*, *Halieaceae*, *Flavitalea* and *Nitrosococcus* were found in group E (Fig. 4a). Commonly, *Deinococcus*, *Thermus* and *Nitrosococcus* are known to be capable of degrading phenolic compounds.[55] Thus, the dominance of these species indicated that they may have important functions in biodegradation. However, even consistently observed in contaminated environments, no significant roles in organic contaminant biodegradation were found for *Halieaceae*, *Phycisphaerae* and *Flavitalea*.

It was noteworthy that *Nocardia* was the dominant taxa as well as the biomarker in group C with Fe_3O_4 nanoparticles, while *Pantoea* and *Promicromonospora* were significantly more abundant in group D with Fe_2O_3 nanoparticles. *Nocardia*, *Pantoea* and *Promicromonospora* species as candidates for the removal of organic contaminants have been widely reported. Notably, *Nocardia* and *Promicromonospora* are actinobacterial[56] and play important roles as recyclers of organic matter.[57] Typically,

Nocardia has been reported to synthesis biosurfactant such as lipopeptides and glycolipids, which is benefit to the potential degradation of hydrocarbon compounds.[58] Chang et al.[59] has isolated *Nocardia* sp. strain CYKS2 capable of degrading dibenzothiophene and thiazole. Zeinali et al[60] also reported that *Nocardia otitidiscaviarum* TSH1 could degrade phenol, n-alkanes and some polycyclic aromatic hydrocarbons. Their pre-dominance in groups C and D suggested that they were selectively enriched with iron oxides incorporation and had an important function related to 4NP removal.

Considering the significant difference in 4NP degradation, LEfSe analysis was further conducted between group D and E (group D vs group E). LefSe analysis showed that the biomarker demonstrating significant difference between group D and group E. As shown in Fig. S4, group E with MWCNTs mainly enriched members of *Flavitalea*, *Lysobacter*, *Deinococcus*, and *Oligoflexales*, consistent with the previous results (Fig. S4). *Enterobacteriales* (o) (including *Enterobacteriaceae* (f) and *Enterobacteriales* (g)), *Promicromonospora* (g), *Aquincola* (g) were the discriminative taxa in group D with Fe_2O_3 nanoparticles. Interestingly, iron-reducing bacteria, including *Pantoea*, *Shewanella* and *Shewanellaceae* were discriminately detected in group D. It may be speculated that iron-reducing bacteria may proliferate in response to Fe_2O_3 nanoparticles incorporation. These iron-reducing bacteria, which are capable of coupling microbial Fe(III) reduction to oxidation of organic matter, play a significant role in the global geochemical cycling.[61, 62] *Pantoea* and *Shewanellaceae* family are well-known ferric iron-respiring microorganisms (FRMs), which use Fe(III) as well as

other metals as terminal electron acceptors.[63] Accordingly, FRMs such as *Pantoea*,[64] *Salinibacterium*[65] and *Shewanella* [66] have been reported capable to transform, detoxify, or immobilize a variety of metallic and organic pollutants. For example, *Pantoea*, such as *P. agglomerans* SP1, is widely reported to participate in microbial Fe(III) reduction[67] and linked to the biodegradation of numerous organic contaminants.[68, 69] Recently, Haleyur et al.[64] reported that *Pantoea* sp. could also utilize substrates from different biochemical categories (i.e., amino acids, phenolic compounds, carbohydrates, carboxylic acids and polymers) as carbon source. This suggested that iron oxides incorporation induced the microorganisms with iron reducing ability and endowed a better advantage in 4NP survival and degradation.

3.5. Predicted functions basing on PICRUSt analysis

We also explored microbial function using the PICRUSt algorithm.[34] A total of 6,372 KEGG functions, corresponding to 304 level 3 KO entries were identified by matching sequences data basing on KEGG enzyme nomenclature. Majority of predicted protein sequences in the tested samples were associated with the functions involved in metabolism (48.87%–52.04%), genetic information processing (15.68%–16.03%), environmental information processing (12.82%–15.31%) and unclassified processes (13.06%–13.40%) (Fig. 5a). Specifically, 4NP contamination resulted in a significant increase in the genes involved in relevant metabolic functions, including amino acid metabolism (9.78%–11.41%), carbohydrate metabolism (9.38%–10.83%), energy metabolism (5.27%–6.03%), xenobiotics biodegradation and metabolism (2.87%–4.64%), replication and repair (6.47%–7.31%), whereas enzyme family (1.89%–

2.15%), cell mobility (2.88%–4.60%) and membrane transport (4.22%–5.95%) were significantly depleted response to 4NP contamination (Fig. 5b). Results demonstrated that 4NP contamination promoted genes for metabolism, genetic information processing, environmental information processing and cellular processes associated with biodegradation pathways. Meanwhile, Fe_2O_3 incorporated samples showed the most significant expression in genes related to carbohydrate metabolism (10.83%) and membrane transport (5.95%). In contrary, MWCNTs incorporation repressed those functional genes, while promoting the genes associated with amino acid metabolism, cell growth and death related pathways.

Typically, 4NP contamination significantly enriched the abundances of xenobiotic biodegradation pathways, including degradation of polycyclic aromatic hydrocarbon, benzoate, bisphenol, xylene and drug metabolism (Fig. 5c). Specially, samples incorporated with Fe_2O_3 nanoparticles possessed the most abundant functional genes corresponding to organic degradation, followed by Fe_3O_4 nanoparticles, while MWCNTs showed the least functional genes. Results demonstrated that ENMs affected bacterial function on cellular processes and metabolic pathways, thus, also affecting the 4NP degradation ability in sediments. Results were quite agreed with Kim et al., who reported that $Fe(OH)_3$ addition increased cell numbers/viability and caused changes in cellular physiology that resulted in enhancement of carbon tetrachloride bioremediation, mainly via stimulating microbial iron reduction and surface-bound Fe(II) production.[70]

Additionally, it was interesting to note that proteins related to iron regulation and transport also varied significantly among the different groups. In responding to changes

in the environment, especially iron oxides incorporation, response regulators usually alter expression of genes that promote iron transport and availability. Here, those related functional proteins were determined by PICRUSt prediction and presented in Fig. 6a. As well known, iron is the most abundant transition metal in the environment and is an essential co-factor for many metabolic enzymes involved in biological reactions.[61] Commonly, iron transport is usually controlled via ferrous iron transport systems (composed of three proteins FeoA, FeoB and FeoC) and iron complex transport system including substrate binding protein, iron complex out-membrane receptor protein, permease protein as well as ATP binding protein.[66] Meanwhile, ABC transporters can also translocate heme and iron-siderophores across the cytoplasmic membrane with important functions in iron metabolism.[71] Meanwhile, iron uptake is usually regulated by the ferric uptake regulator (Fur) and Fur-like protein (including manganese uptake regulator (Mur) and peroxide stress response (PerR)). Diphtheria toxin regulator (DtxR) protein that can also regulate iron uptake in some gram-positive bacteria (such as *streptomyces*, *corynebacteria*, and *mycobacteria*, etc.).[62, 65] As shown in Fig. 6a, iron complex transport system, including permease protein, out-membrane receptor protein and substrate-binding protein, were widely varied in all samples. The mean percentage of these iron complex transport system in group C with Fe_2O_3 nanoparticles was higher than group E with MWCNTs. It was apparent that iron oxides incorporation promoted the expression of Fur family transcriptional protein, phtheria toxin regulator (including Fur, and FhuF and DtxR) and iron complex transport system (especially substrate-binding protein, permease protein and ATP-binding protein) (Fig.

6a), which were responsible for iron transport and iron cycle in the environment. However, PICRUSt is only a means of predicting functional genes; thus, further research is required to confirm the accuracy of gene function information by other biology technologies.

To date, limited studies are available focused on the effects of exogenous iron oxide nanomaterials incorporation on iron regulation pathway and iron cycling in natural sediments. Thus, to deep understand the possible roles of iron oxides incorporation and iron-reduction in 4NP degradation, microbial available Fe(II) concentrations in sediments were determined (Fig. 6b). Apparently, gradual increase in microbial available Fe(II) was found in all samples. In natural sediments without ENMs, Fe(II) concentration was elevated from 77.26 mg kg^{-1} to 140.42 mg kg^{-1}, which could possibly be due to the natural iron oxides in river sediments that are undergoing reduction with increasing available Fe(II).[72] Indeed, significant increase in Fe(II) levels was found in groups C and D with iron oxides incorporation, while sediments incorporated with MWCNTs exhibited the lowest Fe(II) levels during the entire duration of the 30 day study. Results here demonstrated that iron oxide nanoparticles incorporation stimulated iron transport and cycle in sediments. Observations were similar to previous studies reporting that the microbial reduction of Fe(III) to Fe(II) had key roles in the iron cycle and organic matter mineralization in the overlying water-sediments interface.[73] Accordingly, iron oxides could be dissolved in soil or sediment to form Fe(III), and further reduced to Fe(II) via microbial reduction by anaerobic, facultative anaerobic and hyperthermophilic microorganisms. Insoluble Fe(III) oxides

468 are generally the most abundant potential electron acceptors for oxidation of organic
469 matter. Detailly, iron-reducing microbes could transfer electrons to the extracellular
470 surface of iron oxides by respiration, Fe(III) acted as electron acceptors and transferred
471 to Fe(II). Meanwhile, microbially driven Fenton reactions might occur with the
472 alternately production of H_2O_2 (via microbial O_2 respiration) and Fe(II) (via microbial
473 Fe(III) reduction), which might promote the organics degradation.[74] These could
474 promote the organics degradation through the cyclic transformation between Fe(III) and
475 Fe(II).[75]

476 In our study, significant negative correlation was found between residual 4NP
477 levels and Fe(II) concentrations in the sediments (Fig. 6c), suggesting the coherence of
478 lower residual 4NP and higher Fe(II) levels. Results were quite corroborated with
479 previous studies that crystalline iron oxides, including goethite and hematite, could
480 stimulate iron reduction and organic degradation.[76] Although the thermodynamic
481 favorability of crystalline Fe(III) oxides reduction increased compared to amorphous
482 Fe(III) oxides, previous studies have suggested that FRMs were capable of utilizing
483 crystalline solid-phase Fe(III) as electron acceptor.[73, 77] For example, Zhang et al.[78] also
484 found that a variety of iron oxide-mediated iron reduction processes significantly
485 promoted the reduction of nitrate by *Bacillus* sp., among which α-Fe_2O_3 and γ-Fe_2O_3
486 exhibited the most notable promotion. Meanwhile, even with the weak ability of Fe(III)
487 reduction, many fermentative microorganisms were reported to be responsible for the
488 production of fermentation products, serving as electron donors for the iron reduction.[75]
489 For example, *Shewanella* species can transfer electrons from the cell surface to Fe(III)

oxides by releasing soluble electron-shuttling compounds, thus overcoming the insolubility of Fe(III) oxides.[38] Furthermore, redox-reactive organic compounds ubiquitous in sediments, such as humic acids and plant exudates, could serve as electron shuttles, which can transfer electrons between a wide variety of both inorganic and organic compounds in redox reactions.[79] These findings were consistent with the LEfSe analysis, which showed that *Shewanella* species tended to be biomarkers in Fe_2O_3 incorporated sediments. Herein, results suggested that such an enhancement of microbial mediated hematite-Fe(III) reduction to microbial available Fe(II) might be important reason contributing to promotion in 4NP degradation. Accordingly, we inferred that iron oxide nanomaterials promoted 4NP degradation might be attributed to two possible reasons: First, good biocompatibility and low toxicity of iron oxides to sediment microbes. Second, because some microorganisms contributed to iron oxides dissolution and couple growth using Fe(III) as electron acceptors, the exogenous addition of iron oxides might increase cell numbers and viability of iron reducing microbes, and thus stimulating microbial iron reduction and surface-bound Fe(II) production that contributed to enhancement in 4NP degradation.

4. Conclusion

Unlike cellular toxicity studies, which have been widely performed, studies focusing on the environmental behavior and fate of ENMs are still in their infancy. Investigations on the interaction among ENMs, chemicals and environmental organisms remain challenging. In this study, taking 4NP as a target contaminant, we

explored 4NP sedimentation, degradation and bacterial response in a water-sediment interface ecosystem. We found that:

- For the three kind of ENMs used here, iron oxides promoted 4NP degradation in sediments, whereas MWCNTs significantly inhibited 4NP degradation.
- Observed bacterial response and LEfSe analysis results suggested that iron oxides incorporation stimulated iron respiring bacteria participating in iron reduction and thus strengthening 4NP degradation.
- PICRUSt analysis demonstrated that iron oxides elevated the expression of xenobiotic biodegradation and metabolism proteins and also iron regulated proteins including FeoB, Fur and Fur-like family, and iron complex transport systems. Indeed, such an enhanced expression might contribute to the promotion of 4NP degradation in iron oxides incorporated sediments. In contrast, the reduction in those functional proteins and even microbial inactivation led to impede 4NP degradation in MWCNTs incorporated sediments.
- Given this information, we suggested the well biocompatible and stimulation in the predominance of iron-reducing bacteria probably have important functions in sediments and brought about a better performance with 4NP degradation.
- Further study is required to confirm the accuracy of gene function information by biology technologies (e.g., metagenomics, microarrays, single cell genomics).

ACKNOWLEDGMENTS

This study was financially supported by the Program for the National Natural Science Foundation of China (51709101, 51879101, 51609023, 51579098, 51779090,

51709101, 51521006), the National Program for Support of Top–Notch Young Professionals of China (2014), the Program for Changjiang Scholars and Innovative Research Team in University (IRT-13R17), and Hunan Provincial Science and Technology Plan Project (2018SK20410, 2017SK2243, 2016RS3026), and the Fundamental Research Funds for the Central Universities (531118010226, 531119200086, 531118010114, 531107050978).

Appendix A. Supplementary data

Additional information about ENMs properties and bacterial community and function analysis were provided in the supporting information. Content includes Figures S1-S4 and Tables S1.

REFERENCES

1 D. Singh, L. A. Schifman, C. Watson-Wright, G. A. Sotiriou, V. Oyanedel-Craver, W. Wohlleben and P. Demokritou, Nanofiller Presence Enhances Polycyclic Aromatic Hydrocarbon (PAH) Profile on Nanoparticles Released during Thermal Decomposition of Nano-enabled Thermoplastics: Potential Environmental Health Implications, *Environmental Science & Technology* 2017, **51**, (9), 5222-5232.

2 J. H. Deng, X. R. Zhang, G. M. Zeng, J. L. Gong, Q. Y. Niu and J. Liang, Simultaneous removal of Cd (II) and ionic dyes from aqueous solution using magnetic graphene oxide nanocomposite as an adsorbent, *Chem. Eng. J.* 2013, **226**, 189-200.

3 P. Xu, G. M. Zeng, D. L. Huang, C. L. Feng, S. Hu, M. H. Zhao, C. Lai, Z. Wei, C. Huang and G. X. Xie, Use of iron oxide nanomaterials in wastewater treatment: A review, *Sci. Total Environ.* 2012, **424**, 1-10.

4 X. Tan, Y. Liu, G. Zeng, X. Wang, X. Hu, Y. Gu and Z. Yang, Application of biochar for the removal of pollutants from aqueous solutions, *Chemosphere* 2015, **125**, 70-85.

5 A. Parish, Production and applications of carbon nanotubes, carbon nanofibers, fullerenes, graphene and nanodiamonds: A global technology survey and market analysis, *ET-113. Stamford, CT, USA: Innovative Research and Products* 2011.

6 A. Nel, T. Xia, L. Mädler and N. Li, Toxic potential of materials at the nanolevel, *Science* 2006, **311**, (5761), 622-627.

7 V. L. Colvin, The potential environmental impact of engineered nanomaterials, *Nat. Biotechnol.* 2003, **21**, 1166.

8 T. Hofmann and F. Von Der Kammer, Estimating the relevance of engineered carbonaceous nanoparticle facilitated transport of hydrophobic organic contaminants in porous media, *Environ.*

Pollut. 2009, **157**, (4), 1117-1126.
9 A. Soares, B. Guieysse, B. Jefferson, E. Cartmell and J. N. Lester, Nonylphenol in the environment: A critical review on occurrence, fate, toxicity and treatment in wastewaters, *Environ. Inter.* 2008, **34**, (7), 1033-1049.
10 J. Shan, T. Wang, C. Li, E. Klumpp and R. Ji, Bioaccumulation and bound-residue formation of a branched 4-nonylphenol isomer in the geophagous earthworm Metaphire guillelmi in a rrice paddy soil, *Environ. Sci. Technol.* 2010, **44**, (12), 4558-4563.
11 Anonymous, Overview of the US nonylphenol sector, *Anonymous, Focus Surfact. 2007* 2007, **8**, 4.
12 T. Toyama, M. Murashita, K. Kobayashi, S. Kikuchi, K. Sei, Y. Tanaka, M. Ike and K. Mori, Acceleration of nonylphenol and 4-tert-octylphenol degradation in sediment by *Phragmites australis* and associated rhizosphere bacteria, *Environ. Sci. Technol.* 2011, **45**, (15), 6524-6530.
13 W. Giger, P. H. Brunner and C. Schaffner, 4-Nonylphenol in sewage sludge: accumulation of toxic metabolites from nonionic surfactants, *Science* 1984, **225**, (4662), 623.
14 G. G. Ying, B. Williams and R. Kookana, Environmental fate of alkylphenols and alkylphenol ethoxylates—a review, *Environment international* 2002, **28**, (3), 215-226.
15 S. Yuan, C. Yu and B. Chang, Biodegradation of nonylphenol in river sediment, *Environ. Pollut.* 2004, **127**, (3), 425-430.
16 K. Fujii, N. Urano, H. Ushio, M. Satomi and S. Kimura, *Sphingomonas cloacae* sp. nov., a nonylphenol-degrading bacterium isolated from wastewater of a sewage-treatment plant in Tokyo, *Int. J. Syst. Evol. Microbiol.* 2001, **51**, (2), 603-611.
17 W. Pluemsab, Y. Fukazawa, T. Furuike, Y. Nodasaka and N. Sakairi, Cyclodextrin-linked alginate beads as supporting materials for *Sphingomonas cloacae*, a nonylphenol degrading bacteria, *Bioresour. Technol.* 2007, **98**, (11), 2076-2081.
18 A. Soares, B. Guieysse, O. Delgado and B. Mattiasson, Aerobic biodegradation of nonylphenol by cold-adapted bacteria, *Biotechnol. Lett.* 2003, **25**, (9), 731-738.
19 J. H. Writer, J. N. Ryan, S. H. Keefe and L. B. Barber, Fate of 4-nonylphenol and 17β-estradiol in the Redwood River of Minnesota, *Environ. Sci. Technol.* 2011, **46**, (2), 860-868.
20 H. Wu, C. Lai, G. Zeng, J. Liang, J. Chen, J. Xu, J. Dai, X. Li, J. Liu and M. Chen, The interactions of composting and biochar and their implications for soil amendment and pollution remediation: a review, *Crit. Rev. Biotechnol.* 2017, **37**, (6), 754-764.
21 R. Margesin, A. Zimmerbauer and F. Schinner, Monitoring of bioremediation by soil biological activities, *Chemosphere* 2000, **40**, (4), 339-346.
22 X. Ren, G. Zeng, L. Tang, J. Wang, J. Wan, Y. Liu, J. Yu, H. Yi, S. Ye and R. Deng, Sorption, transport and biodegradation–an insight into bioavailability of persistent organic pollutants in soil, *Sci. Total Environ.* 2018, **610**, 1154-1163.
23 P. Xu, G. M. Zeng, D. L. Huang, M. Yan, M. Chen, C. Lai, H. Jiang, H. P. Wu, G. M. Chen and J. Wan, Fabrication of reduced glutathione functionalized iron oxide nanoparticles for magnetic removal of Pb (II) from wastewater, *J. Taiwan Inst. Chem. Eng.* 2017, **71**, 165-173.
24 J. L. Gong, B. Wang, G. M. Zeng, C. P. Yang, C. G. Niu, Q. Y. Niu, W. J. Zhou and Y. Liang, Removal of cationic dyes from aqueous solution using magnetic multi-wall carbon nanotube nanocomposite as adsorbent, *J. Hazard. Mater.* 2009, **164**, (2), 1517-1522.
25 J. Wan, G. Zeng, D. Huang, L. Hu, P. Xu, C. Huang, R. Deng, W. Xue, C. Lai and C. Zhou, Rhamnolipid stabilized nano-chlorapatite: Synthesis and enhancement effect on Pb-and Cd-

immobilization in polluted sediment, *J. Hazard. Mater.* 2018, **343**, 332-339.
26 P. Xu, C. Lai, G. Zeng, D. Huang, M. Chen, B. Song, X. Peng, J. Wan, L. Hu, A. Duan and W. Tang, Enhanced bioremediation of 4-nonylphenol and cadmium co-contaminated sediment by composting with *Phanerochaete chrysosporium* inocula, *Bioresour. Technol.* 2018, **250**, (Supplement C), 625-634.
27 X. Li, S. Zhou, F. Li, C. Wu, L. Zhuang, W. Xu and L. Liu, Fe(III)oxide reduction and carbon tetrachloride dechlorination by a newly isolated *Klebsiella pneumoniae* strain L17, *J. Appl. Microbiol.* 2009, **106**, 130-139.
28 E. Kandeler and H. Gerber, Short term assay of soil urease activity using colorimetric determination of ammonium, *Biol. Fert. Soils* 1988, **6**, 68-72.
29 C. Tu, Y. Teng, Y. Luo, X. Sun, S. Deng, Z. Li, W. Liu and Z. Xu, PCB removal, soil enzyme activities, and microbial community structures during the phytoremediation by alfalfa in field soils, *J. Soil. Sediment.* 2011, **11**, (4), 649-656.
30 P. Perucci, C. Casucci and S. Dumontet, An improved method to evaluate the o-diphenol oxidase activity of soil, *Soil Biol. Biochem.* 2000, **32**, (13), 1927-1933.
31 S. Ratering and S. Schnell, Localization of iron-reducing activity in paddy soil by profile studies, *Biogeochemistry* 2000, **48**, (3), 341-365.
32 H. Fadrus and J. Malý, Rapid extraction-photometricdetermination of traces of iron(II) and iron (III) in water with 1, 10-phenanthroline, *Anal. Chim. Acta.* 1975, **77**, 315-316.
33 J. G. Caporaso, J. Kuczynski, J. Stombaugh, K. Bittinger, F. D. Bushman, E. K. Costello, N. Fierer, A. G. Peña, J. K. Goodrich, J. I. Gordon, G. A. Huttley, S. T. Kelley, D. Knights, J. E. Koenig, R. E. Ley, C. A. Lozupone, D. McDonald, B. D. Muegge, M. Pirrung, J. Reeder, J. R. Sevinsky, P. J. Turnbaugh, W. A. Walters, J. Widmann, T. Yatsunenko, J. Zaneveld and R. Knight, QIIME allows analysis of high-throughput community sequencing data, *Nat. Methods* 2010, **7**, 335.
34 M. G. Langille, J. Zaneveld, J. G. Caporaso, D. McDonald, D. Knights, J. A. Reyes, J. C. Clemente, D. E. Burkepile, R. L. V. Thurber, R. Knight, R. G. Beiko and C. Huttenhower, Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences., *Nat. Biotechnol.* 2013, **31**, 814-821.
35 C. Lozupone and R. Knight, UniFrac: a new phylogenetic method for comparing microbial communities, *Appl. Environ. Microbiol.* 2005, **71**, (12), 8228-8235.
36 J. Oksanen, R. Kindt, P. Legendre, B. O'Hara, M. H. H. Stevens, M. J. Oksanen and M. Suggests, The vegan package, *Community Ecology Package* 2007, **10**, 631-637.
37 Hansel C.M., Benner S.G., Nico P. and F. S., Structural constraints of ferric (hydr)oxides on dissimilatory iron reduction and the fate of Fe(II). *Geochim. Cosmochim. Acta* 2004, **68**, (15), 3217-3229.
38 S. Bonneville, T. Behrends, P. V. Cappellen, C. Hyacinthe and W. F. M. Roling, Reduction of Fe(III) colloids by Shewanella putrefaciens: A kinetic model, *Geochim. Cosmochim. Ac.* 2006, **70**, 5842-5854.
39 S. B. Li, U. Turaga, B. Shrestha, T. A. Anderson, S. S. Ramkumar, M. J. Green, S. Das and J. E. Cañas-Carrell, Mobility of polyaromatic hydrocarbons (PAHs) in soil in the presence of carbon nanotubes, *Ecotoxicol. Environ. Saf.* 2013, **96**, 168-174.
40 S. Kang, M. Herzberg and D. F. Rodrigues, Antibacterial effects of carbon nanotubes: Size does matter, *Langmuir* 2008, **24**, (13), 6409-6413.
41 P. Xu, M. Chen, G. Zeng, D. Huang, C. Lai, Z. Wang, M. Yan, Z. Huang, X. Gong, B. Song, T. Li

and A. Duan, Effects of multi-walled carbon nanotubes on metal transformation and natural organic matters in riverine sediment, *J. Hazard. Mater.* 2019, **doi.org/10.1016/j.jhazmat.2019.04.037**.

42 T. Chen, C. Sun, G. Lin and W. Chen. In *Change in enzymatic activity in Tween80-enhanced phytoremediation of polychlorinated biphenyl-contaminated soil*, Application of Materials Science and Environmental Materials (AMSEM2015): Proceedings of The 3rd International Conference, 2016; World Scientific: 2016; p 179.

43 W. Xue, D. Huang, G. Zeng, J. Wan, C. Zhang, R. Xu, M. Cheng and R. Deng, Nanoscale zero-valent iron coated with rhamnolipid as an effective stabilizer for immobilization of Cd and Pb in river sediments, *J. Hazard. Mater.* 2018, **341**, 381-389.

44 M. Sylvestre, Prospects for using combined engineered bacterial enzymes and plant systems to rhizoremediate polychlorinated biphenyls, *Environ. Microbiol.* 2013, **115**, (3), 907-915.

45 M. B. Hinojosa, J. A. Carreira, J. M. Rodríguez-Maroto and R. García-Ruíz, Effects of pyrite sludge pollution on soil enzyme activities: Ecological dose–response model, *Sci. Total Environ.* 2008, **396**, (2), 89-99.

46 F. Gottschalk, T. Sonderer, R. W. Scholz and B. Nowack, Modeled Environmental Concentrations of Engineered Nanomaterials (TiO_2, ZnO, Ag, CNT, Fullerenes) for Different Regions, *Environ. Sci. Technol.* 2009, **43**, (24), 9216-9222.

47 N. L. Ward, J. F. Challacombe, P. H. Janssen, B. Henrissat, P. M. Coutinho, M. Wu, G. Xie, D. H. Haft, M. Sait and J. Badger, Three genomes from the phylum *Acidobacteria* provide insight into the lifestyles of these microorganisms in soils, *Appl. Environ. Microbiol.* 2009, **75**, (7), 2046-2056.

48 T. A. Pankratov, A. O. Ivanova, S. N. Dedysh and W. Liesack, Bacterial populations and environmental factors controlling cellulose degradation in an acidic *Sphagnum* peat, *Environ. Microbiol.* 2011, **13**, (7), 1800-1814.

49 S. A. Eichorst, C. R. Kuske and T. M. Schmidt, Influence of plant polymers on the distribution and cultivation of bacteria in the phylum *Acidobacteria*, *Appl. Environ. Microbiol.* 2011, **77**, (2), 586-596.

50 D. Fang, G. Zhao, X. Xu, C. Zhang, Q. Shen, Z. Fang, L. Huang and F. Ji, Microbial community structures and functions of wastewater treatment systems in plateau and cold regions, *Bioresour. Technol.* 2018, **249**, 684-693.

51 H. M. Jang, J. H. Ha, J. M. Park, M.-S. Kim and S. G. Sommer, Comprehensive microbial analysis of combined mesophilic anaerobic–thermophilic aerobic process treating high-strength food wastewater, *Water Research* 2015, **73**, 291-303.

52 Z. Yan, N. Song, H. Cai, J. H. Tay and H. Jiang, Enhanced degradation of phenanthrene and pyrene in freshwater sediments by combined employment of sediment microbial fuel cell and amorphous ferric hydroxide, *J. Hazard. Mater.* 2012, **199**, 217-225.

53 H. M. Dionisi, A. C. Layton, G. Harms, I. R. Gregory, K. G. Robinson and G. S. Sayler, Quantification of *Nitrosomonas oligotropha*-like ammonia-oxidizing bacteria and *Nitrospira* spp. from full-scale wastewater treatment plants by competitive PCR, *Appl. Environ. Microbiol.* 2002, **68**, (1), 245-253.

54 A. B. Caracciolo, P. Grenni, R. Ciccoli, G. Di Landa and C. Cremisini, Simazine biodegradation in soil: analysis of bacterial community structure by in situ hybridization, *Pest Manag. Sci.* 2005, **61**, (9), 863-869.

55 J. Boonnorat, S. Techkarnjanaruk, R. Honda and P. Prachanurak, Effects of hydraulic retention

time and carbon to nitrogen ratio on micro-pollutant biodegradation in membrane bioreactor for leachate treatment, *Bioresour. Technol.* 2016, **219**, 53-63.
56 N. M. Henderson and R. K. Sutherland, *Nocardia* and *Actinomyces*, *Medicine* 2017, **45**, (12), 753-756.
57 J. Ishikawa, A. Yamashita, Y. Mikami, Y. Hoshino, H. Kurita, K. Hotta, T. Shiba and M. Hattori, The complete genomic sequence of *Nocardia farcinica* IFM 10152, *P. Natl. Acad. Sci. USA* 2004, **101**, 14925-14930.
58 R. Yang, G. Liu, T. Chen, S. Li, L. An, G. Zhang, G. Li, S. Chang, W. Zhang, X. Chen, X. Wu and B. Zhang, Characterization of the genome of a *Nocardia* strain isolated from soils in the Qinghai-Tibetan Plateau that specifically degrades crude oil and of this biodegradation, *Genomics* 2018, **doi.org/10.1016/j.ygeno.2018.02.010**.
59 J. H. Chang, S. K. Rhee, Y. K. Chang and H. N. Chang, Desulfurization of diesel oils by a newly isolated dibenzothiophene-degrading *Nocardia* sp. strain CYKS2, *Biotechnol. Prog.* 1998, **14**, 851-855.
60 M. Zeinali, M. Vossoughi, S. K. Ardestani, E. Babanezhad and M. Masoumian, Hydrocarbon degradation by thermophilic *Nocardia otitidiscaviarum* strain TSH1: physiological aspects, *J. Basic Microbiol.* 2007, **47**, 534-539.
61 G. Porcheron and C. M. Dozois, Interplay between iron homeostasis and virulence: Fur and RyhB as major regulators of bacterial pathogenicity, *Vet. Microbiol.* 2015, **179**, (1), 2-14.
62 K. N. Raymond, E. A. Dertz and S. S. Kim, *Enterobactin*: An archetype for microbial iron transport, *P. Natl. Acad. Sci. USA* 2003, **100**, (7), 3584-3588.
63 A. Sikora, J. Wojtowicz-Sienko, P. Piela, U. Zielenkiewicz, K. Tomczyk-Zak, A. Chojnacka, R. Sikora, P. Kowalczyk, E. Grzesiuk and M. Blaszczyk, Selection of bacteria capable of dissimilatory reduction of Fe(III) from a long-term continuous culture on molasses and their use in a microbial fuel cell, *J. Microbio. Biotechnol.* 2011, **21**, (3), 305-316.
64 N. Haleyur, E. Shahsavari, M. Taha, L. S. Khudur, E. Koshlaf, A. M. Osborn and A. S. Ball, Assessing the degradation efficacy of native PAH-degrading bacteria from aged, weathered soils in an Australian former gasworks site, *Geoderma* 2018, **321**, 110-117.
65 M. F. Fillat, The FUR (ferric uptake regulator) superfamily: Diversity and versatility of key transcriptional regulators, *Arch. Biochem. Biophys.* 2014, **546**, 41-52.
66 S. C. Andrews, A. K. Robinson and F. Rodriguez-Quinones, Bacterial iron homeostasis, *FEMS Microbiol. Rev.* 2003, **27**, 215-237.
67 C. A. Francis, A. Y. Obraztsova and B. M. Tebo, Dissimilatory metal reduction by the facultative anaerobe *Pantoea agglomerans* Sp1, *Appl. Environ. Microbiol.* 2000, **66**, 543-548.
68 M. M. Abboud, K. M. Khleifat, M. Batarseh, K. A. Tarawneh, A. Al-Mustafa and M. Al-Madadhah, Different optimization conditions required for enhancing the biodegradation of linear alkylbenzosulfonate and sodium dodecyl sulfate surfactants by novel consortium of *Acinetobacter calcoaceticus* and *Pantoea agglomerans*, *Enzyme Microb. Technol.* 2007, **41**, (4), 432-439.
69 J. Schäfer, U. Jäckel and P. Kämpfer, Analysis of *Actinobacteria* from mould-colonized water damaged building material, *Syst. Appl. Microbiol.* 2010, **33**, (5), 260-268.
70 S. Kim and F. W. Picardal, Enhanced anaerobic biotransformation of carbon tetrachloride in the presence of reduced iron oxides, *Environ. Toxicol. Chem.* 1999, **18**, (10), 2142-2150.
71 V. Braun and K. Hantke, Recent insights into iron import by bacteria, *Curr. Opin. Chem.. Biol.* 2011, **15**, (2), 328-334.

72 R. Karlin and S. Levi, Diagenesis of magnetic minerals in recent haemipelagic sediments, *Nature* 1983, **303**, 327.

73 K. A. Weber, L. A. Achenbach and J. D. Coates, Microorganisms pumping iron: anaerobic microbial iron oxidation and reduction, *Nat. Rev. Microbiol.* 2006, **4**, 752.

74 R. Sekar, H. D. Shin and T. J. DiChristina, Direct conversion of cellulose and hemicellulose to fermentable sugars by a microbially-driven Fenton reaction, *Bioresource Technology* 2016, **218**, 1133-1139.

75 C. J. Lentini, S. D. Wankel and C. M. Hansel, Enriched iron(III)-reducing bacterial communities are shaped by carbon substrate and iron oxide mineralogy, *Front. Microbiol.* 2012, **3**, 1-14.

76 D. R. Lovley and E. J. P. Phillips, Organic matter mineralization with reduction of ferric Iron in anaerobic sediments, *Appl. Environ. Microbiol.* 1986, **51**, (4), 683.

77 J. E. Kostka and K. H. Nealson, Dissolution and reduction of magnetite by bacteria, *Environ. Sci. Technol.* 1995, **29**, (10), 2535-2540.

78 W. Zhang, X. M. Li, T. X. Liu and L. F.B., Enhanced nitrate reduction and current generation by *Bacillus* sp. in the presence of iron oxides, *J. Soil. Sediment.* 2012, **12**, 364-365.

79 K. P. Nevin and D. R. Lovley, Potential for nonenzymatic reduction of Fe(III) via electron shuttling in subsurface sediments, *Environ. Sci. Technol.* 2000, **34**, 2472-2478.

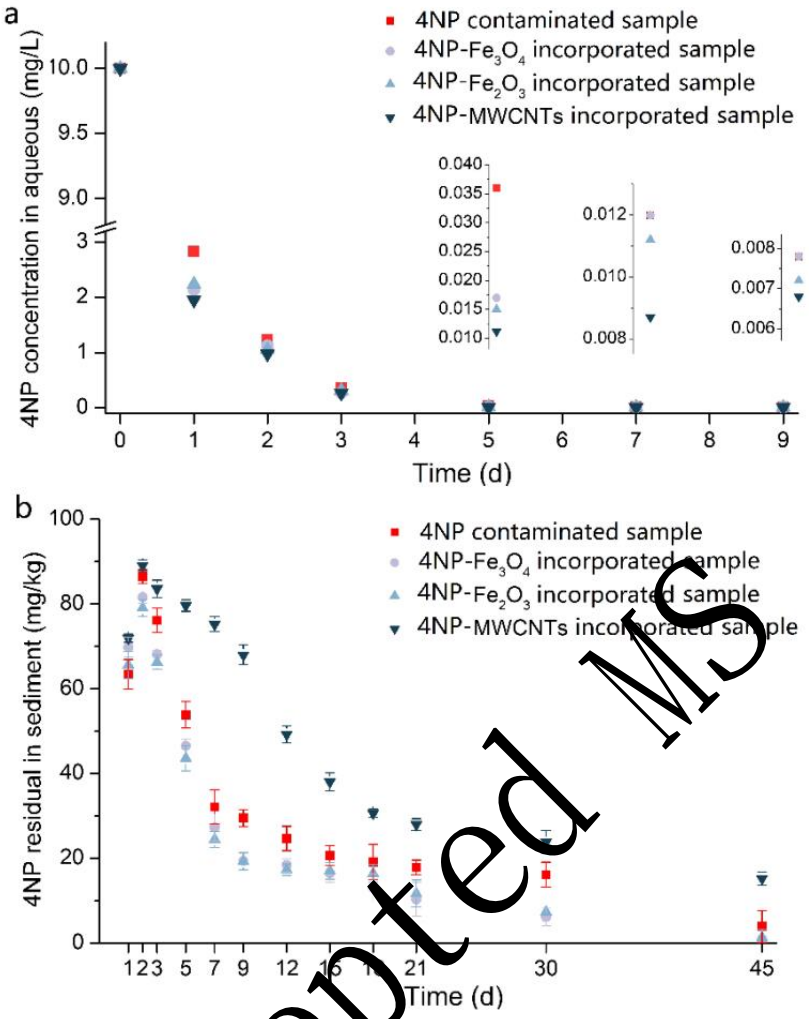

Figure 1. (a) Residual 4NP concentration in overlying water; (b) Residual 4NP concentration in sediments incorporated with different engineering nanomaterials. (Experimental conditions: initial 4NP concentration: 10 mg L^{-1}; ENMs dosage: 0.5%; Solid-to-liquid ratio: 1:10; Temperature: 25 ℃, pH: 7.0).

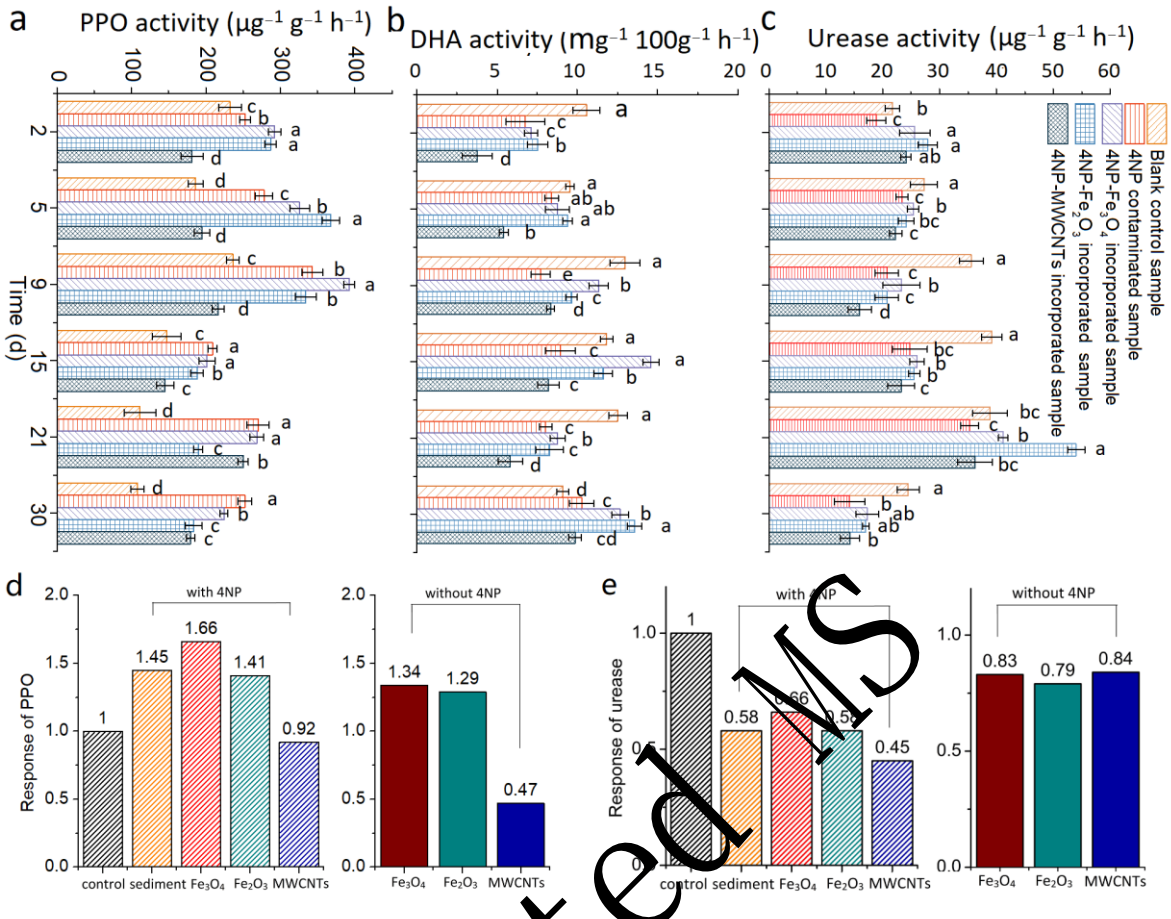

Figure 2. Dynamic variations in the activity of polyphenol oxidase (PPO, a), dehydrogenase (DHA, b) and urease (c), response of PPO (d) and urease activity (e) to Fe_3O_4 nanoparticles, Fe_2O_3 nanoparticles and MWCNTs at day 9 (d). Different letters indicate significant differences ($p < 0.05$). (Experimental conditions: initial 4NP concentration: 10 mg L^{-1}; ENMs dosage: 0.5%; Solid-to-liquid ratio: 1:10; Temperature: 25 ℃, pH: 7.0).

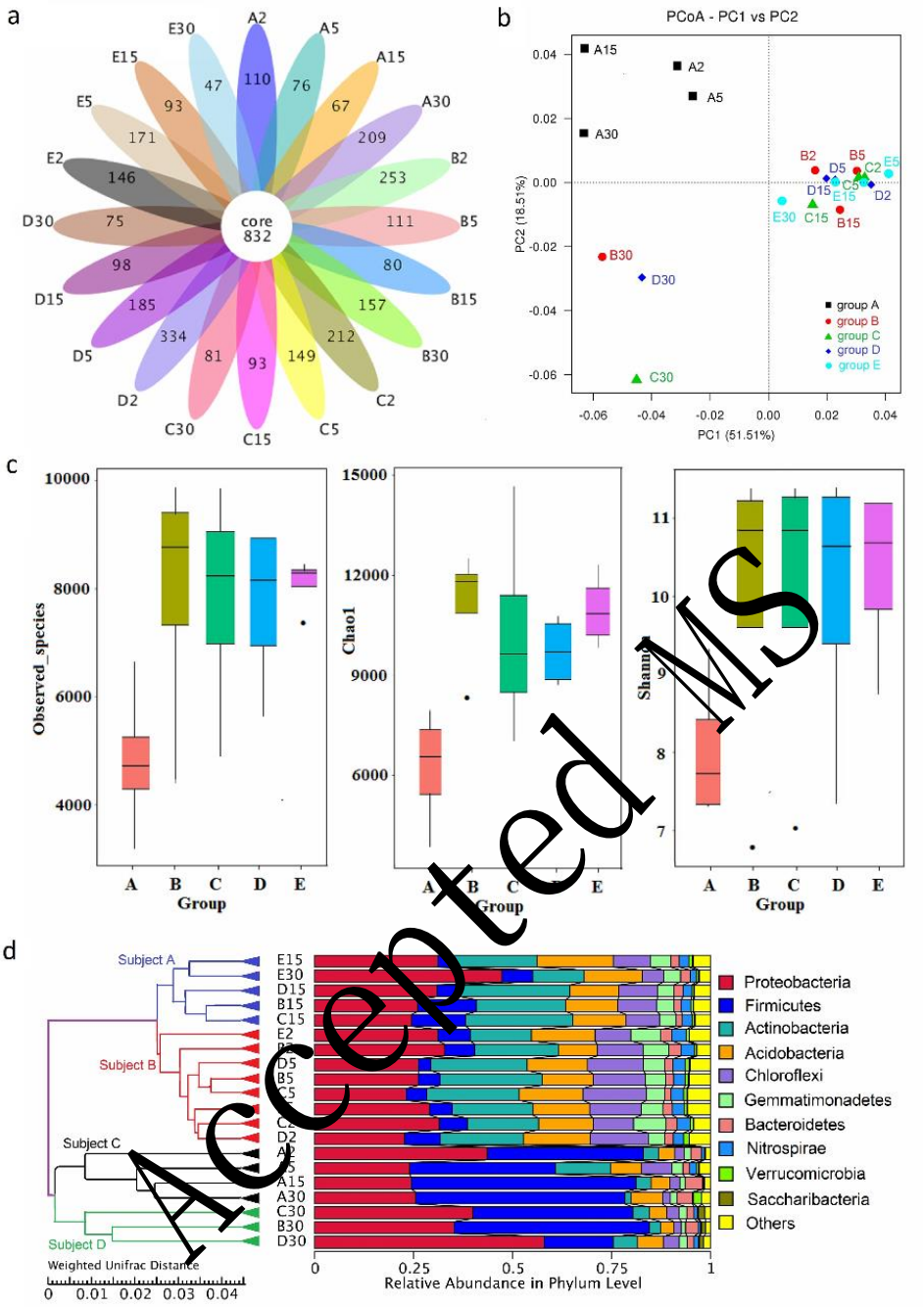

Figure 3. (a) OTUs analysis in the tested samples at day 2, 5, 15 and 30; (b) α-diversity analysis in the five tested groups; (c) PCoA analysis based on the distance of Weighted Unifrac; (d) Hierarchical cluster analysis based on Weighted Unifrac distance in Phylum level in the tested samples. (Group A: control samples without 4NP and ENMs; group B: samples contaminated with 4NP; group C: samples incorporated with 4NP

and Fe_3O_4; group D: samples incorporated with 4NP and Fe_2O_3; group E: samples incorporated with 4NP and MWCNTs).

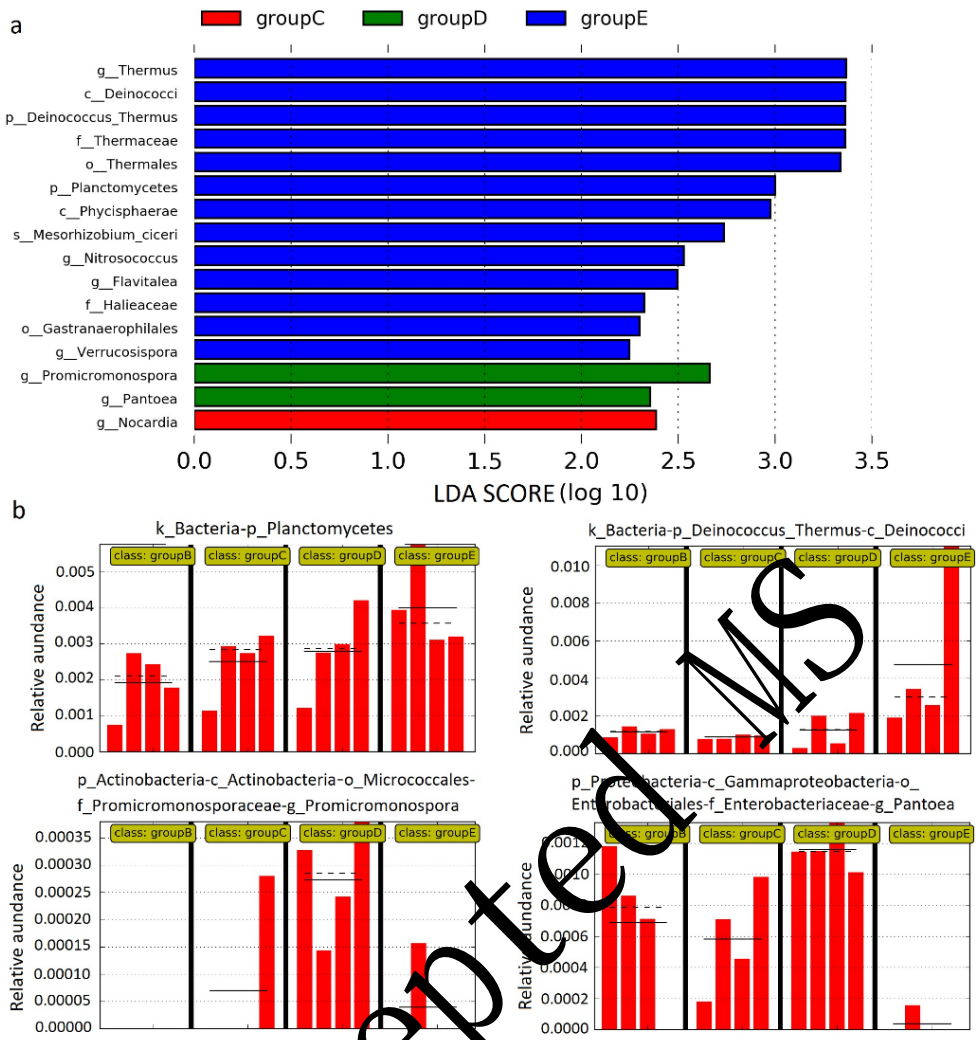

Figure 4. LEfSe results and Biomarker detection analysis of bacteria among the five tested groups. (Group A: control samples without 4NP and ENMs; group B: samples contaminated with 4NP; group C: samples incorporated with 4NP and Fe_3O_4; group D: samples incorporated with 4NP and Fe_2O_3; group E: samples incorporated with 4NP and MWCNTs).

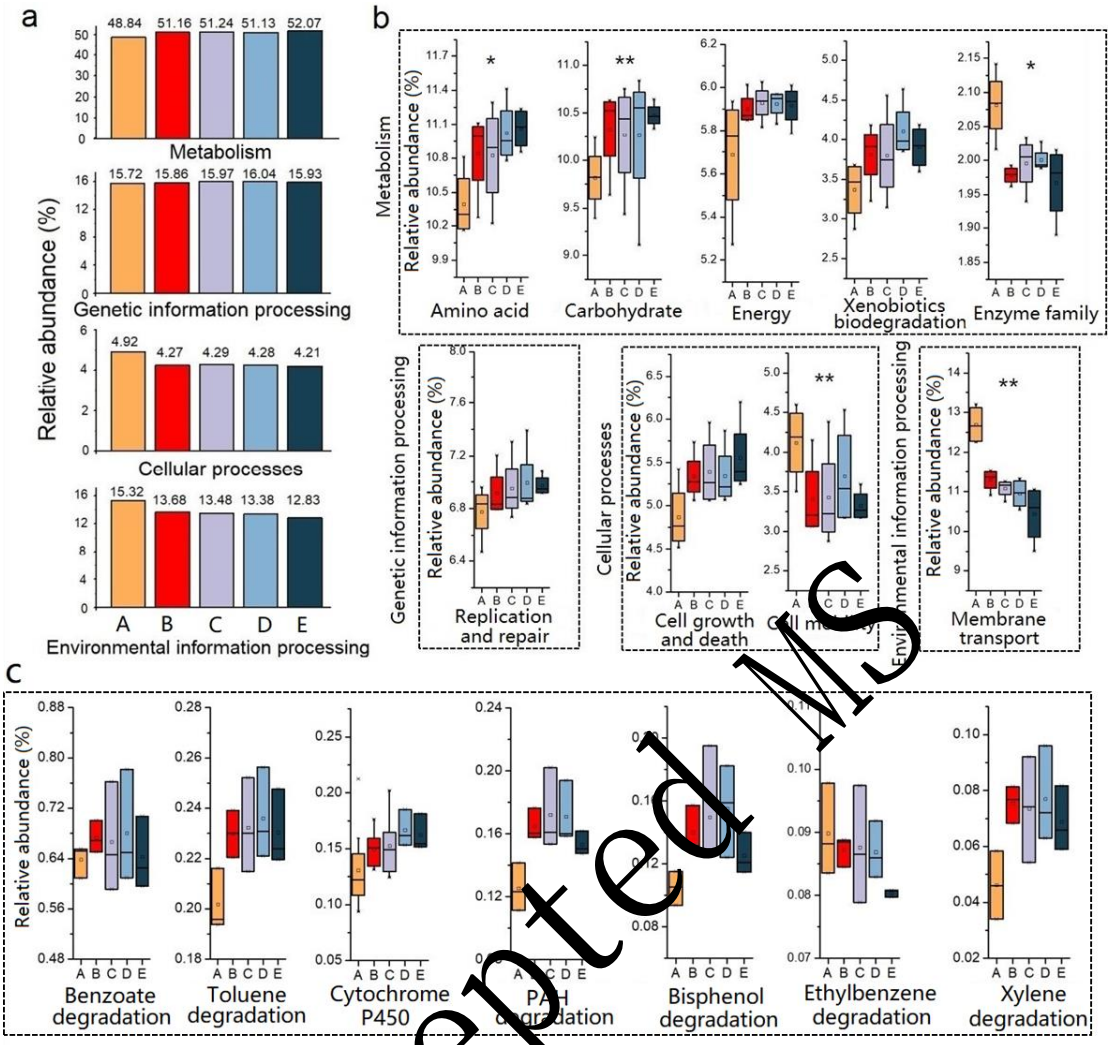

Figure 5. (a) Variation of bacterial functions relating to metabolism, genetic information processing, cellular processes and environmental information processing at KEGG level 1; (b) Variation of bacterial functions at KEGG level 2; (c) Variation of bacterial functions relating to xenobiotics biodegradation and metabolism at KEGG level 3. Bacterial gene functions were predicted from 16S rRNA gene-based microbial compositions using the PICRUSt algorithm to make inferences from KEGG annotated databases. (Group A: control samples without 4NP and ENMs; group B: samples contaminated with 4NP; group C: samples incorporated with 4NP and Fe_3O_4; group D:

797 samples incorporated with 4NP and Fe_2O_3; group E: samples incorporated with 4NP

798 and MWCNTs).

799

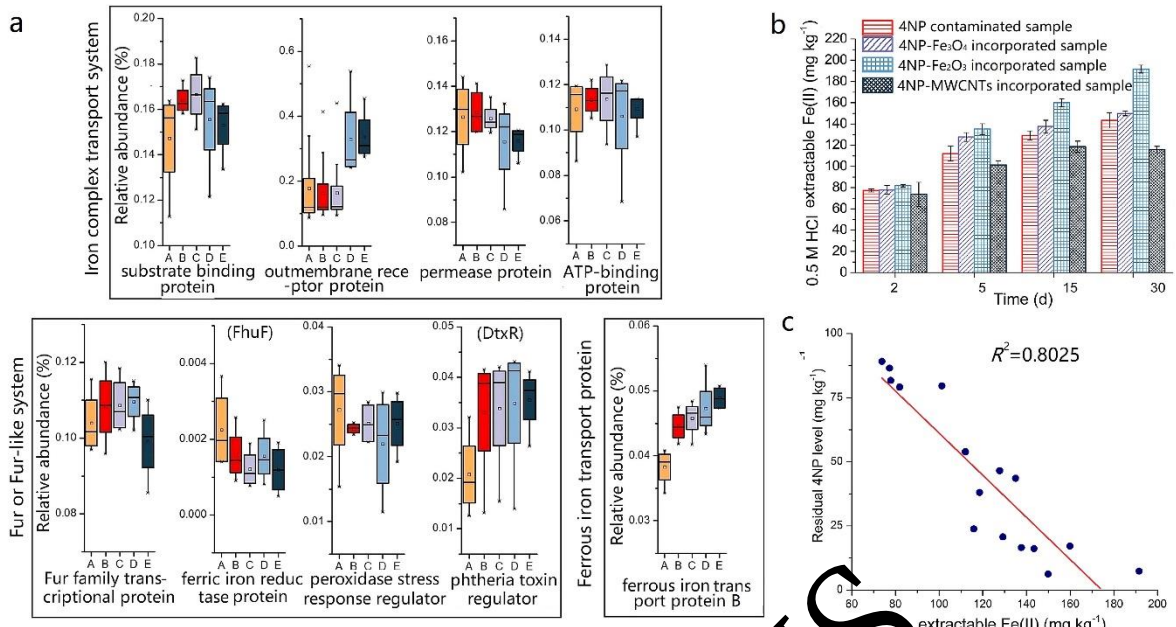

Figure 6. (a) HCl-extracted Fe(II) concentration in sediments; (b) Relationship between residual 4NP concentration and extracted Fe(II) concentrations; (c) Variation of bacterial functions relating to iron regulated proteins. (Group A: control samples without 4NP and ENMs; group B: samples contaminated with 4NP; group C: samples incorporated with 4NP and Fe_3O_4; group D: samples incorporated with 4NP and Fe_2O_3; group E: samples incorporated with 4NP and MWCNTs).