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Enhanced bioremediation of 4-nonylphenol and cadmium co-contaminated sediment by composting with *Phanerochaete chrysosporium* inocula

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Abstract

Composting is identified as an effective approach for solid waste disposal. The bioremediation of 4-nonylphenol (4NP) and cadmium (Cd) co-contaminated sediment was investigated by composting with *Phanerochaete chrysosporium* (*P. chrysosporium*) inocula. *P. chrysosporium* inocula and proper C/N ratios (25.51) accelerated the composting process accompanied with faster total organic carbon loss, 4NP degradation and Cd passivation. Microbiological analysis demonstrated that elevated activities of lignocellulolytic enzymes and sediment enzymes was conducive to organic chemical transformation. Bacterial community diversity results illustrated that *Firmicutes* and *Proteobacteria* were predominant species during the whole composting process. Aerobic cellulolytic bacteria and organic degrading species played significant roles. Toxicity characteristic leaching procedure (TCLP) extraction and germination indices results indicated the efficient detoxification of 4NP and Cd co-contaminated sediment after 120 days of composting. Overall, results demonstrated that *P. chrysosporium* enhanced composting was available for the bioremediation of 4NP and Cd co-contaminated sediment.

Keywords: Composting; 4-nonylphenol; cadmium; bacteria diversity; detoxification.

1. Introduction

4-Nonylphenol (4NP), a central degradation product and either a raw material of nonylphenol ethoxylates (NPEs) surfactants, exists widely in most environmental samples, including water, soils and sediments (Karahan et al., 2010; Zeng et al., 2013). 4NP is a representative environmental endocrine disruptor (EED) with estrogenic responses, carcinogenicity, teratogenesis and mutagenicity (Gerent & Spinelli, 2016; Gong et al., 2009). 4NP is easily adsorbed on particulate matter due to its low solubility in aqueous solution, and thus soils and sediments are crucial destination. Field surveys revealed that 4NP existed at a level of ng L^{-1} to μg L^{-1} in water and mg kg^{-1} concentrations in sediments (Gong et al., 2011). Accordingly, wide range of NP has been detected in surface sediment, a mean value of 73.42 and 115.90 ng g^{-1} dw was reported in East Dongting Lake and Honghu Lake, respectively (Yang et al., 2015). Luo et al. (2017) further reported a maximal bioaccumulation of 3.27 ng g^{-1} in fish samples from lake and rivers within Hunan Province, China. Additionally, significant amounts of heavy metals released to aquatic and terrestrial environments, commonly resulting in the co-contamination of heavy metals and organic pollutants (Xu et al., 2012a; Xu et al., 2017). Numerous studies demonstrated the wide occurrence and great threat of heavy metals in sediment. Recent study reported a range of 2.95-29.15 mg kg^{-1} Cd, especially accompanied with a relative high bioavailable fraction (66.93%), in sediment from Xiangjiang river, Hunan province (Liu et al., 2017a). As such, in consideration of the potential toxicity of 4NP and heavy metals, complete remediation is crucial to the environment.

Microbial biodegradation is a principle mechanism for organic carbon transformation and degradation. Biodegradation, through various biological process, might modify organic molecules or result in microbial destruction of organic pollutants (Alexander, 1999; Yang et al., 2010). Microorganism with available catabolic potential to accessible organic chemicals is essential to the biodegradation. Microorganism is the significant component in sediments, which tends to be major or occasionally sole means in biodegradation in the case of sediment self-purification. However, biodegradation ability of indigenous microorganisms in sediment is limited. Furthermore, as structural and natural diversity, specific degradation is necessary due to the degradative requirement of microbial accessibility to chemicals (Alexander, 1999; Carboneras et al., 2017). Enhanced biodegradation is imperative to complete destruction of organic chemicals.

Composting is an appropriate enhanced biodegradation approach for organic contaminant removal and heavy metal passivation (Jonkers et al., 2001; Rawoteea et al., 2017). How to reduce active metals and organic contaminants to alleviate the toxicity of the composting products needs to broaden concerns. Our previous studies suggested that composting with white rot fungi inocula is one of the most prospective approaches for soil and solid waste treatment (Huang et al., 2008). *Phanerochaete chrysosporium* (*P. chrysosporium*) are extensively studied in environmental remediation (Xu et al., 2012b). Degradation of NPs or 4NP by *P. chrysosporium* has been reported previously (Cajthaml et al., 2009; Subramanian & Yadav, 2009). Cajthaml et al. (2009) reported that *P. chrysosporium* was efficient to degrade 4NP in

aqueous solution within 14 d with the acceptable activity of lignin peroxidase (LiP) and manganese peroxidase (MnP). However, scarce study focused on the *P. chrysosporium* enhanced bioremediation of 4NP in sediment, especially in the metal co-contaminated sediment. Hence, the aim of this study was to investigate the bioremediation of 4NP and Cd co-contaminated sediment via composting with *P. chrysosporium* inocula. An attempt was made to determine the potential roles of oxidoreductase in organic chemical biodegradation and the possibility in composting maturity assessment accompanied with physico-chemical parameters. Importantly, bacterial community analysis was conducted to investigate the bacterial species participated in composting and bioremediation.

2. Materials and methods

2.1. Materials preparation and fungal strain

Sediment samples were collected from the 5–15 cm layer from Xiangjiang River in Changsha, China. After air dried, sediment samples were grinded and sieved to 2 mm prior to the experiments. The sediment had a neutral pH (6.76) and an organic carbon content of 24.9 g kg^{-1}. The 4NP concentration was detected at 31.5 μg kg^{-1} in the prepared sediments. 500 mL of 4NP methanol solution (200 mg L^{-1}) was added to 1 kg of dry sediment to prepare the 4NP contaminated sediment. The slurry was then stirred for 12 h in dark and left to stewing in fume cupboard until dried. Thereafter, the contaminated sediments were grinded and sieved to 2 mm for further use. The straw was air-dried and cut into about 20 mm length. Rice bran and vegetable leaves were also air dried as composting substrate.

The *P. chrysosporium* (BKMF-1767) obtained from China Center for type Culture Collection (Wuhan, China). Spore suspensions were prepared by scraping and blending in the sterile distilled water. All reagents were analytical grade and used without further purification. Distilled water was used for the preparation of all the solutions throughout this study.

2.2. Composting setup

Four piles with each about 3 kg of composting materials (dry weight) were set up indoors in 70 L polystyrene boxes with the internal dimensions of 0.58 × 0.42 × 0.38 m (length × width × height). In detail, sediment, straw, vegetable leaves and rice bran were mixed in the designed ratios to obtained four piles with C/N ratios at the value of 9.53 (Pile A), 18.77 (Pile B), 25.51 (Pile C) and 25.46 (Pile D), respectively. Initial 4NP concentration was 45.86 mg kg^{-1} (Pile A), 37.01 mg kg^{-1} (Pile B), 30.59 mg kg^{-1} (Pile C) and 29.87 mg kg^{-1} (Pile D). Initial total Cd concentration was 79.28 mg kg^{-1} (Pile A), 73.51 mg kg^{-1} (Pile B), 52.34 mg kg^{-1} (Pile C) and 51.91 mg kg^{-1} (Pile D). The moisture content was adjusted to about 70% with the distilled water. Pile A, B and C was inoculated with 1% of *P. chrysosporium* spore suspensions, pile D was used as the control without the inoculants. The moisture was controlled by addition of distilled ultrapure water, and the piles were turned over twice a week for eventual oxygen supply and temperature distribution.

2.3. 4NP analysis and Cd sequential extraction

For chemical and microbiological analysis, triplicate samples were collected and mixed from four symmetrical locations in each pile at designated time intervals. 4NP

was extracted by ultrasonic extraction method modified from Yang et al. (2014). 2.0 g composting samples were dried via vacuum freeze-drying equipment for 24 h, and then 15 mL of acetone and n-hexane (1:1, v/v) extracting solution and ultrasonic extracted for 30 min. The supernatant was collected by centrifugation at 4000 rpm for 20 min at 4 °C. 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. All the extracts were filtrated through 0.45 μm membrane. 4NP concentration was detected by HPLC as described by Gabriel et al. (2005). Detection was carried out at 277 nm, and acetonitrile/water (85:15, v/v) was used as the mobile phase at a flow rate of 1.0 mL min^{-1}. The limit of detection (LOD) for NP was 0.01 mg kg^{-1} sediment in this study.

Metal speciation was investigated by improved BCR procedure according to the previous study (Rauret et al., 1999). Metal fraction was classified as exchangeable fraction (F1), reducible fraction (F2), oxidizable fraction (F3) and residual fraction (F4). The TCLP tests were conducted with the TCLP leachant (0.1 M glacial acetic acid solution, pH 2.88±0.05). The TCLP leachant was mixed with the samples with a leachant-to-sample ratio of 20:1, vibrated at 30 rpm for 16 h. The supernatant was centrifuged and filtrated by 0.45 μm membrane for the Cd testing. TCLP levels were normalized to mg kg^{-1} by multiplying the TCLP values by the mass/volume ratios.

2.4. Chemical and microbiological analysis

The temperature was recorded at a depth of 20 cm in each pile. For TOC and microbial activity analysis, 5.0 g fresh composting sample was extracted with 50 mL

distilled water, vibrating for 45 min and then centrifuging for 10 min to get the composting extract. Total organic carbon (TOC) was determined in the aqueous extracts via TOC 5050 (Shimadzu Europe GmbH, Germany). Typical degradation enzymes secreted by *P. chrysosporium*, including LiP and MnP, were determined according to our previous study (Huang et al., 2016). Representative enzymes participating in organic pollutants degradation in sediment were also estimated. Polyphenol oxidase (PPO) activity was determined via evaluating the oxidation of catechol in the presence of phosphate buffer (Perucci et al., 2000), and expressed as μg of catechol oxidized h^{-1} g^{-1} of composting matter (on dry weight basis). Catalase (CAT) activity was determined by investigation the degradation ability of H_2O_2 in composition extraction according to previous study (Huang et al., 2016).

2.5. DNA extraction and sequence analysis of the 16S rRNA amplicons

Bacterial species and community structures in pile C were explored to explain bacterial diversity and functional species contributed to organic matter degradation and metal passivation. The total microbial genomic DNA was extracted from 0.5 g of compost matter (in pile C at day 1, 6, 14, 21, 40, 70 and 90) using the E.Z.N.A.TM Soil DNA Kit (Omega Biotek, USA) according to the manufacturer's instructions. DNA extracted from compost samples was used for the PCR amplification of the 16S rRNA genes. The PCR amplification was run on a MyCycler thermal cycle (Bio-Rad, Hercules, CA, USA). The primers for Bacterial rRNA coding genes were 515F and 806R. The purified PCR amplicons were sequenced using the Illumina Miseq (300-b p paired-end reads) platform at Novogene Bioinformatics Technology Co., Ltd.

(Beijing, China). Paired-end reads were merged using FLASH (V1.2.7, http://ccb.jhu.edu/software/FLASH/), and quality filtering of reads was performed according to Matsumura et al. (2015). The 16S rRNA gene sequences were edited and assembled using the Uparse software (Uparse v7.0.1001) and sequences with ≥ 97% similarity were grouped to OTUs. OTUs were compared against the Unit database (https://unite.ut.ee/) using the FASTA program. Bootstrapped trees were created using the Unweighted Pair Group Method with Arithmetic mean (UPGMA) based on 10 hierarchical clusters in the QIIME package.

2.6. Seed germination indices analysis

Seed germination indices (GI) were investigated as previously described (Liu et al., 2017b). 5.0 g fresh composting samples were extracted via 50 mL sterile ultrapure water shaken for 1 h and filtered for seed germination experiments. 20 selected ryegrass seeds were well greased at culture dishes containing two pieces of filter paper. Then 10 mL composting extract was added, incubated at 25 °C for 72 h. 10 mL sterile ultrapure water was added as control. Each sample was performed in duplicate, seed germination and root length were recorded. GI was calculated as following:

$$GI\ (\%) = (\text{Seed germination} \times L_{sample}) / (\text{Seed germination} \times L_{control}) \times 100\%$$

2.7. Statistical analysis

Statistical analysis was conducted by SPSS16.0 software. One-way analysis of variance (ANOVA) using a 95% confidence level was used. Linear discriminant analysis coupled with effect size (LEfSe) was applied to identify the bacterial taxa differentially represented in the tested samples (White et al., 2009). Correlation

between environmental variables and composting assessment was estimated by Pearson's correlation coefficient analysis (SPSS16.0 software) and principal component analysis (PCA) using CANOCO software (Version 4.5).

3. Results and discussion

3.1. Total organic carbon change during composting

Table 1 shows the time-course of TOC change during 90 days of composting. The TOC content decreased continuously, especially with dramatically depletion during the initial 21 days, suggesting the organic materials decomposition during the composting process. The most significant TOC reduction occurred in pile C, which varied appreciably from 74.15% to 31.53% after 90 days of composting. Results suggested that appropriate C/N ratios and *P. chrysosporium* inocula contributed to the TOC degradation, most probably due to the potential lignin degradation ability of *P. chrysosporium* at nitrogen limited condition.

3.2. 4NP degradation and Cd passivation

Evaluating extractable concentration of 4NP and Cd transformation during aerobic composting is important to bioremediation assessment. Fig. 1a plots a stimulated 4NP biodegradation at higher C/N ratios. Biodegradation rate at the initial 3 day was calculated to be 0.18, 0.20, 0.29, 0.17 mg $(kg\ d)^{-1}$ in pile A, B, C and D, respectively. Results represented that biodegradation of 4NP also occurred in pile D without *P. chrysosporium*, which might be attributed to the bioremediation of sediment and composting matrix introduced microorganisms. These biological processes substantially degraded 4NP during the initial 40 days of composting stage.

At day 40, the residual 4NP concentration was 0.251, 0.245, 0.008 and 0.586 mg kg^{-1} in pile A, B, C and D, respectively. This represented almost complete reduction in the total 4NP of 99.97% in pile C, and a relatively high degradation efficiency above 98% in all tested piles. Pseudo-first-order model was further applied to evaluate the NP degradation in this research. Model fitting results represent that 4NP biodegradation during 40 days of composting fitted the pseudo-first-order model quite well with the relative high correlation coefficients (R^2>0.98). $t_{1/2}$ was calculated to be 3.306 d, 3.239 d, 2.485 d and 3.588 d in pile A, B, C and D, respectively.

Practically, as displayed in Fig. 1b, Cd existed mainly in the exchangeable fraction and reducible fraction initially, over 90% of the total Cd was distributed in the F1 and F2 with high bioavailability. Obviously, gradual decrease in exchangeable fraction was observed in the four composting piles, particularly the variation of Cd contents in F1 was relatively evident in pile C with *P. chrysosporium* incubation. The F1 contents in pile C varied gradually from 26.73 mg kg^{-1} to 8.31 mg kg^{-1} from day 0 to day 120. Cd transformation to F4 contents occurred mainly in the later stage. The F4 contents increased from 2.23 mg kg^{-1} to 10.59 mg kg^{-1} in pile C and from 2.17 mg kg^{-1} to 5.67 mg kg^{-1} in pile D. Metal speciation analysis illustrated that composting process, especially *P. chrysosporium* incubation might promote Cd passivation.

3.3. Response of microbial activity during composting

The unique biodegradation ability is on account of the non-specific mineralizing extracellular enzymes (Asgher et al., 2008). Ligninolytic enzymes are reported to be the effective mediators of biodegradation processes and control the biodegradation

rate of *P. chrysosporium* (Xu et al., 2016). In the present study, both LiP and MnP showed a prominent increase during the early composting stage, then a descend tendency to stabilization occurred (Fig. 2). After enzyme peak achieved, decrease of LiP and MnP activity in the late composting process. The LiP and MnP activity might be limited by the excessive production of glucose, polysaccharide during the late composting period which hampered the diffusion of oxygen and other nutrient (Rothschild et al., 1999). It is obvious that pile C showed relatively higher LiP and MnP activities in four piles. Besides, relatively low LiP and MnP activities were observed in pile D without *P. chrysosporium* inocula. The highest LiP and MnP activity was both observed in pile C at the value of 86.78 U g^{-1} and 70.73 U g^{-1} at day 14 and day 21, respectively. Results suggested that pile C at proper C/N ratios was benefit to the ligninolytic enzyme secretion, which was consistent to previous studies that nitrogen-limited medium was appropriate for higher ligninolytic enzymes production (Kersten & Cullen, 2007).

4NP was reported to be efficiently removed by LiP, MnP and Lac (Hirai et al., 2004; Tsutsumi et al., 2001). LiP is classic heme enzymes with widespread application in organic pollution control, and plays a unique role in the geochemical cycling of carbon by mediating lignin depolymerization (Dong et al., 2014). Similarly, MnP is a heme peroxidase that oxidizes phenolic compounds and participate in the organic degradation in the presence of Mn(II) and H_2O_2 (Loredano Pollegioni et al., 2015). LiP has strong oxidizing property and can enable to catalyze one- and two-electron oxidations of numerous chemicals (Mester T et al., 2001). Dong et al.

(2014) reported that possible pathway leading to 4NP transformation was polymerization through radical–radical coupling mechanism catalyzed by LiP and horseradish peroxidase. In the present study, the explode in LiP and MnP at the initial 21 days also contributed to the rapid degradation of 4NP, indicating the participation of lignin degrading enzymes in 4NP bioremediation.

Besides *P. chrysosporium*, bacteria with microbial activity acted curial roles in carbon and other nutrient elements cycling. An effective approach for microbial activity analysis is the determination of enzyme activity. Morel et al. (1985) proposed that the pile biological activity could support the composting maturity assessment. As the most important and widely existed enzyme in sediments, oxidoreductase constructively participated in the oxidative degradation, and also, as an important biochemical parameter for biological activity assessment. Dynamic changes in oxidoreductase activities, including PPO and CAT, are shown in Fig. 2. Accordingly, CAT activity reflected the respiration intensity and microbial activity. In the early stage, the presence of biodegradable organic matters might promote the enzyme synthesis and activity, hence, gradually promotion of CAT occurred before day 21. Results were consistent with TOC content variation, accompanied with the rapid TOC lost before day 30 (Table 1). Then CAT activity tended to be stabilized, mainly due to the composting maturity and almost conversion of biodegradable organic matters to stable products or intermediates (Tiquia et al., 1996). Besides, results based on the CAT activity referred that piles with *P. chrysosporium* inocula at appropriate C/N ratios also promoted the microbial activity of the sediment microbes throughout the

composting.

PPO is a typical oxidoreductase widely reported in soils and sediments, which accounted for the phenol/polyphenol oxidation and organic matters conversion. Similar as CAT activity, samples in pile C possessed the highest PPO activity among the four piles. Persistent elevation of PPO activities before 55 days was detected in the all four piles. The gradual increase in PPO activities was also in accordance with the persistent loss of TOC and 4NP level. Indisputably, the most important role of PPO was the involvement in oxidation of phenolic compounds (Yoruk & Marshall, 2003). Commonly, PPO catalyzed phenolic degradation occurs following two steps: enzymatic transformation of monophenols or *o*-diphenols to *o*-quinones occurs firstly, then follows by condensation or polymerization reactions (Walker & Ferrar, 1998). A sharp decrease occurred from day 55 to day 70, which suggested the promotion of composting maturity Morel et al. (1985).

Apparently, highest ligninolytic enzyme and oxidoreductase activities were observed in pile C, which suggested that *P. chrysosporium* inoculation promoted the microbial activity of indigenous microorganisms. Meanwhile, previous study also reported that *P. chrysosporium* inocula could stimulate the growth of other microbe and improve the biological activities in soils (Yu et al., 2009). And according to Andersson et al. (2000), *P. chrysosporium* inocula also elevated the total bacterial PLFA in the soil and further promoted the PAH degradation in non-autoclaved soils. As such, results suggested that pile C with *P. chrysosporium* inocula at proper C/N ratios was benefit to the ligninolytic enzyme secretion, oxidoreductase activity, and

ultimately the promoted biodegradation ability.

3.4. Response of bacterial diversity and bacterial communities

According to the temperature variation, samples in pile C were classified as thermophilic stage (TS, day 1 and 6), constant temperature stage (CS, day 14, 21 and 40) and mature stage (MS, day 70 and 90). There were noteworthy overlaps in differentially abundant OTUs between the compartments (Fig. 3a). The similar OTUs among TS, CS and MS were about 1431, and the unique amount of OTUs in TS, CS and MS were 56, 482 and 205, respectively. Comparison among TS, CS and MS, it was obviously that the observed species and diversity at CS was quite higher than at TS and MS, CS was a microbial reactive phase during composting. The results of Beta diversity (weighted unifrac) are shown in Fig. 3b. The beta diversity between TS group and CS and that between TS group and MS group varied widely. The results illustrated that the species varied during the composting process.

Bacteria species and their proportions at phylum levels are shown in Fig. 3c. In total, more than 10 bacterial phyla were detected from the experimental pile C. As shown in Fig. 3c, *Firmicutes* and *Proteobacteria* were dominant species in all composting phase, especially, *Firmicutes* occupied almost 60% of bacteria in the initial phase. With the composting process, proportion of *Firmucutes* decreased from 59.53% to 31.09% from TS to CS process. However, *Proteobacteria* increased from 29.36% to 41.47% along with TS phase to CS phase. Importantly, *Bacteroidetes, Chloroflexi* and *Acidobacteria* also increased with the composting proceeding. For example, *Acidobacteria* significantly increased from 0.59% to 2.89% along with TS

phase to CS phase.

In order to illustrate the bacterial communities functioned in organic matter transformation, partial dominant bacteria at family or genus levels is listed in Fig. 4. As expected, bacterial community composition varied along the composting process. In the initial TS phase, *Planococcaceae*, *Bacillaceae* and *Lactobacillaceae* were dominant *Firmicutes*, then depleted during the composting proceeding. *Brucellaceae* and *Moraxellaceae*, which were possible pathogenic, depleted during the initial 6 days of composting. Obviously, proportion of aerobic cellulolytic bacteria, including *Cellulomonas, Pseudomonas*, *Bacillaceae*, *Cellvibrionaceae* and *Cytophagaceae*, gradually increased during composting due to the demand driven of cellulose decomposition. For example, *Cytophaga*, a major cellulolytic aerobic soil bacterium bound to cellulose during growth. The proportion of *Cytophagaceae* increased from 0.038% to 0.58% from day 1 to day 40. *Bacillaceae* increased rapidly from day 1 (1.37%) to day 6 (7.62%).

Meanwhile, we identified the gradually increase in *Pseudomonas*, *Comamonadaceae, Lactobacillaceae* and *Sphingomonas*, which were main bacterial for phenols degradation and use phenols as carbon and energy source (Matsumura et al., 2015). *Pseudomonas,* with catabolic potential and ability to break down recalcitrant xenobiotics, was reported contributed to 4NP degradation. Ajithkumar et al. (2003) found that *Pseudomonas* strain INA06, isolated the from activated sludge, could degrade 4NP via phenol hydroxylases. Similarly, *Sphingomonas* is also efficient xenobiotic-degrading species. Previous studies reported that *Sphingomonas* sp. strain

TTNP3 contributed to 4NP degradation mainly via synthesizing nonanols as end-metabolites from the alkyl chain moiety of nonylphenol, by replacing the aromatic ring with a hydroxyl group (Corvini et al., 2005). The relative high large number of *Pseudomonas*, *Comamonadaceae* and *Sphingomonas* explained the demand-driven of *Pseudomonas* and *Sphingomonas* utilizing 4NP as source carbon, accompanied with the rapid degradation of 4NP. At the later stage of composting (MS), *Oxalobacteraceae*, *Lactobacillaceae*, *Hyphomicrobiaceae*, and *Acetobacteraceae*, which use the simple and small organics as carbon source, increased remarkably. For example, *Oxalobacteraceae* are known to metabolize oxalate as carbon source, and could degrade oxalate to CO_2 and methanoic acid (Chapelle et al., 2016). The multiplication of these bacterial at the MS stage suggested the transformation of the complex organics, such as cellulose, hemicellulose and 4NP, to the simple organics, possibly oxalate, methanol and ethanol.

Besides participation in organic transformation, microorganisms in sediment also contribute to the metal passivation, commonly owing to the cell surface adsorption, extracellular polymeric substance chelation and accumulation (Ledin, 2000). *P. chrysosporium, Pseudomonas*, *Rhizobiaceae*, *Bacillaceae* and *Micrococcaceae* are important microorganisms involved in metal passivation (McEldowney, 2000; Volesky, 1994). During the composting process, extensive *Bacillaceae*, *Pseudomonadaceae* and *Rhizobiaceae* species were observed in the CS and MS stage, which was quite agreed with the rapid transformation of Cd in CS and MS stage.

3.5. Detoxification of co-contaminated sediments via composting

As shown in BCR variation of metals, Cd appeared to have lower bioavailability and ecotoxicity potential after composting. To comparatively evaluate the metal toxicity and bioavailability in composting samples, the Cd leachability ratio was calculated by investigating the TCLP-Cd value (Fig. 5a). During the whole composting process, TCLP-Cd declined gradually, probably due to the lower mobility in the composting samples. After 120 days of composting, the TCLP-Cd fraction was 24.17% of the total Cd in composting pile C, compared to 56.36%, 57.58% and 49.62% of the total Cd in pile A, B and D respectively. As reported in this study and previous study, it was quite widely accepted that *P. chrysosporium* was responsible for the lignin degradation and carbon cycle of straw, promoting the composting maturity and humus formation. Commonly, metal mobility and transport in the environment were influenced by the association with organic matter (Feng et al., 2010). Previous study verified that *P. chrysosporium* inocula reduced active metal and metal toxicity (Huang et al., 2017; Xu et al., 2016). Besides biosorption ability, *P. chrysosporium* inocula accelerated carbon cycle in agricultural wastes (such as straw, bran, et al.) via degradation of lignin, cellulose and hemicellulose, facilitated humus formation during composting, also contributed to the Cd passivation. Results demonstrated that composting with *P. chrysosporium* inocula was an efficient approach for metal passivation and detoxification.

In order to further investigate the bioavailability of 4NP and metals, germination index was determined. As shown in Fig. 5b, at the initial composting process, relatively low germination index was observed, which might account for the high 4NP

concentration and active metal speciation. With the composting process, germination index increased gradually, identified with the 4NP biodegradation and metal passivation. Obviously, Pile C with proper C/N ratios exerted relatively higher germination index after 14 days of composting. At day 40, the highest germination index (83.64%) was observed in pile C, followed by pile B (79.25%), pile A (76.25%) and pile D (71.57%), result was agreed with 4NP biodegradation and metal speciation variation.

3.6. Interactions among composting process, bioremediation and bioavailability

Table 2 lists the Pearson correlation coefficient among the tested indexes. Apparently, 4NP degradation were significant positive correlated with MnP, PPO and CAT (P<0.01), demonstrating the participation of those enzymes in 4NP degradation. Meanwhile, TOC degradation was correlated with LiP and PPO (p<0.05). Exchangeable Cd contents (F1) and TCLP-Cd had a relative negative correlation with PPO, GI and degradation rate of 4NP and TOC (p<0.01), suggesting that Cd contamination inhibited the microbial activity accounting for organic contaminant degradation. No significant relationship was found between bioavailable Cd (F1 and TCLP-Cd) with LiP and MnP. Interestingly, GI was found to be negative related with 4NP, Cd contents in F1 and TCLP-Cd (p<0.01), and positive related with residual Cd content (F4), PPO and degradation rate of 4NP and TOC (p<0.01). Results attested that depleted toxicity of 4NP and Cd contaminated sediments occurred via composting, the mature compost showed even no toxicity to ryegrass seeds.

The results of PCA are shown in the form of a correlation biplot in Fig. 6. Quantitative environmental variables are indicated by arrows and individual samples by circles. PCA analysis illustrated that the first two PCA axis explained the 83.9% of variation, with the eigenvalues of λ_1=0.645 and λ_2=0.188. The PCA component 1 is mainly determined by LiP, MnP and CAT, the PCA component 2 was account for TOC, TCLP-Cd, 4NP, and also PPO, GI. Many of the variables were mutually highly correlated, as indicated by acute angles between the arrows representing these variables. For example, notably high inter-correlations were found among the variables among PPO, GI, Cd in F2, F3 and F4, and also degradation rate of 4NP and TOC. Significant negative correlations among variables between 4NP, Cd contents in F1, TCLP-Cd, and enzymes/organic degradation, pointing to opposite directions, indicated the inhibition effect of 4NP and bioavailable Cd on the composting maturity and microbial activity, mainly due to the potential toxicity of 4NP and Cd. Interesting, lower impacts were found among bioavailable Cd (TCLP-Cd, Cd in F1 and F2) and LiP, MnP, CAT, based on both the PCA ordination and Pearson correlation analysis. Results suggested the lower toxicity to these enzymes, which are particularly secreted by *P. chrysosporium*, possible reason might be the admirable tolerance and resistance of *P. chrysosporium* as reported in our previous studies (Xu et al., 2016).

PCA analysis classified three major groups according to distinctive geochemical characteristics. Group 1 included the initial variables before the initial 6 days, group 2 contained samples at day 14 and 21, and group 3 clustered samples at the later stage of composting from day 30 to day 90. Interestingly, samples in group 3 were

consistently correlated with GI, suggesting the relatively low toxicity of mature compost matters in the later stage of composting. As such, results represented in this study illustrated that composting was an effective approach for metal and organic co-contaminated sediment bioremediation, accomplished with 4NP degradation, Cd passivation and lower toxicity.

4. Conclusion

This study corroborated the feasibility of composting in organic pollutants and metal co-contaminated sediment bioremediation. Analysis of 4NP level and Cd transformation in the tested four types of composting piles indicated that C/N ratios at 25.51 with *P. chrysosporium* inoculation showed the optimal remediation efficiency and composting maturity, and also reduced the Cd bioavailability. Aerobic cellulolytic bacteria and organic degrading species played significant roles during the composting process. Statistical analysis and bioavailability evaluation confirmed the availability of composting approach in the co-contaminated sediment bioremediation.

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Figure captions

Fig. 1. (a) Dynamic variation and degradation kinetics of 4-nonylphenolin (4-NP) different composting piles; (b) Dynamic variation of four-fractions Cd percent in composting piles in the four fractions following the improved BCR procedure. The four phases were exchangeable, water- and acid-soluble fraction (F1), reducible fraction (F2), oxidizable fraction (F3), and residual fraction (F4).

Fig. 2. Dynamic changes of microbial enzyme activities during composting: (a) Lignin peroxidase (LiP); (b) Manganese peroxidase (MnP); (c) Catalase (CAT) and (d) Polyphenol oxidase (PPO).

Fig. 3. (a) Venn diagram of OUTs in plie C samples; (b) Beta diversity (weighted unifrac) among all tested the samples; (c) Weighted unifrac distance among all the tested samples and their relative abundance in phylum level.

Fig. 4. Hierarchical cluster analysis of bacterial community at genus level in plie C samples.

Fig. 5. (a) Germination index of the four types of composting piles; (b) TCLP-Cd level of composting piles.

Fig. 6. PCA ordination plots between environment variables and composting samples using CANOCO software. Axis 1 and axis 2 account for 64.5% and 18.8% of the variance, respectively ($4NP_{-Red}$ represents reduction rate of 4NP, and TOC_{-Red} represents reduction rate of TOC).

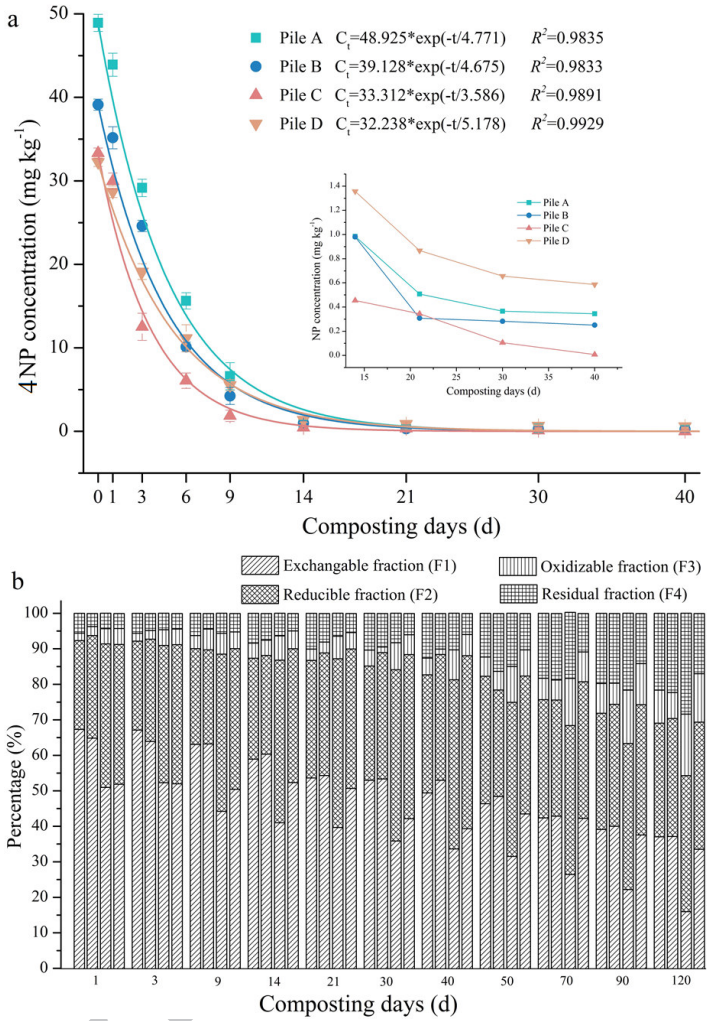


Fig. 1

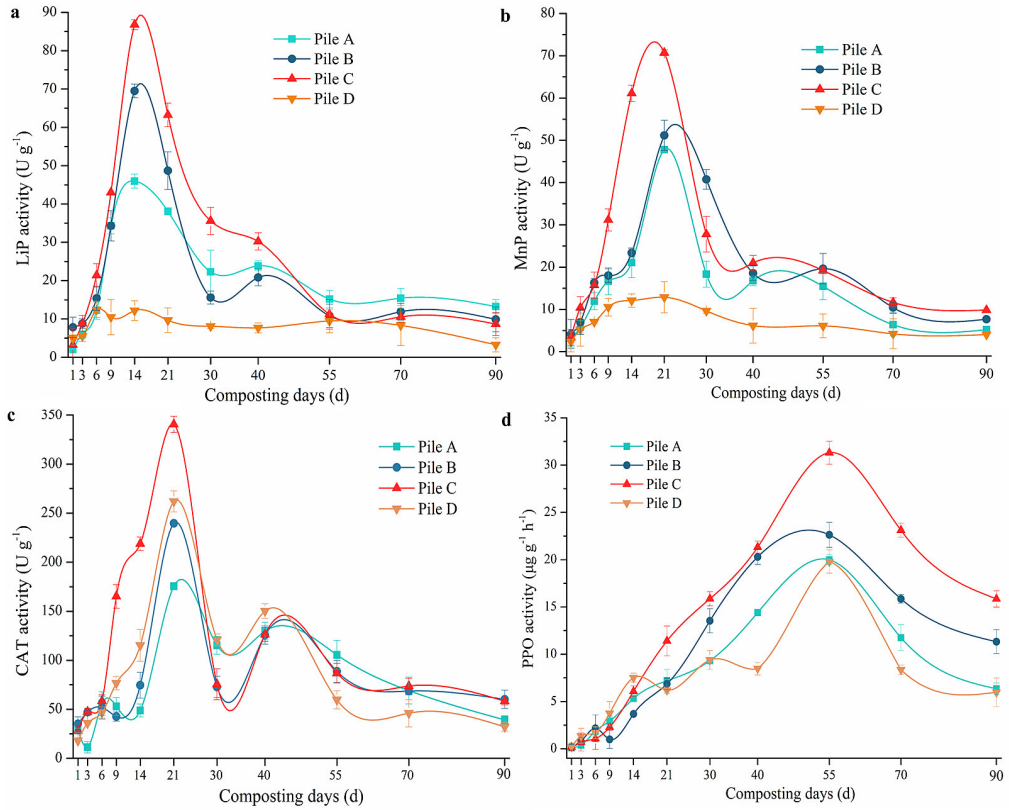


Fig. 2

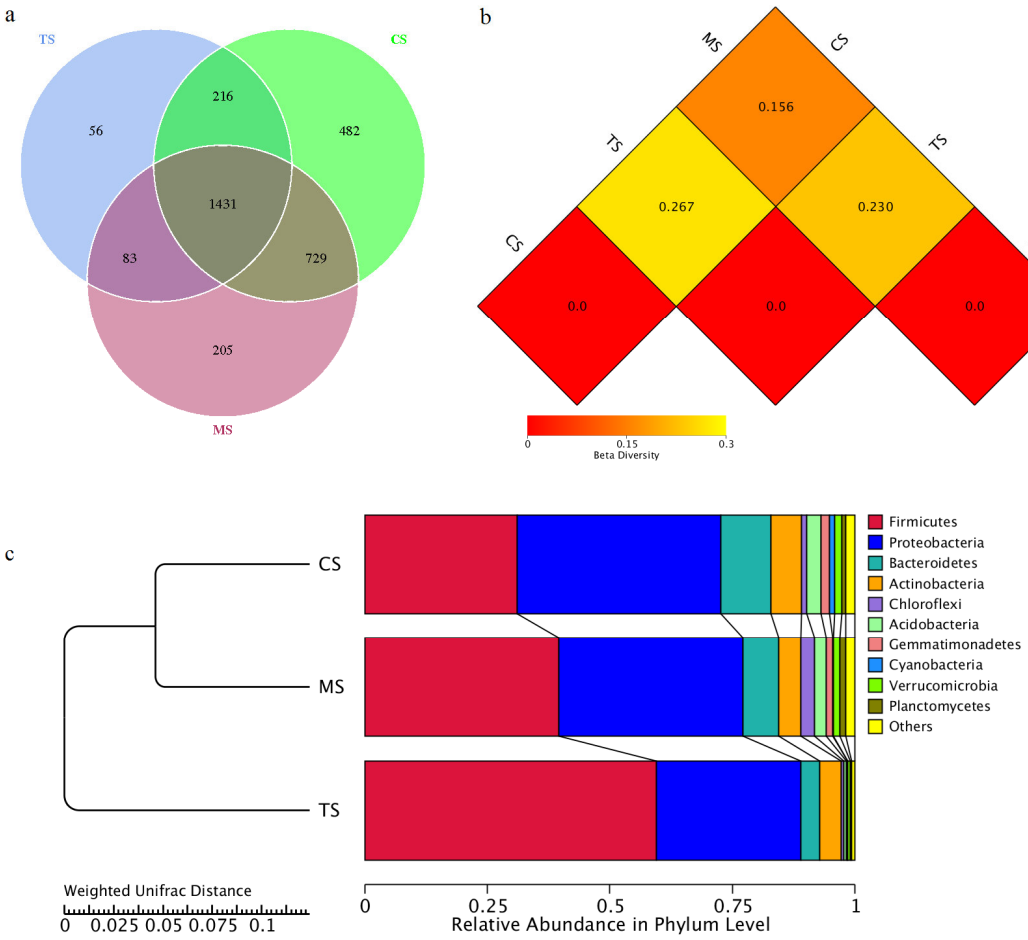


Fig. 3

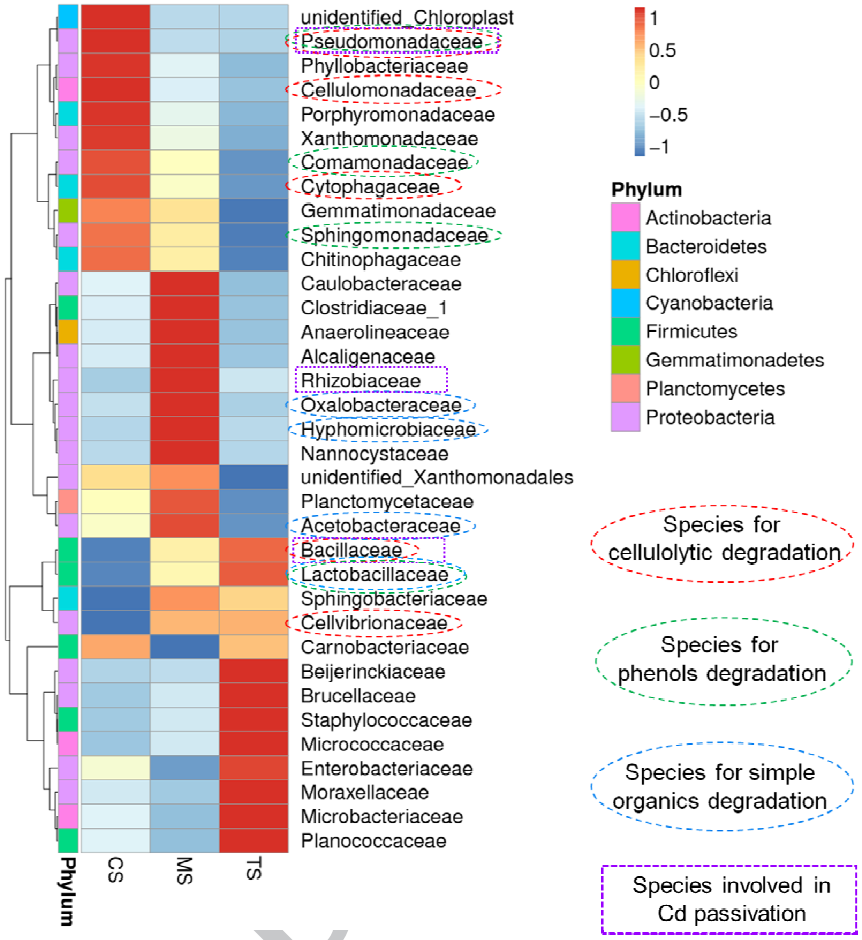


Fig. 4

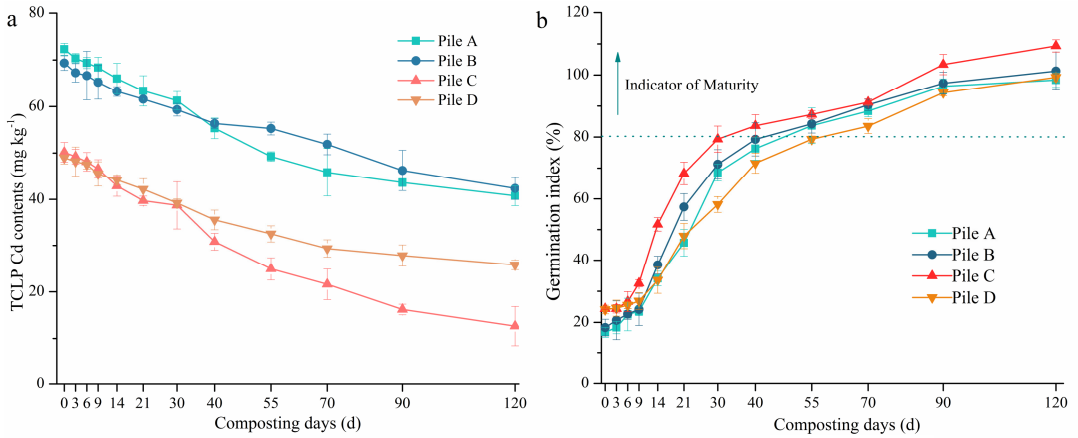


Fig. 5

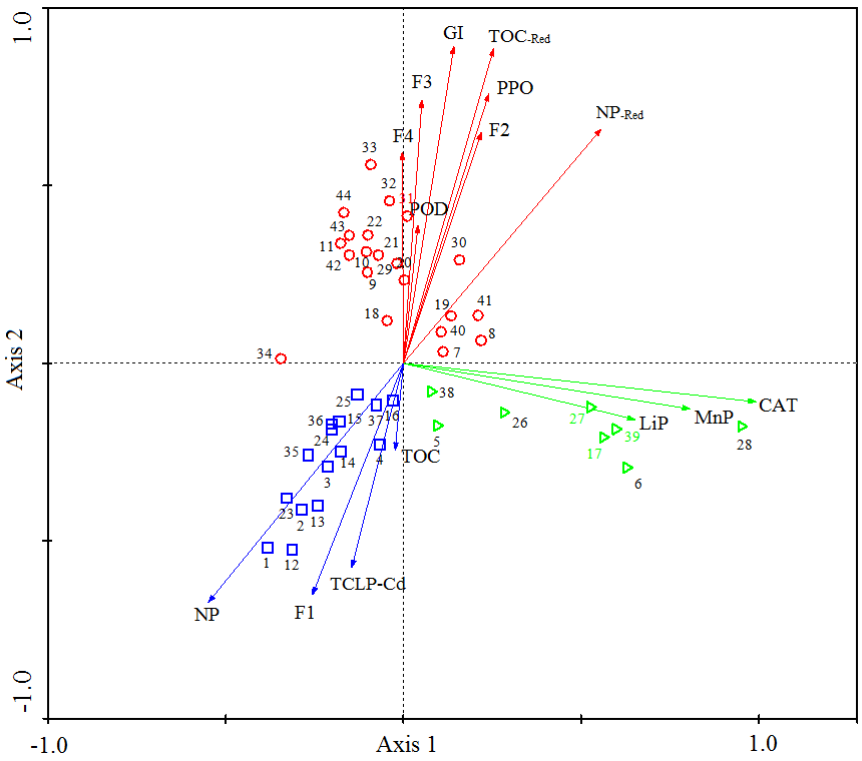


Fig. 6

Table 1 Total organic carbon (TOC, %) variation in different composting piles with various initial C/N ratios.

Piles	1 d	3 d	6 d	9 d	14 d	21 d	30 d	40 d	55 d	70 d	90 d
Pile A	27.85	26.98	26.35	25.86	25.43	24.11	22.41	21.20	20.35	19.32	18.68
Pile B	42.14	39.81	38.95	37.41	35.01	33.75	30.98	27.23	25.89	23.98	23.21
Pile C	74.25	69.82	61.67	57.37	55.28	52.34	47.28	40.83	36.38	34.12	31.53
Pile D	71.99	70.10	69.14	67.24	64.38	60.81	54.71	52.37	51.18	48.32	47.11

	TOC	4NP	F1	F4	LiP	MnP	PPO	CAT	$4NP_{-Red}$ [a]	TOC_{-Red} [b]	TCLP-Cd	GI
TOC	1											
4NP	.227	1										
F1	-.436**	.478**	1									
F4	-.792**	-.415**	.014	1								
LiP	-.082	-.374*	.026	-.081	1							
MnP	-.083	-.385**	-.054	-.032	.820**	1						
PPO	-.375*	-.585**	-.455**	.579**	-.029	.143	1					
CAT	.060	-.453**	-.258	-.060	.583**	.760**	.211	1				
$4NP_{-Red}$	-.323*	-.986**	-.415**	.450**	.383*	.394**	.600**	.458**	1			
TOC_{-Red}	-.358*	-.682**	-.604**	.621**	.315*	.147	.839**	.213	.698**	1		
TCLP-Cd	-.302*	.475**	.945**	-.070	.141	.080	-.534**	-.153	-.433**	-.704**	1	
GI	-.292	-.530**	-.562**	.631**	-.102	.007	.720**	.120	.509**	.808**	-.634**	1

Table 2 Pearson correlation analysis among the tested indexes

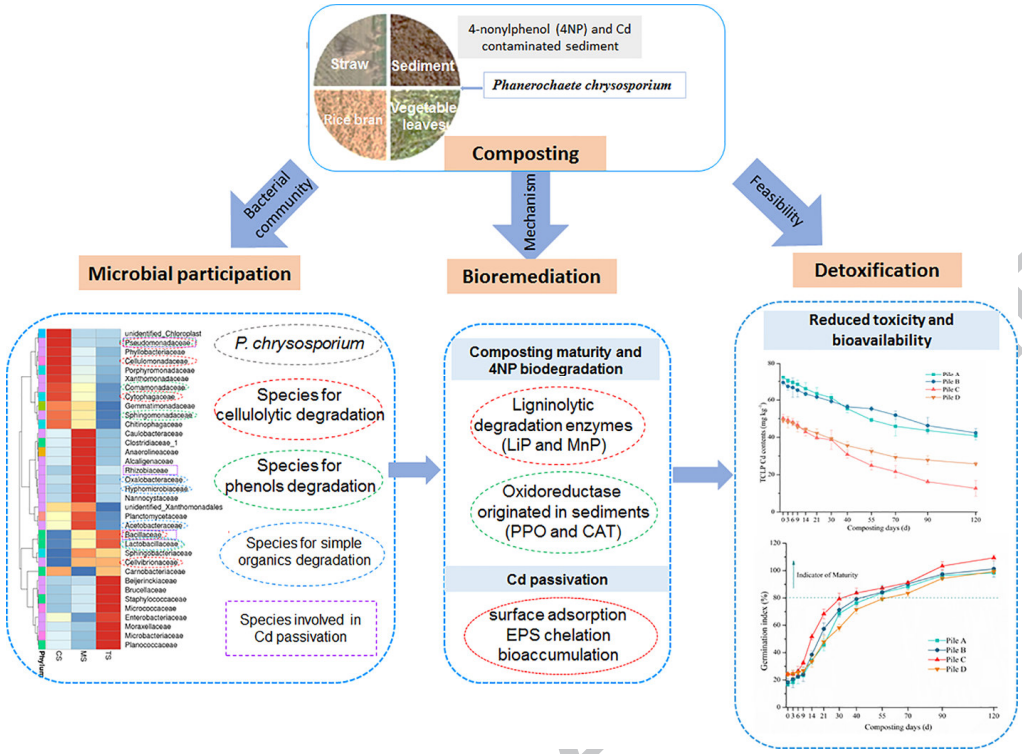
a $4NP_{-Red}$, Reduction rate of 4NP; b TOC_{-Red}, Reduction rate of TOC

*Correlation is significant at the 0.05 level.

**Correlation is significant at the 0.01 level.

Highlights

- *P. chrysosporium* inocula and proper C/N ratios accelerated the composting process
- *P. chrysosporium* inocula promoted TOC loss, NP degradation and Cd passivation
- LiP, MnP, CAT and PPO participated in organic chemical degradation
- Microbial community structure variation contributed to chemical transformation
- Depleted in bioavailability occurred in contaminated sediment via composting



Graphical abstract