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Corresponding Author: Prof. Yongtao Li, Ph.D.

Corresponding Author's Institution: South China Agriculture University

First Author: Zhong Lin

Order of Authors: Zhong Lin; Jing Bai; Shiqi Lao; Wenyan Li; Zhihao Wu; Yongtao Li, Ph.D.; Baruch Spiro; Dayi Zhang

College of Natural Resources and Environment
South China Agricultural University
No. 483, Wushan Road, Guangzhou, 510642, P. R. China
Tel.: 86-20-38297890, Fax: 86-20-85280292, Web: www.scau.edu.cn

Date: Oct. 25th, 2015

Dear Editor,

I would like to revise this manuscript, entitled "*Enhancing pentachlorophenol degradation by vermicomposting associated bioremediation*", for the consideration as Short Communication in *Ecological Engineering*. This study investigated the roles and mechanisms of earthworm (*Eisenia foetida*) on soil pentachlorophenol (PCP) degradation under sterile and non-sterile soil-compost condition. The vermicomposting technology has the potential to enhance the bioremediation of PCP contaminated soil, which was first time revealed in this study to our knowledge.

All the comments are accordingly considered in the revised version.

I affirm that

(1) All of the reported work is original.

(2) All authors have seen and approved the final version submitted.

(3) All prevailing local, national and international regulations and conventions, and normal scientific ethical practices, have been respected.

(4) Consent is given for publication in *The Journal of Hazardous Materials*, if accepted.

The total words number is 3280.

Main context pages are 10.

Figure number is 3.

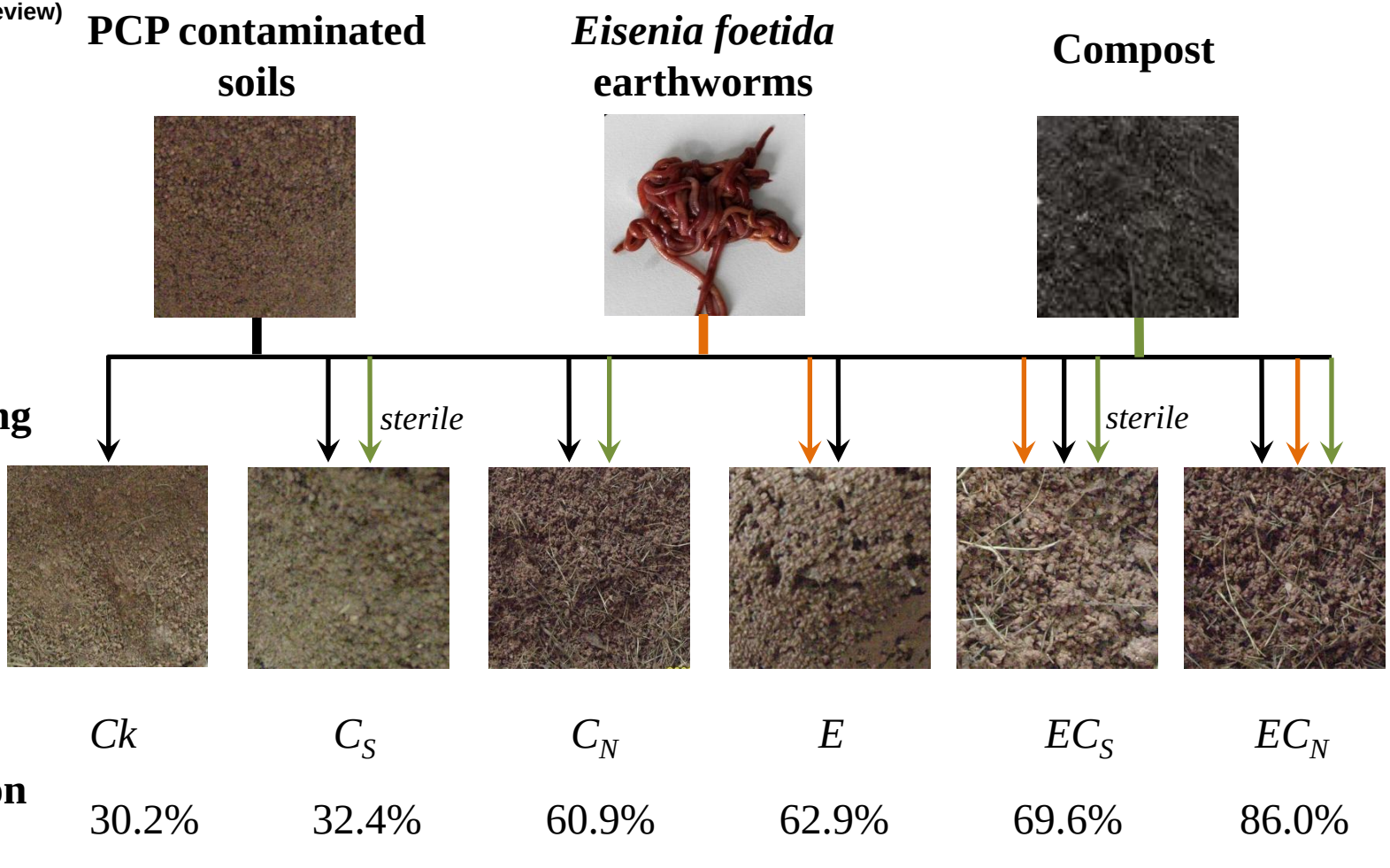
Table number is 1.

I look forward to receiving feedback on the revised.

Yours sincerely

Dr. Yongtao Li

Graphical Abstract (for review)



Enhancing pentachlorophenol degradation by vermicomposting associated bioremediation

Zhong Lin, Jing Bai, Shiqi Lao, Wenyan Li, Zhihao Wu, Yongtao Li, Baruch Spiro, Dayi Zhang

Highlights

- We enhanced soil PCP biodegradation from 14.0% to 71.9% by vermicomposting
- Main roles of vermicomposting are pH neutralization and humus-PCP decomposition
- Vermicomposting stimulated microbial biomass and bacterial activities *in situ*
- Enrichment of indigenous bacteria and fungi responsible for PCP degradation

Response to comments

Editor's comments:

We have published many vermiculture over the years. But I am willing to consider your paper if you willing to resubmit it as a Short Communication. Short Communications are less than or equal to 10 manuscript pages, double-spaced, with no more than 4 Figures + Tables.

I would like you to cite at least 3 recent papers from Ecological Engineering to better tie your paper to the fields of ecological engineering and ecosystem restoration.

Answer: Thanks for editor's consideration for our revision and possible publication on Ecological engineering. The authors would like to publish this paper as short communication, with appropriate modification to shorten the manuscript with required number of Figures/Tables. The recent papers published on Ecological Engineering are also cited in the revised version.

Reviewer #1:

It is a very interesting paper with a lot of data tables and figures which give a good idea of the evolution of microbial activity and diversity in polluted soil treated with compost. My only problem is that we have no comparison with a non-polluted soil then it is sometimes difficult to link the results to the bioremediation.

Answer: Thanks for the comments. Our recent published paper has compared the microbial community change between PCP contaminated and non-contaminated samples, and the results indicated significant change in the two cases. Due to the limitation of space for a short communication, we did not discuss this part in the manuscript. Alternatively, based on the DGGE bands comparison, we selected the unique bands in the vermicomposting treatments, with appropriate citation of the publication.

Reviewer #2: This manuscript (ECOLENG-D-15-00183) entitled "Enhancing pentachlorophenol degradation by vermicomposting associated bioremediation "is a research paper for sustainably mineralization of organic compound in the composted material can be accepted by major revision. Specific comments are given below

Specific comments:

1. After conversion of compost to non toxic compost. Please author check the plant growth attributes of any crops in that compost under natural condition, then this research will be applied for sustainable agricultural and farmers field because large quantity of sludge dump as waste on the field which causes the environmental pollution.

   Answer: Thanks for the comments. We did not carry out further work on the growth test of the treated soil samples, mainly for two reasons: 1) the soil amount is too small even for pot plant; 2) the soil is not nutrient rich (Supplementary materials) and there is limited plants growing around the sampling point, and we cannot find appropriate ones for the growth test. Though many previous and similar works have focused on the organic contaminants degradation with

vermicomposting, the growth test is seldom applied. The reviewer gave us some good ideas to address further the applicability and practices of applying the vermicomposting in the field and evaluate whether it can really helped the plant growth or reduce PCP uptaken by the plant. It will be our plan.

2. Please give some figures regarding your experimental setup for composition. And also give the clear figure of sludge and compost after conversion by earthworm.
   Answer: Thanks for the comments and we have prepared a graphic abstract illustrating all the key information during and after treatment.
3. Use more recent references (at least more 5 paper after 2010), you definitely know that recent years scientists have done numerous research regarding vermicomposting especially with chlorophenols (PCP and Others).
   Answer: The authors have revised the whole manuscript, with appropriate new citations of the recent work. In the revised version, we believe that most of the most recent and valuable work has been cited in the manuscript (but we have to short the list to about 30 references as short communication criteria).
4. Author has taken sterile soil for the experiments: further microbial community analysis basically or initially soil does not have any microorganism but later microbes are grown which comes naturally under open condition…so how author communicate about this
   Answer: Thanks and the author needs to clarify there is no sterile soil treatment. The sterile treatment (Cs) included soil and sterile compost. Therefore, there are huge amount of active microorganisms in the soils (from the start to the end of experiment) and the potential contamination from open air can be ignored.
5. If author have checked the microbial diversity in the sterile compost after inoculation and beginning of the degradation than he should also check diversity of the microorganism in the non sterile compost and than he can compare that how much same and different microorganism are growing.
   Answer: Thanks for the comments. The author has noticed the significant change of microbial diversity before and after non-sterile treatments. However, such change was caused by both compost itself and the microbes remaining activities in the compost. We have pointed out the increasing abundance of bacterial *Flavobacteriaceae* and fungal *Hypocreaceae* (please see Section 3.3 and 4.3). Due to the limitation of short communication, we cannot provide more data and deeper discussion, but further work will be carried out addressing the specific effects of non-sterile compost on soil microbial diversities.
6. What was the physiochemical parameter kept for the survival of the microorganism?
   Answer: Many soil parameters can affect the survival and growth of soil microorganisms, including temperature, pH, carbon sources, etc. In this study, the author identified the two effects of compost assisted by earthworm activities, including: 1) pH neutralization to release PCP and provide better pH condition for microbes; 2) compost decomposition with more smaller carbon substrates to simulate microbial activities. We believe these are the key parameters in vermicomposting treatments to improve PCP degradation.

7. Author writing in conclusion part -The contribution of vermicomposting was identified as the earthworms' consuming humus and neutralizing soil pH, releasing the humus-PCP complex and increasing PCP solubility. How author has observed humus-PCP complex and where the PCP solubility?

   Answer: The humus-PCP was analysed by Scelza's method. The author has corrected manuscript for clearer demonstration, in line 99. The author mis-presented the PCP bioavailability in the previous version as "solubility". In fact, we mean the change of PCP bioavailability by converting humus-fixed PCP to extractable PCP. The author has modified all the places for clearer demonstration.

8. In conclusion line 387-The phylogenetic classification demonstrated that the soil PCP biodegradation was improved by stimulating the growth of the indigenous bacterial families

   Answer: Thanks for the comments and the author has modified the whole conclusion part for a better statement.

Enhancing pentachlorophenol degradation by vermicomposting associated bioremediation

Zhong Lin[1, 2], Jing Bai[1], Zhen Zhen[2], Shiqi Lao[1], Wenyan Li[1], Zhihao Wu[1], Yongtao Li[1, 3]*, Baruch Spiro[4], Dayi Zhang[5]

[1]College of Natural Resources and Environment, South China Agricultural University, Guangzhou, 510642, P.R. China

[2]College of agriculture, Guangdong Ocean University, Zhanjiang, 524088, P.R. China

[3]Institute of Environmental Protection of the Ministry of Agriculture, Tianjin, 300191, P.R. China

[4]Department of Mineralogy, The Natural History Museum, Cromwell Road, London, SW7 5BD, UK

[5]Lancaster Environment Centre, Lancaster University, Lancaster, LA1 2YW, UK

Correspondence author:

Professor Yongtao Li

Address: College of Natural Resources and Environment, South China Agricultural University, No. 483, Wushan Road, Guangzhou 510642, P.R. China.

Phone: +86-20-38297890

Fax: +86-20-85280292

E-mail: yongtao@scau.edu.cn

ABSTRACT

Vermicomposting is an effective and environmentally friendly approach for soil organic contamination clean-up. This study investigated the roles and mechanisms of earthworm (*Eisenia foetida*) on soil pentachlorophenol (PCP) degradation with sterile and non-sterile soil-compost treatment. Limited soil PCP degradation was observed in the control and sterile compost treatments, whereas the synergetic effects of earthworm and compost contribute to the PCP biodegradation acceleration by significantly improving microbial biomass and activities. Sequence analysis and phylogentic classification of soil bacterial and fungal community structure after 42 days treatment identified the dominancy of indigenous bacterial families *Pseudomonadaceae*, *Sphingobacteriaceae* and *Xanthomonadaceae*, and fungal family *Trichocomaceae*, which were responsible for PCP biodegradation and stimulated by vermicomposting. Further investigation revealed the dominant roles of sterile compost during PCP biodegradation as the formation of humus-PCP in soil rather than neutralizing soil pH and increasing PCP availability. The mechanisms of vermicomposting include humus-PCP complex degradation, humus consumption and soil pH neutralization. This study provides a comprehensive understanding of the synergetic effect of vermicomposting on microbial community functions and PCP degradation enhancement in soils.

Keywords: Pentachlorophenol; biodegradation; Earthworm; Compost; Microbial community

1. Introduction

Pentachlorophenol (PCP) has been widely used in agricultural and industrial applications as herbicide, pesticide, and broad-spectrum biocide over the world (Fukushima and Tatsumi, 2007). Its use has been banned due to acute toxicity, poor biodegradability and chemical stability (Puglisi et al., 2009). Nevertheless, a considerable amount of PCP residues still exist in soils, directly affecting soil quality and agricultural product safety (Gao et al., 2008). The remediation of soil PCP residues is therefore important and widely applied for environmental safety around the world.

Bioaugmentation is a promising technology to clean up soil organic pollutants in a cost-effective and environmentally friendly manner. Currently, bioremediation methods mainly focus on adding exogenous degrading strains or compost (Sayara et al., 2009). Adding exogenous degrading strains directly affects soil PCP residues (Walter et al., 2004). Compost additives can change soil structure and nutrient content, ameliorating indigenous microbes for PCP biodegradation (Lau et al., 2003). However, these techniques suffer from the competition between the inoculated and autochthonous microbes, facing the challenges as low efficiency of growth substrates, low pollutants bioavailability and insufficient oxygen supply for aerobic biodegradation (Cea et al., 2010). Hence, vermicomposting is viewed as an implemented biological tool in strengthening bioremediation.

Earthworms are common soil organisms, with strong environmental adaptability and reproductive capacity, and show high resistance to organic pollutants (Reid and Watson, 2005; Rajpal, et al., 2014). Through their mucilaginous secretions and soil organic matter transformation, earthworms can increase microbial activity and nutrient availability (Tripathi et al., 2014). Their movement and burrowing activities enhance soil aeration and increase the contact opportunities between microorganisms and reactants (Luepromchai et al., 2002). Through these ecological functions, earthworm can ameliorate soil properties and offset the limitations on bioremediation (Ravindran, et al., 2015). So far, several studies reported that earthworms could enhance the degradation of organic pollutants. Lin (Lin et al., 2012) found that earthworms significantly enhanced DDT dechlorination. Luepromchai (Luepromchai et al., 2002) reported that earthworms accelerated polychlorinated biphenyl removal by increasing polychlorinated biphenyl-degrading microorganisms. Regardless, there was little research on whether vermicomposting can affect the PCP biodegradation, and its functions in PCP degradation and roles in soil microbial community structure remain unclear.

This study aims to investigate the effects of vermicomposting on the PCP degradation in soils. The results uncovered the changes in bacterial/fungal community influenced by vermicomposting and identified the roles of the microbial community in PCP biodegradation process.

2. Materials and methods

2.1 Sites and sampling

Upland soils were collected from South China Agricultural University, China (23°14'22"N, 113°37'51"E). Soil samples were collected from 0 to 20 cm depth, dried and passed through a 2 mm sieve, and moistened to 60% of their water holding capacity for a week. The fresh cow dung compost was sampled from the cattle ranch in South China Agricultural University (23°09'29"N, 113°21'37"E). The dung was mixed with lime, transferred into the fermentation cylinder (1 m × 1 m), and covered by 10 cm soils. The cylinder was subsequently wrapped tightly within plastic film and fermented at 40^{o}C for 20 days. The compost was then passed through a 2 mm sieve before use. PCP was not detectable in soils and compost used in the experiment. The chemical properties of soils and compost are presented in Table S1. The *Eisenia foetida* earthworms were purchased from Pengcheng Farm, Jiangmen. PCP, acetone and ethanol were analytic grade purchased from Sigma Aldrich (USA). Other chemicals are purchased from Chengshuo Company (China).

2.2 Experiment design and procedure

The vermicomposting treatments for PCP biodegradation are listed in Table 1. Considering PCP contamination level in heavily polluted areas in China (Gao et al., 2008) and earthworm tolerance test (Supplementary Fig. S1), an initial concentration of 40 mg kg^{-1} of soil PCP was set for all the treatments. Each treatment was carried out on 0.5 kg PCP contaminated soil. Earthworms were added to the soils with the density of 16 individuals kg^{-1}. The initial compost dosage was 4.5% as a common dosage for adequate earthworm survival (Puglisi et al., 2009). Sterile compost was produced by γ-irradiation with a total dose of 60 kGy. The preparation of PCP contaminated soils followed previous protocol (Scelza et al., 2008), as described in Supplementary materials.

2.3 Chemical analysis

PCP residue extraction and analysis followed Khodadoust's method (Khodadoust et al., 1999). Soil samples were freeze-dried, and the samples (2 g, dry weight) were transferred into polycarbonate centrifuge tubes and added with 40 mL water:ethanol (50:50, v/v). The tubes were sealed, shaked at 180 rpm for 1 h, and centrifuged at 3,000 g for 15 min. The supernatant passed through 0.45 μm

filter and the precipitate was separated to humic acid, fulvic acid, and humin fractions using 0.5 mol L^{-1} NaOH or HCl (Nieman et al., 2005). The earthworm-accumulated PCP was extracted according to Parrish (Parrish et al., 2006). The humus-fixed PCP was extracted according to Scelza (Scelza et al., 2008). PCP residues and soil properties were analysed by high-performance liquid chromatography (HPLC, Waters 1525/2487, USA), as described in Supplementary materials.

2.4 Soil microbial community analysis

Total soil microbial biomass (15 g soil, dry weight) was determined by the fumigation-extraction method for biomass carbon and nitrogen (Wu et al., 1990). Total microbial activity (25 g soil, dry weight) was measured by Basal respiration after 0, 14, 28, and 42 d (Bhattacharyya et al., 2005). The soil microbial community structure was evaluated at 14, 28 and 42 d by DGGE with 16S rRNA and 18S rRNA amplification for bacteria and fungi, respectively. Briefly, soil DNA was extracted with Powersoil DNA extraction kit (MoBio Laboratories) following manufacturer's instructions. For bacterial community, the V3 region of 16S rRNA gene was amplified by touchdown polymerase chain reaction (PCR) with the primer set 518R (5'-ATTACCGCGGCTGCTGG) and GC-338F (5'-CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGGCCTACGGGAGGCAGC AG). For fungal community, the fragments of 18S rRNA gene were amplified with primer sets GC-fung (5'-CGCCCGCCGCGCCCCGCGCCCGGCCCGCCGCCCCCGCCCCATTCCCCGTTA CCCGTTG-3') and NS1 (5'-GTAGTCATATGCTTGTCTC-3').

PCR mixtures contained 25 μL of Premix Taq (Takara Biotechnologies), 1.5 μL of each primer and 2 μL of DNA template, made up to 50 μL with Milli-Q water. The samples were amplified in a PTC-200 (Bio-Rad Laboratories, USA) with modification from previous research (Cea et al., 2010) as initial denaturation for 5 min at 94°C, followed by 25 cycles of 30 s at 94°C, 30 s at 55°C, 30 s at 72°C, and 10 cycles of 30 s at 92°C, 30 s at 55°C, 45 s at 72°C, final extension at 72 °C for 10 min. Blank controls were carried out through all steps. Bacterial and fungi community analysis was carried out by DGGE with a DCode universal mutation detection system (Bio-Rad Laboratories, USA) (Supplementary materials). The relative abundance of each band was analyzed by Quantity

One, and the bands were further re-amplified, sequenced and compared to GenBank database from the National Center for Biotechnology Information (NCBI) by BLAST tools (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The neighbor joining phylogenetic tree was analysed with software package MEGA 4.0, evaluated by the bootstrap values based on 1000 replicates.

2.5 Data analysis

Analysis of variance followed by a one-way analysis of variance (ANOVA) at the 0.05 level was used to determine significant differences between treatments. All statistics were carried out in SPSS (Version 13.0). Significant difference (p<0.05) was marked with different alphabet letters.

3. Results

3.1 Effects of vermicomposting on soil PCP residues

PCP residues have significantly decreased in the compost and vermicomposting treatments (Fig. 1). Residual PCP in C_N, E, EC_S and EC_N treatments was significantly lower than in *Ck* and *Cs* (p<0.05). After 42 days, only 14.0% and 30.4% of residual PCP were found in EC_N and EC_S, while 39.1% and 37.1% in C_N and E. The PCP half-life in EC_S and EC_N was 15 and 22 days respectively, lower than C_N (31 d), E (29 d), *Ck* (87 d) and C_S (88 d). The results suggested that vermicomposting with non-sterile compost enhances soil PCP biodegradation via stimulating local microbial communities. The humus-fixed PCP is the dominant PCP bound residue in soils (Fig. S3), significantly decreased after 42 days in E and EC_N, lower than EC_S, C_S and *Ck*. The humus-fixed PCP contents in *Ck* and C_N were lower than C_S, while no significant difference was observed between other treatments. Additionally, PCP concentrations accumulated in earthworms were 0.03, 0.03 and 0.04 mg kg^{-1} in E, EC_S and EC_N treatments, only accounting for 0.08%−0.10% of initial PCP addition. Little PCP accumulated in earthworms, although they could ingest PCP-contaminated soil. Meanwhile, high *E. foetida* survival rates were observed as 96.67-100% during the vermicomposting remediation.

3.2 Effects of vermicomposting on soil properties and microbial activities

The pH and organic matter content after treatment are presented in Table S2. Soil pH in EC_S and

150 EC_N was significantly higher than E, C_S and C_N, all of which were significantly higher than Ck. The
151 compost addition evidently increased the organic matters. TOC in C_S, S_N, EC_S and EC_N were at the
152 same level (p>0.05), all significantly higher than E and Ck. Humus was the lowest in E treatment.
153 Humin in C_S was significantly higher than that in C_N and Ck, all significantly higher than that in
154 EC_S and EC_N. Humic acid in C_S was significantly higher than that in EC_S, EC_N, C_N and Ck, and
155 fulvic acid in C_N and EC_N were significantly higher than EC_S, C_S and Ck.
156 Soil respiration rates showed high variability for all vermicomposting treatments, especially for EC_S
157 and EC_N (Fig. S4). After 42 days, EC_S and EC_N had the highest soil respiration rates as 6.87 mg kg^{-1}
158 and 7.16 mg kg^{-1}, and the lowest in Ck (1.98 mg kg^{-1}) and C_S (2.27 mg kg^{-1}). Fig. S5a further
159 demonstrated higher biomass carbon in E, EC_S and EC_N than C_N. Similarly, the biomass nitrogen in
160 EC_S and EC_N were significantly higher than E, C_S and C_N, and Ck was the lowest of all the
161 treatments (Fig. S5b). The results suggested that vermicomposting showed positive impacts on
162 microbial activity and biomass, consequently resulting in soil PCP biodegradation improvement.

163 ### *3.3 Soil microbial community change in vermicomposting treatment*

164 The difference and succession of microbial community in various vermicomposting treatments was
165 evaluated by PCR-DGGE (Fig. 2). The observable changes involved the possible bacterial and
166 fungal PCP degraders in vermicomposting treatments, originally from the initial PCP contaminated
167 soils or the new exogenous species driving from the earthworm and compost.
168 The bacterial community changed substantially and the dominant bands were unique in different
169 treatment (Fig. 2A). The bands $B_B/B_D/B_E/B_F$ were enhanced by either earthworm or compost, while
170 bands B_A/B_C were enhanced only by earthworm and non-sterile compost, respectively (Fig. 2A).
171 After 14 days, the bands B_e/B_f in vermicomposting treatments have higher abundance than those in
172 Ck. Vermicomposting stimulated the bands $B_a/B_b/B_c$ in E, EC_S and EC_N, while C_N enhanced the
173 band B_d. Cluster analysis showed that the bacterial community in C_S and C_N gathered for a class,
174 and then with Ck, while E, EC_S and EC_N had a higher degree of similarity (Fig. 2A). The bacterial
175 community was the most diverse after 28 days degradation, and cluster analysis indicated similar

176 category within C_S and C_N, followed by *Ck*, whereas earthworm treatments (*E*, EC_S and EC_N) had
177 high similarity. Compared to *Ck*, the similarity order of treatments is C_S>C_N>(*E*/EC_S/EC_N),
178 suggesting vermicomposting significantly influenced the soil bacterial community.
179 Soil fungal community also had noticeable change with the additive of earthworm or/and compost.
180 The more significance of relative abundance of specific bands at 42 days indicated the stimulation
181 of vermicomposting treatments (Fig. 2B). The most significant enhancement occurred in EC_S and
182 EC_N. The enhanced bands F_a/F_b/F_c/F_d at 14 days were also found at 28 and 42 days, marked as
183 F_A/F_B/F_C/F_D and F_1/F_2/F_3/F_4, respectively. Cluster analysis showed that the fungi community in
184 earthworm treatments (*E*, EC_S and EC_N) had higher similarity, whereas compost treatments (C_S and
185 C_N) formed another similar category and *Ck* in separate one (Fig. 2B).
186 DGGE results indicated that earthworm and compost significantly affected bacterial and fungal
187 community structure, and their synergic effects stimulated more microbial populations compared to
188 the treatments with only earthworm or compost. The targeted bacterial and fungal bands were
189 sequenced and phylogenetically classified, as shown in Table S3. Compared with *Ck*, compost
190 stimulated bacterial band B_5 assigned to *Sphingobacteriaceae*. The bands B_2/B_7 have higher
191 abundance in *E*, EC_S and EC_N with phylogenetic similarity to *Sphingobacteriaceae* and
192 *Xanthomonadaceae*, whereas less in C_S and C_N. The band B_1 (C_N) was assigned to
193 *Flavobacteriaceae* and stimulated by earthworm. It is noteworthy that, in EC_S and EC_N treatments,
194 the bands B_3/B_4/B_6 were of higher abundance than in *Ck* and close to *TM7*, *Pseudomonadaceae* and
195 *Opitutaceae* (Fig. 2A and Table S3). Earthworm and compost significantly stimulated the fungal
196 bands F_1/F_2/F_3, assigned to *Mucoraceae*, *Tremellaceae* and *Trichocomaceae* respectively. The
197 addition of non-sterile compost promoted *Hypocreaceae* (F_4) (Fig. 2B and Table S3).

198 ## 4. Discussions

199 ### *4.1 Roles of compost on soil PCP biodegradation*

200 After 42 days, the PCP residual concentration in *Ck* was 28.66 mg kg^{-1}, suggesting that
201 autochthonous microorganism had a poor ability on PCP degradation. The PCP residual in C_S was

similar as 28.75 mg kg^{-1} and sterile compost did not improve soil PCP removal by itself. Sterile compost is mainly composed of macromolecular compounds, hard for soil indigenous microorganisms to utilize. Only after decomposition into smaller compounds, they can stimulate the growth of degrading microorganisms as carbon sources (Purnomo et al., 2010). The soil respirations and microbial biomass had no significant difference between these two treatments (Fig. S4 and S5), further proving the limited stimulation effects of sterile compost on soil biota.

The sterile compost treatment (*Cs*) significant enhanced the soil humus and humus-fixed PCP (Fig. S3 and Table S2), suggesting PCP was fixed against degradation. The compost addition increased PCP stability and decreased its mineralization by increasing soil organic matters and their adsorption of PCP (Lau et al., 2003; Cea et al., 2010; Sayara et al., 2009). It also prevented the access and utilization of soil microbes to the PCP (Cea et al., 2010; Sayara et al., 2010).

C_N showed significantly higher removal of PCP than C_S and *Ck* (Fig. 1). The results indicated that non-sterile compost effectively enhanced soil PCP removal via more substrates from compost decomposition by compost microorganisms. The metabolites secretion from non-sterile compost stimulates the activities of indigenous microbes, and consequently accelerates PCP degradation (Bhattacharyya et al., 2005). Further evidence was observed from the increasing soil respirations and microbial biomass carbon/nitrogen in C_N (Fig. S4 and S5). Non-sterile compost was reported to help soil organic pollutants mineralization by encouraging mesophilic and thermophilic bacteria, ligninolytic fungi and actinomycetes (Sayara et al., 2010). Lau (Lau et al., 2003) found that microbes in mushroom compost have the capability to bioremediate PAH-contaminated soil.

4.2 Vermicomposting promoting soil PCP biodegradation

Vermicomposting treatment (*E*) remarkably reduced soil PCP residue (Fig. 1). It is possibly explained by stimulated indigenous microbial activities from the increasing soil basal respiration and biomass carbon/nitrogen after earthworm addition. Earthworms could optimize soil properties, offset bioremediation limitations, and accelerate soil PCP biodegradation. Earthworms increase soil porosity and enlarge soil surface area to improve mineral-bacterial interaction (Natal-da-Luz et al.,

2012). Additionally, earthworm has rich mucus, urine and cast, also promoting the activities of indigenous microorganisms (Eijsackers et al., 2001; Shan et al., 2011). All these factors contributed to soil amelioration, PCP homogenous dissipation and biodegradation (Natal-da-Luz et al., 2012). Vermicomposting with compost (E_{CS} and E_{CN}) showed significantly higher PCP removal than others. The compost provides adequate food to earthworms and enhances their growth. During the compost digestion, earthworms crush compost into tiny particles, accelerate compost decomposition, and improve nutrients availability, favoring the growth of indigenous microorganisms (Lin et al., 2012). Earthworm can also consume soil humus and release humus-fixed PCP to enhance PCP bioavailability (Fig. S3), and the inert and inaccessible PCP was then available for soil microbes (Shan et al., 2011). Moreover, earthworms also neutralize soil pH to desorb anionic PCP, which is mainly of molecular state in acidic soil (pH=4.71 in this study). The pH neutralization transfers PCP from hydrophobic molecular state to hydrophilic anionic state and improves its availability.

4.3 Vermicomposting assistant bacterial and fungi biodegradation

The bacteria and fungi diversity was different during PCP biodegradation process between negative control and vermicomposting treatments (Fig. 2 and Fig. 3). The significant differences of DGGE bands between PCP contaminated and non-contaminated soils proved the roles of targeted microbes in PCP degradation (Lin et al., 2016). Trees for bacterial 16S rRNA genes showed that the sequenced bands belonged to six families as *Sphingobacteriaceae*, *Flavobacteriaceae*, *Pseudomonadaceae*, *TM7*, *Xanthomonadaceae* and *Opitutaceae* (Table S3), stimulated by vermicomposting to enhance the soil PCP biodegradation. Similarly, previous research also showed that these bacteria could degrade pentachlorophenol, chlorinated phenols, benzoic acid and salicylic acid (Basta et al., 2005; Liu et al., 2011). Basta (2005) suggested that *Sphingobacteriaceae* used available polycyclic or monocyclic aromatic hydrocarbons. Liu (2011) reported that earthworm stimulated soil MCPA-degrading bacteria, and *Xanthomonadaceae* was an important herbicide MCPA consumer. *Flavobacteriaceae* was observed in non-sterile compost treatments (C_N and E_{CN}) and stimulated by earthworm. Oh (2011) found that *Flavobacteriaceae* degraded macromolecule

organic compounds and performed anaerobic respiration. Lange (1996) also reported that the enzyme PCP 4-monooxygenase from *Flavobacterium* could catalyze the oxygenolytic removal of the first chlorine from pentachlorophenol. Our study further suggested that *Flavobacteriaceae* contributed to macromolecular compost decomposition and associated with PCP degradation. Our fungal sequenced bands belonged to *Mucoraceae*, *Tremellaceae*, *Trichocomaceae* and *Hypocreaceae*. Besides direct PCP degradation, *Mucoraceae* and *Tremellaceae* have been reported to degrade plant-derived cellulose (Kuramae et al., 2013). These results indicated that vermicomposting treatment enhanced *Mucoraceae* and *Tremellaceae* to decompose compost, further secreting small molecular metabolites to stimulate soil microorganisms and accelerate PCP degradation. It is the first time to report *Hypocreaceae* as organic pollutants degrading fungus.

5. Conclusions

This study revealed that earthworm or non-sterile compost had unique roles in enhancing soil PCP removal. Vermicomposting contributed to humus consumption and soil pH neutralization, releasing humus-PCP complex and increasing PCP availability. Sterile compost slowed PCP mineralization by increasing humus-fixed PCP in soil. Soil bacterial and fungal community structure was also significantly affected by vermicomposting, and the phylogenetic classification uncovered some indigenous microorganisms mineralizing PCP, including bacterial *Pseudomonadaceae*, *Sphingobacteriaceae* and *Xanthomonadaceae* and fungal *Trichocomaceae*. Compost decomposition also provided microbial available substrates to stimulate PCP degradation, supported by the activities of bacterial family *Flavobacteriaceae* in non-sterile compost, and fungal families *Mucoraceae*a and *Tremellaceae*. Vermicomposting has the potential to enhance the bioremediation of PCP contaminated soil, which was first time revealed in this study to our knowledge.

6. Acknowledgements

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8. Table

Table 1 Experimental treatment designs.

Treatment	PCP (mg kg^{-1})	Upland soil (kg)	Earthworm (individuals)	Compost (%)	
				Sterile	Non-sterile
Ck	40	0.5	−	−	−
C_S	40	0.5	−	4.5	−
C_N	40	0.5	−	−	4.5
E	40	0.5	8	−	−
EC_S	40	0.5	8	4.5	−
EC_N	40	0.5	8	−	4.5

Note: *Ck* = Blank control, C_S = Sterile compost treatment, C_N = Non-sterile compost treatment, *E* = *Eisenia fetida* vermicomposting treatment, EC_S = *Eisenia fetida* vermicomposting with sterile compost, EC_N = *Eisenia fetida* vermicomposting with non-sterile compost.

9. Figures

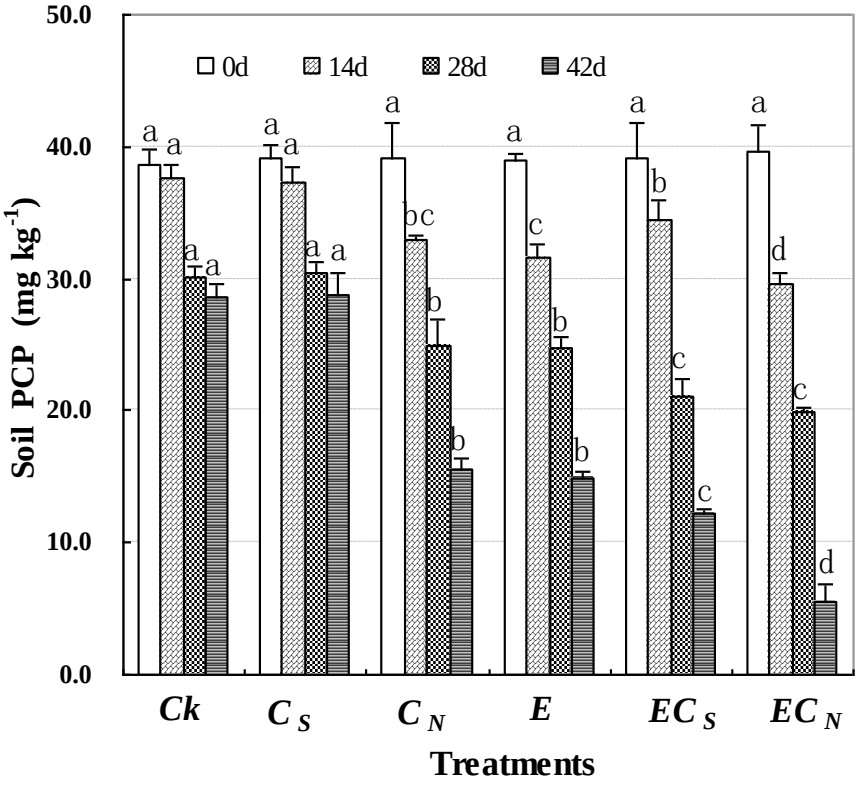
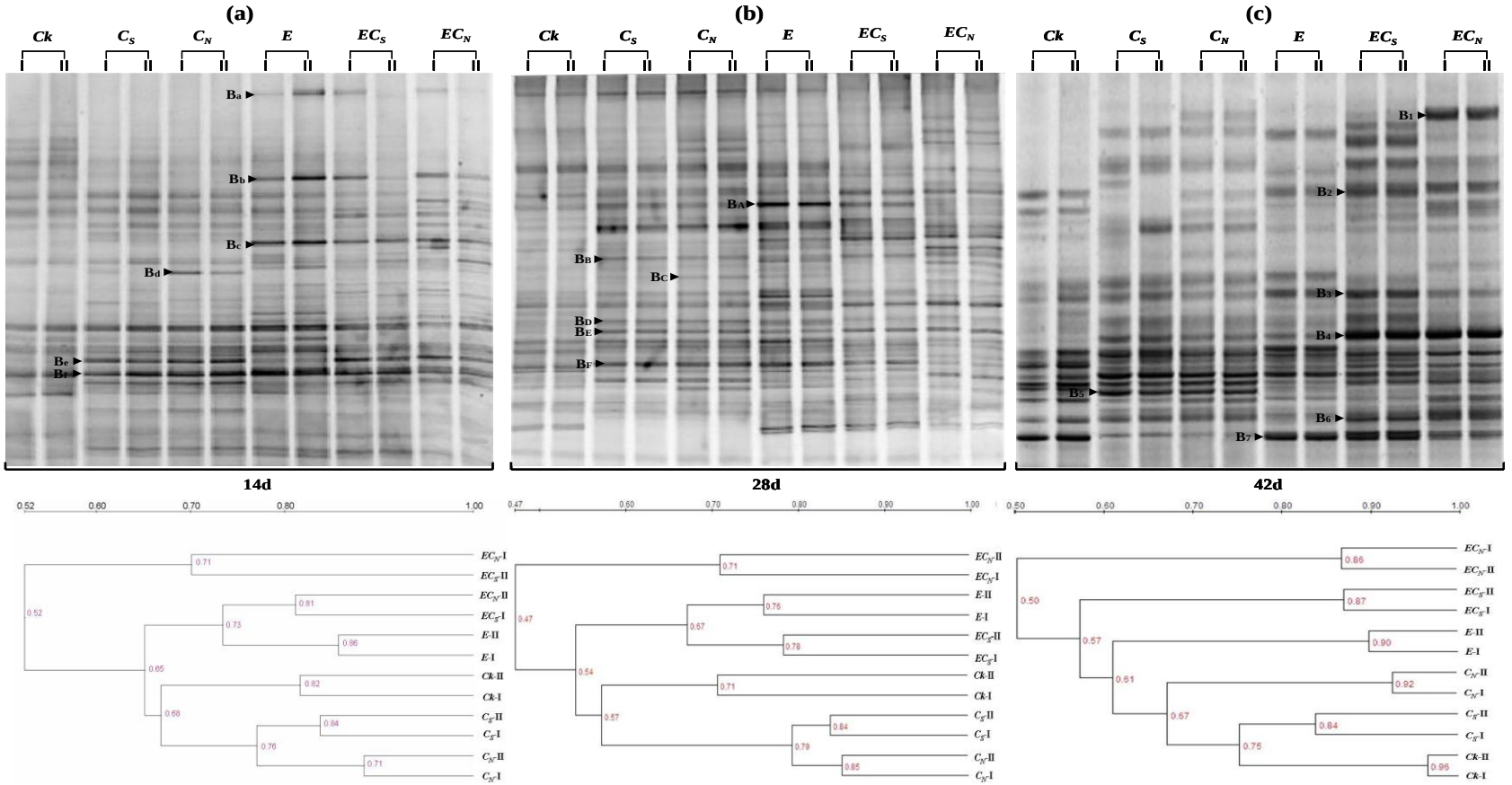
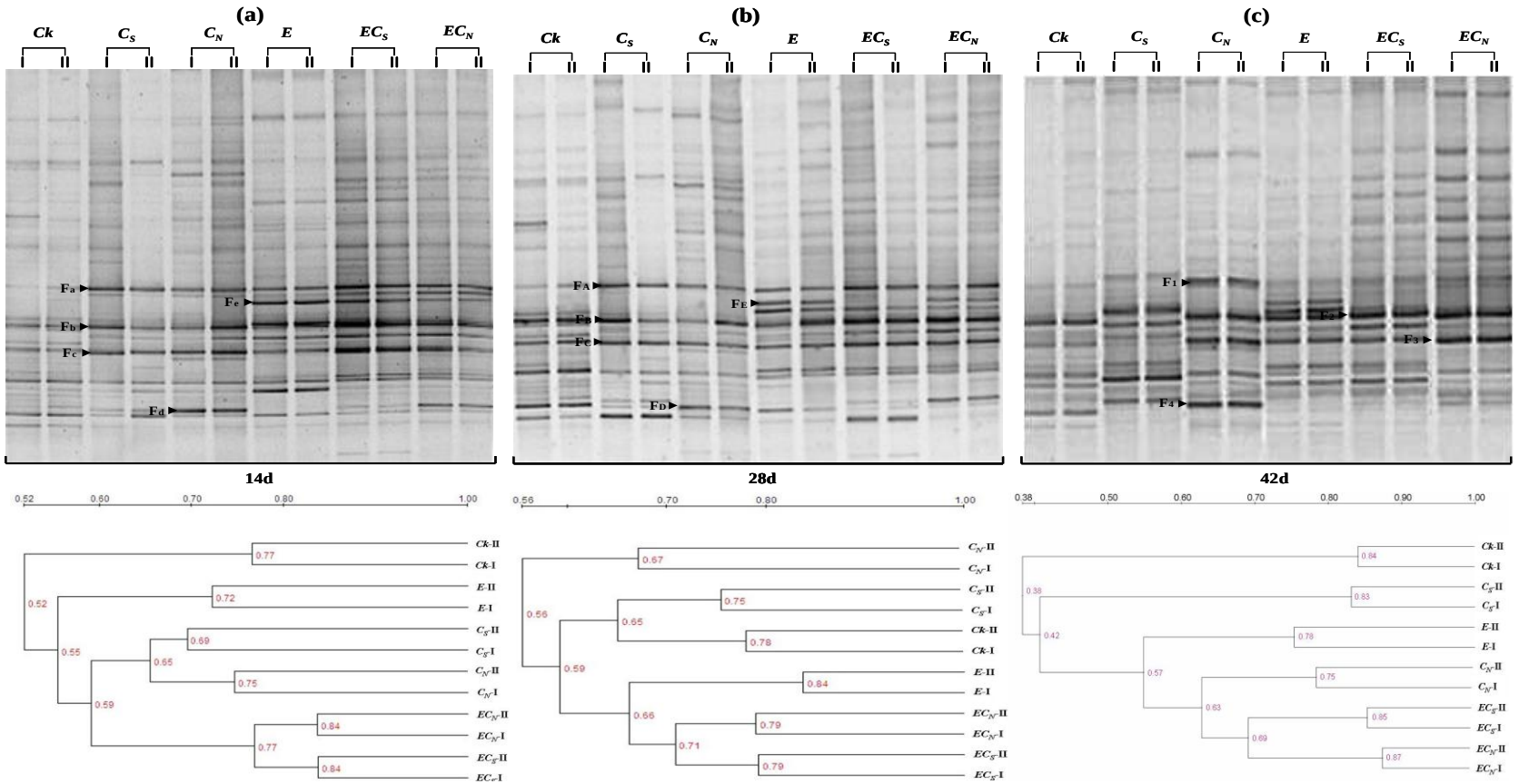


Fig. 1. Soil PCP residue concentrations with time dependence in different treatments. Data are mean ± SD (n=3). Different lower case letters refer to significantly differences (ANOVA, Duncan's test, $p<0.05$) among the treatments.

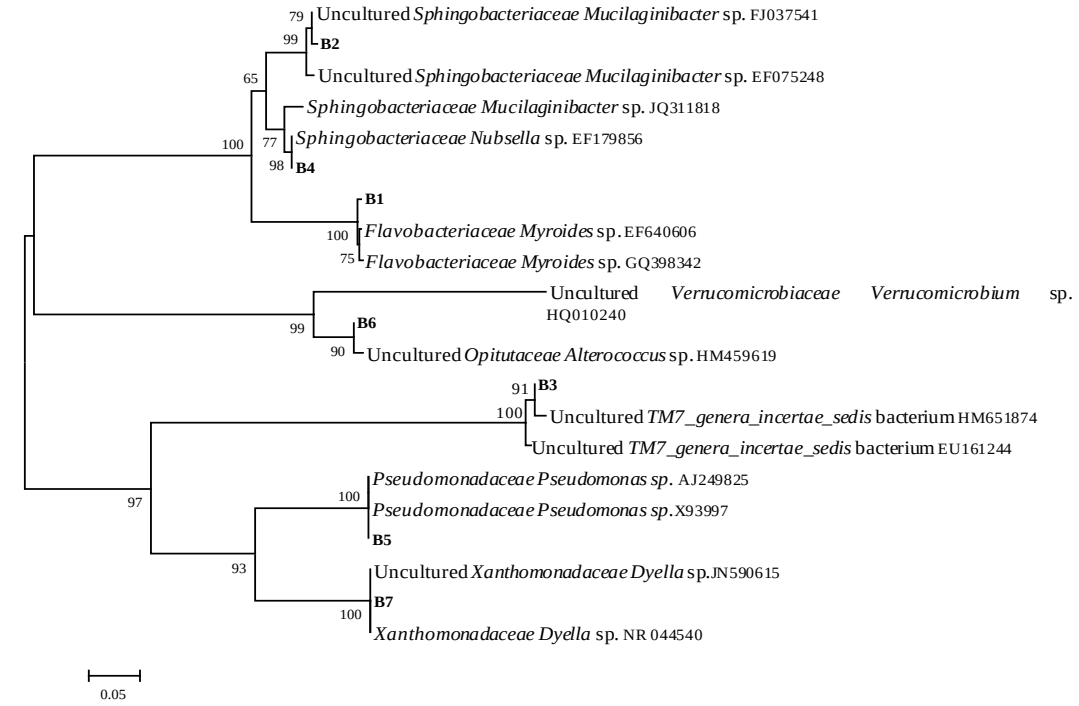

(a)
(b)
(c)
Ck
C_S
C_N
E
EC_S
EC_N
Ba
Bb
Bc
Bd
Be
Bf
BA
BB
BC
BD
BE
BF
B1
B2
B3
B4
B5
B6
B7
14d
28d
42d
0.52 0.60 0.70 0.80 1.00
0.47 0.60 0.70 0.80 0.90 1.00
0.50 0.60 0.70 0.80 0.90 1.00
EC_N-I
EC_S-II
EC_N-II
EC_S-I
E-II
E-I
Ck-II
Ck-I
C_S-II
C_S-I
C_N-II
C_N-I
0.71
0.81
0.73
0.86
0.52
0.65
0.82
0.68
0.84
0.76
0.71
0.71
0.76
0.67
0.78
0.47
0.54
0.71
0.57
0.84
0.79
0.85
0.86
0.87
0.50
0.90
0.57
0.61
0.92
0.67
0.84
0.75
0.96

(A)

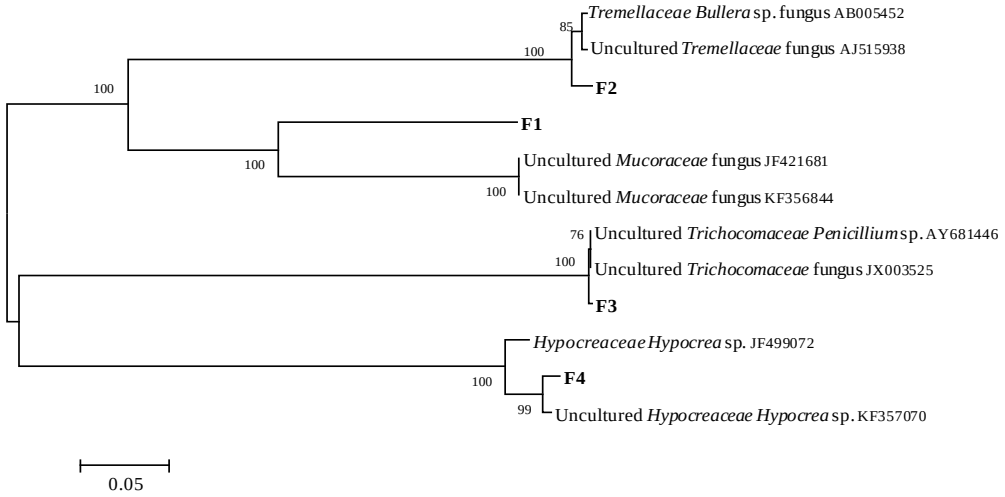


(B)

Fig. 2. Cluster analysis of bacterial (16S rRNA, A) and fungal (18S rRNA, B) community structure in different treatments by DGGE. (a), (b) and (c) represents the communities at 14, 28 and 42 days respectively.



(A)



(B)

Fig. 3. Neighbor-joining phylogenetic trees of the bacterial 16S (A) and fungal 18S (B) rRNA genes. The neighbor-joining trees were constructed based on their closest relatives taken from the NCBI database and by using ClustalX software. Scale of bar indicated 5 % sequence divergence and a bootstrap analysis was performed with 1000 trials.

Supplementary Material

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