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**Aerobic co-composting degradation of highly PCDD/F-contaminated field soil. A study of bacterial community.**

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**Abstract**

This study investigated bacterial communities during aerobic food waste co-composting degradation of highly PCDD/F-contaminated field soil. The total initial toxic equivalent quantity (TEQ) of the soil was 16,004 ng-TEQ kg<sup>-1</sup> dry weight. After 42-day composting and bioactivity-enhanced monitored natural attenuation (MNA), the final compost product's TEQ reduced to 1,916 ng-TEQ kg<sup>-1</sup> dry weight (approximately 75% degradation) with a degradation rate of 136.33 ng-TEQ kg<sup>-1</sup> day<sup>-1</sup>. Variations in bacterial communities and PCDD/F degraders were identified by next-generation sequencing (NGS). Thermophilic conditions of the co-composting process resulted in fewer observed bacteria and PCDD/F concentrations. Numerous organic compound degraders were identified by NGS, supporting the conclusion that PCDD/Fs were degraded during food waste co-composting. Bacterial communities of the composting process was defined by four phyla (*Proteobacteria*, *Actinobacteria*, *Bacteroidetes* and *Firmicutes*). At the genus level, *Bacillus* (Firmicutes) emerged as the most dominant phylotype. Further studies on specific roles of these bacterial strains are needed, especially for the thermophiles which contributed to the high degradation rate of the co-co-composting treatment's first 14 days.

**Keywords:** PCDD/F-contaminated field soil; aerobic food waste co-composting; next-generation sequencing; PCDD/F degraders; catabolic activity.

## 1. Introduction

Polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) represent a group of persistent environmental pollutants and are known to be carcinogens and potent endocrine disruptors (van den Berg *et al.* 1998). The remediation of PCDD/F-contaminated sites is a major concern, especially in areas where industrial activities once thrived (Lee *et al.* 2006). Their high molecular stability, low water solubility (hydrophobicity/lipophilicity) and high tendency to adsorb onto particulate phase make PCDD/Fs less bioavailable and difficult to remove from soil and sediment matrices (Fukushi *et al.* 2016).

Thermal treatment is the most popular technique for degrading PCDD/Fs since it can efficiently reduce PCDD/F content in a soil matrix. However, thermal treatment is energy-consuming, making it a costly and less affordable remediation technology (Mattei *et al.* 2016). Bioremediation strategies for contaminated soil have been reported to be environmentally friendly, energy- and cost-efficient, and create less or no secondary pollutants (Meinen *et al.* 2014, Huang *et al.* 2016). Co-composting is defined using more than one feedstock, e.g. food waste and contaminated soil/sediment, to aerobically degrade organic contaminants. Co-composting is a widely employed biological method for the treatment of recalcitrant contaminants (Covino *et al.* 2016, Mattei *et al.* 2016). Co-composting technologies have been reported to successfully address PCDD/F contamination in laboratory-scale studies (Yoshida *et al.* 2005, Narihiro *et al.* 2010, Kaiya *et al.* 2012). Food waste is common organic waste that can be utilized as an effective carbon and energy source, facilitating the co-composting process, which also needs readily available wastes as bulking agents (Mehariya *et al.* 2018, Tran *et al.* 2018, Wong *et al.* 2018). The co-composting method takes advantage of the catabolic ability of naturally occurring microorganisms for the degradation of organic contaminants (Barje *et al.* 2013, Mattei *et al.*

2016). Suitable environmental parameters, for example, moisture content, pH, temperature, nutrients, oxygen feeding and ratio of bulking agents determine the efficiency of the co-composting process (Lin *et al.* 2012).

Previous successful composting-based biodegradation studies have been published, for example: Xu *et al.* (2018) with 4-nonylphenol; Siebielska and Sidelko (2015) with polychlorinated biphenyls (PCBs); Covino *et al.* (2016) with polycyclic aromatic hydrocarbons (PAHs); and Lin *et al.* (2012) with diesel oil. These analyses support the technical feasibility employing co-composting degradation to remediate highly PCDD/F-contaminated field soil. However, to date, most co-composting studies have been conducted as laboratory-scale bioreactors with simulated contaminated soil materials (Chen *et al.* 2015, Siebielska and Sidelko 2015). Larger scale studies of co-composting degradation of field soil contaminated with organic compounds are scarce in the literature.

To better understand the catabolic mechanism underlying organic pollutant degradation, it is necessary to characterize the inherently occurring microorganisms in the co-composting system. Next-generation sequencing (NGS) methods can be employed to identify and taxonomically categorize complex microbial communities (Covino *et al.* 2016, Kalkan Aktan *et al.* 2018). Millions of sequences of a sample in a single run can be executed using NGS, providing in-depth insights into the taxonomic diversity of microorganisms in soil or sediment matrices. To date, there is a shortage of published studies employing 454-pyrosequencing or Illumina sequencing to investigate the structure and dynamics of microbial communities in the co-composting degradation of recalcitrant contaminants (Llado *et al.* 2015). Therefore, in designing this study, the NGS approach is believed to better help us understand the bacterial communities and dynamics of co-composting degradation of highly PCDD/F-contaminated field soil.

The contaminated field soil sample was retrieved from a hazardous waste site in southern

Taiwan, where pentachlorophenol (PCP) was manufactured but the operation has not been in production for more than thirty years. After soil sieving pre-treatment (the initial TEQ of the soil with all particle sizes was  $7,730 \pm 3.16$  ng-TEQ kg<sup>-1</sup> dry weight), the size of 0.105 – 0.250 mm (or fine sand) was selected for the co-composting experiment because it constituted 67% of the total mass. It should be noted that at present, the current treatment method used at the contaminated site in Tainan City is incineration. Although incineration is effective in removing PCDD/Fs from the contaminated soil, its high cost means that the remediation management board should seek more economical alternatives. Our opinion is that if the soil fraction with the largest volume could be remediated by co-composting bioremediation, the other fractions of even higher PCDD/F TEQs could then be treated with incineration, reducing the total remediation costs considerably. This study was therefore conducted to determine whether a co-composting process might be able to degrade highly PCDD/F-contaminated field soil. Also, the bacterial communities of the co-composting degradation process were investigated by NGS approach. In this study, the correlation between the degradation efficiency and control parameters (temperature, moisture, pH and odor) were identified. Illumina sequencing was carried out to provide qualitative insights into changes in bacterial communities at different stages of the co-composting process. The most influential bacteria in the degradation process as well as possible degradation mechanisms were proposed and discussed.

## 2. Methodology

### 2.1 Chemicals and materials

Standard solutions of 17 PCDD/F compounds (2,3,7,8-TCDD; 2,3,7,8-TCDF; 1,2,3,7,8-PeCDD; 1,2,3,7,8-PeCDF; 2,3,4,7,8-PeCDF; 1,2,3,4,7,8-HxCDD; 1,2,3,6,7,8-HxCDD; 1,2,3,7,8,9-HxCDD; 1,2,3,4,7,8-HxCDF; 1,2,3,6,7,8-HxCDF; 1,2,3,7,8,9-HxCDF; 2,3,4,6,7,8-

HxCDF; 1,2,3,4,6,7,8-HpCDD; 1,2,3,4,6,7,8-HpCDF; 1,2,3,4,7,8,9-HpCDF; OCDD; OCDF) mixed in Nonane were purchased from Sigma-Aldrich (Missouri, USA). Deionized (DI) water was used in the co-composting experiment and sample analysis unless otherwise noted. Other chemicals and reagents used in the experiment, sample extraction and instrumental analysis, e.g. sodium sulfate, toluene, *n*-hexane, sulfuric acid and silica gel, were purchased from Sigma-Aldrich and/or Fisher Scientific and satisfied standards for laboratory use.

## 2.2 Co-composting experiment

### 2.2.1 Wet sieving pre-treatment of the contaminated soil

Soil wet screening was employed to prepare the soil for the co-composting experiment. The soil wet screening preparation has been described in detail in our previous study (Hung *et al.* 2017). Briefly, the soil wet screening method developed by the American Society for Testing and Materials (ASTM) - E276–13 – served to separate different particle sizes into homogenous materials for subsequent experimental design. This study used ASTM E276–13 for soil sieving pre-treatment. The field soil was manually sieved through six different sized meshes (2, 1, 0.5, 0.25, 0.105, 0.053 mm) with the addition of tap water (wet screening) and then sun-dried. Following the wet screening stage, the soil was mixed homogeneously in a mixer (50 L, 50 rpm). Soil characteristics (pH, total organic carbon, total nitrogen and electrical conductivity) were determined (pH: ASTM D4972-13 method; organic matter: titration method; total Kjeldahl nitrogen: ISO 11261; electrical conductivity: Singh and Shah (2004) method). The characteristics and PCDD/F TEQs of the soil's particle sizes were reported in our previous publication (Hung *et al.* 2017).

### 2.2.2. Composting reactor

The design of the bench-scale reactor (volume 0.2 m<sup>3</sup>, 0.7 m in height and 0.45 in width) is depicted in Fig. 1. The aerobic reactor had clockwise and counterclockwise rotation control,

180-degree turnover wheel control and air injection. On the lid, there were channels for sampling, air injection and visual observation. Representative compost samples (100 g dry wt.) were collected and analyzed after the experimental setup and every 7 days until the experiment was completed. The co-composting experiment was performed over 28 days. After the co-composting experiment finished (at the time the temperature of the reactor decreased to around ambient values indicating that the nutrient was totally used up and bacterial activity stopped functioning), the follow-up MNA was conducted and finished at day 42 since the beginning of the co-composting experiment. The co-composting experiment was conducted in the compost facility at the Center of Environmental Analysis Services, National Kaohsiung University of Science and Technology (CEAS - NKUST).

### 2.2.3 Food waste co-composting and follow-up monitored natural attenuation (MNA)

Food waste used for the co-composting in the current study consisted of discarded dairy products, grains, bread, fruits, vegetables, red meat, seafood and kitchen waste collected from local schools and restaurants. The pH of the food waste was recorded at  $4.80 \pm 0.05$  and the starting pH of the mixed compost materials was  $5.14 \pm 0.04$ . The food waste ingredient composition was 62% of red meat and seafood, 22% of vegetables, and 16% of others. The C/N ratio, salinity and bulk density of the food waste were, respectively, 13.6, 0.25% and  $1018 \text{ kg/m}^3$ . The food waste's water content, ash content and combustible matter were 81.3%, 2% and 16.7%, respectively. Fresh food waste was shredded into fractions of less than 5 mm diameter in order to make it easily well-mixed with other ingredients. Sawdust was used as a bulking agent to adjust the compost mixture's moisture content (Chang *et al.* 2016). Mature compost was added to enrich the microbial population and subsequently enhance the fermentation process. The reactor was packed with 50 kg wet wt. of food waste, 5 kg wet wt. of sawdust, 10 kg wet wt. of mature compost, and 20 kg dry weight of highly PCDD/F-contaminated field soil.



Since the moisture content of food waste, mature compost, sawdust and the contaminated soil was 80.3%, 39.1%, 27.6% and 1.8%, respectively, the dry weight of them should be 9.85, 6.09, 3.62 and 19.64 kg, also respectively. The moisture content of final waste mixture is 50%. From the dry weight calculation, the ratio of the contaminated soil to the compost material was approximately 1:1. Initial moisture was adjusted to be 60%, which should favor organic degradation (Lin *et al.* 2012, Barje *et al.* 2013, Llado *et al.* 2015). The reactor was set up inside a greenhouse and operated with continuous aeration. The mechanical mixer was fixed within the reactor to help maintain a homogenous aerobic state. Daily operations included mixing (25 rpm), oxygen feeding (aeration rate at 8.48 vvm - liter of air/liter of medium/minute) and measuring operational parameters [temperature (K Type TES 1310 Digital Thermocouple Thermometer), pH (Hanna Instruments HI99121) and moisture content (gravimetric method), odorous gaseous ammonia (GASTEC GV-100)] (Lin *et al.* 2012, Barje *et al.* 2013). Also, before the experiment was conducted using the PCDD/F-contaminated soil, we undertook a preliminary study, in which the composting process was done with only food waste, saw dust and mature compost, and without the PCDD/F-contaminated field soil. Changes in bacterial communities at different stages of the preliminary study were determined using Denaturing Gradient Gel Electrophoresis (DGGE) method and the results are shown in Table S1.

The follow-up MNA was then employed to take advantage of the co-composting treatment since the soil had been bio-stimulated with bacterial organic degraders during the co-composting process and this approach could help save the cost of food waste, bulking agents, and associated operational costs. Compost samples were taken at intervals for analysis until day 42 since the beginning of the co-composting experiment.

### *2.3 Sample extraction, clean-up and instrumental analysis*

Details of sample extraction, clean-up and analysis can be viewed in our previous analysis

(Hung *et al.* 2017). Briefly, ultrasonic extraction was performed following US EPA method 3550C. The samples (10 g each) were lyophilized within a vacuum freeze dryer (Eyela FDU-1200, Tokyo Rikakikai Co. Ltd., Japan, -50 °C, 10 Pa, 24 hours). Then, the soil matrix was mixed with anhydrous sodium sulfate and toluene. A horn-type sonicator (VCX 750, Sonics Materials, USA) was used for the extraction. Sonication was performed in three cycles (3 min/cycle) under the same conditions (amplitude 62%, pulse on 6.0 and off 3.0).

Clean-up was performed following a modified USEPA method 3630C. After ultrasonic extraction, the aliquots were eluted through columns comprising three layers, these being glass wool, acid silica gel (90 g silica gel mixed with 20 mL sulfuric acid 98.08%) and anhydrous sodium sulfate. The eluates were concentrated in a Turbo Vap II (Biotage, USA) using nitrogen (10 psi) until dry. Solvent exchange involved the use of *n*-hexane 95%, acid silica gel and glass wool filter. Final extracts (1 mL) were added with sulfuric acid and maintained in 1.5 mL glass vials at -4 °C until required for analysis.

US EPA method 8290 was used for PCDD/Fs analysis. A 6890-5973 gas chromatography-mass selective detector (GC-MSD) system with an HP-5MS (30 m x 0.25 mm x 0.5 µm) capillary column was used to quantify PCDD/Fs. The injection volume and carrier gas (Helium) flow rate were set at 1 µL and 1 mL/min, respectively. Oven temperature began at an initial 40 °C for 4 mins before ramping up to 300 °C at the rate of 10 °C/min and this was maintained for 20 mins. Selective ion monitoring (SIM) mode was employed. All calculations of concentrations and calibrations were performed based on a previously built standard calibration curve (average response factors < 20%,  $R^2 > 0.990$ , with a midpoint recovery of 100±25% for OCDD and OCDF and 100±20% for other species). The details of PCDD/F analysis are given in Table S2. The reduction in PCDD/F content was expressed as toxic equivalent quantities (TEQs) based on Stockholm Convention - World Health Organization (van den Berg *et al.* 1998).

#### 2.4 QA/QC

QA/QC included internal standard recovery, standard calibration curve, ion intensity ratio, blank and midpoint recovery. Built standard calibration curve was accepted when average response factors were less than 20% or correlation coefficients ( $R^2$ ) were more than 0.990. Every sample batch included blank, midpoint check, sample duplicate and matrix spike. Blanks showed non-detected PCDD/F concentrations, indicating that no contamination occurred during sampling storage and the extraction procedure. Sample duplicates revealed relative percent difference (RPD) less than 20%. Matrix spike duplicate recovery was  $80\pm 26\%$ , which is within a reasonable range.

#### 2.5 DNA extraction, PCR amplification and Illumina sequencing

Three parallel compost samples (0.3 g each) were extracted and the resulting aliquots were pooled into a single representative sample. Metagenomic DNA was extracted using WelPrep DNA kit (Welgene Biotech., Cat No. D001) according to the instructions of the manufacturer. DNA purity was tested with a NanoDrop spectrophotometer. DNA samples with an optical density of 260/280 fell within the 1.8-2.0 range, indicating very good purity. Portions of 16S rRNA genes, involving the variables V3-V4 region were amplified from the extracted DNA samples with OD 260/280 in the 1.8-2.0 range.

PCR amplifications were performed with SureCycler 8800 (Agilent Technologies) for the bacterial-specific primers S17 (5' TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG 3') and A21 (5' GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC 3') (Klindworth *et al.* 2013), which were included in the preparation solution (Nextera XT Index Kit). The amplified DNA sizing was checked by 4200 TapeStation (Agilent Technologies). Amplicon

sequencing was done using an Illumina MiSeq sequencer (2 x 300 bp). Details concerning quality control (QC) summary of sequencing data are shown in Table S3.

### 2.6 Identification and taxonomic classification

Long reference sequences were aligned from sequencing reads using the Bowtie 2, which is an ultrafast and memory-efficient tool for aligning sequencing reads to long reference sequences (<http://bowtie-bio.sourceforge.net/bowtie2/index.shtml>). Bowtie 2 describes the genome in indices with an FM Index. Sequence filtering was performed, and inferior quality sequences (quality score mean of 25) were removed, and bar-codes and primers were trimmed off. FLASH was used to merge paired-end reads from next-generation sequencing results. Noise was decreased employing the PyroNoise algorithm developed by Mothur (<https://www.mothur.org/>). The sequences were grouped into operational taxonomic units (OTUs) involving the UPARSE algorithm derived from USEARCH v. 7.0.1090 (<http://www.drive5.com/usearch/>) and agreeing sequences from each OTU were created. After the bacterial community analysis, OTU sequences were classified into taxa employing the Ribosomal Database Project (RDP) classifier. From the kingdom of bacteria, the analyzed taxonomic levels were phylum, class, order, family, genus and species. Rank abundance and diversity indices of 16S data ( $\alpha$ -diversity: Shannon, Inverse Simpson, Chao1, ACE;  $\beta$ -diversity: Bray-Curtis dissimilarity matrix) were calculated using an open-source bioinformatics pipeline known as Qiime (Caporaso *et al.* 2010), which allows raw sequencing data to be statistically categorized and graphically displayed. Details of the calculation and the results are provided in the Supporting Information (SI).

### 2.7 Statistical analysis

SPSS for Windows, Version 22, was employed for statistical operations. ANOVA post-hoc Turkey test was used to compare statistical differences between two data groups of gaseous ammonia and temperature. Principal component analysis (PCA) was employed for identifying the

difference in bacterial composition. Differences were regarded as significant with 95% confidence interval.

### 3. Results

#### 3.1 Temperature, moisture content, pH and gaseous ammonia profiles of the co-composting degradation process

Fig. 2A presents the temperature and moisture content profile of the co-composting process. Moisture content was maintained at 30-60% during the operation. The temperature increased rapidly from the beginning 30 °C to 50 °C on day one and continued increasing to more than 70 °C on day 5. The temperature then remained at 70 °C until day 16 before falling to the ambient temperature on day 21. Fig. 2B shows the pH profile of the co-composting process. The initial pH of the contaminated soil was 8.9, which then decreased substantially to 5.14 after the mixing with bulking agents. After three days, the pH value increased to neutral and remained constant at about 8.0 until the end of the experiment. The gaseous ammonia (NH<sub>3</sub>) profile is presented in Fig. 2C. Gaseous ammonia rapidly peaked at 500 mg L<sup>-1</sup> on day 6 and gradually decreased to 0 mg L<sup>-1</sup> on day 16. After day 14, gaseous ammonia remained constant at 0 mg L<sup>-1</sup>, although there was a slight increase on day 17.

#### 3.2 Degradation of PCDD/Fs in contaminated field soil

Fig. 3 shows the degradation of PCDD/F TEQs throughout the experiment. The initial TEQ value of the soil was 16,004 ng-TEQ kg<sup>-1</sup> dry weight, which then decreased by around 50% to 7,642 ng-TEQ kg<sup>-1</sup> dry weight after mixing with bulking agents. During the first 14 days, the degradation rate was 283 ng-TEQ kg<sup>-1</sup> day<sup>-1</sup>. Then, as the temperature gradually returned to ambient values (20 – 25°C) indicating the maturation stage, PCDD/F degradation rate decreased and the TEQ after 28 days of co-composting was 3,713 ng-TEQ kg<sup>-1</sup> dry weight. The follow-up

MNA was then conducted and after 42 days since the experiment started, PCDD/F TEQs was recorded to be 1,916 ng-TEQ kg<sup>-1</sup> dry weight, making an overall PCDD/F degradation rate of 136.33 ng-TEQ kg<sup>-1</sup> day<sup>-1</sup>.

### 3.3 Bacterial catabolic activity during the co-composting process

Fig. 4A illustrates the changes in the number of OTUs (at phylum level) at different stages of the co-composting process. At the beginning (day 0 and day 1), although there was a slight increase, the number of OTUs did not alter much, staying at around 700. Then, it decreased to approximately 250 on day 10 before increasing back to 700 on day 14.

Fig. 4B shows the structure and dynamics of the bacterial community on days 0, 1, 7, 10 and 14 of the bench-scale co-composting experiment. At the Phylum level, bacterial composition almost remained unchanged between day 0 and day 10. A similarity in bacterial structure of day 0 and day 1 (when the temperature was below 50°C), and those of day 7 and day 10 (when the temperature reached its peak) was observed. Although the incubation time was different, the overall bacterial communities from day 0 to day 10 were largely occupied by *Firmicutes* (gram-positive bacteria), accounting for 78%, 70%, 99% and 80% on days 0, 1, 7 and 10, respectively.

On day 0 and day 1, another type of gram-positive bacteria, *Actinobacteria*, was present with relatively high percentages, 9% and 15%, respectively. However, its relative abundance was reduced to less than 1% during the thermophilic stage. Gram-negative prokaryotes (*Proteobacteria*) showed up in abundance at 9%, 11% and 10% on days 0, 1 and 10, respectively. *Pseudoxanthomonas* sp. was found in both our preliminary study (at 70 °C and later at 55 °C, Table S1) and in this co-composting study (on days 0, 1 and 10). On day 10, *Bacteroidetes* made up 8% of the total bacterial composition. This phylum was also observed in the preliminary study at the thermophilic stage (70 °C; Table S1).

On day 14, when the composting moved into the maturation stage, a remarkable shift in

the structure of bacterial communities was observed. At this stage, the bacterial composition seemed to be more equally occupied by different bacterial phyla, except for *Proteobacteria*, which dominated with a slightly high proportion (45%). Other phyla formed relatively even portions of bacterial composition on day 14, i.e. *Actinobacteria* (13%), *Firmicutes* (12%) and *Bacteroidetes* (11%). PCA resulted in no significant similarity in the bacterial communities of day 14 and other days of the experiment (Fig. S2). Through the analysis of the bacterial composition, the co-composting process was dominated by certain phyla: two gram-positive (*Firmicutes* and *Actinobacteria*) and two gram-negative (*Proteobacteria* and *Bacteroidetes*).

At the genus level, the most thermophilic abundant phylotypes (thermophile) was *Bacillus* (84% on day 7 and 54% on day 10) within the endospore-producing *Bacillales* (*Firmicutes*). Other abundant thermophiles of *Firmicutes* were *Bacillaceae* (14% on day 10) and *Lactobacillus* (7% on day 0 and 4% on day 1). The phylotype of *Pseudomonas* (*Proteobacteria*) was detected in relatively high abundance levels (10%) on day 14. The phylotype, *Sphingobacterium*, of *Bacteroidetes* was present on day 10 at a slightly high relative abundance (8%).

#### 4. Discussion

At early period, the fact that the temperature of the co-composting rapidly increased to 50 °C (thermophilic stage) on day one indicates that bioactivity was not strongly inhibited by the high PCDD/F concentration (approximately 7600 ng TEQ kg<sup>-1</sup> dry wt.). Overall the thermophilic stage (> 40°C) last from day 1 to day 20. Thermophilic period is believed to be beneficial for microbial growth during the composting process and thus the degradation of organic contaminants (Lin *et al.* 2012, Llado *et al.* 2015, Covino *et al.* 2016).

Our observation of pH value remaining unchanged at about 8.0 from day 3 onward is in agreement with similar studies of aerobic co-composting degradation of organic compounds and neutral pH values (5.5–8.5) were found to be favored by aerobic microbial communities (Narihiro

*et al.* 2010, Lin *et al.* 2012). Gaseous ammonia emission profile was recorded since gaseous ammonia represents odor released by microbial activity during the co-composting process as the mineralization of organic contaminants results in the formation of nitrogen (He *et al.* 2018, Wang *et al.* 2018). A gaseous ammonia profile can also represent the developmental stages of microorganisms within the co-composting (Kim *et al.* 2017, He *et al.* 2018). In this study, gaseous ammonia reached a peak at 500 mg L<sup>-1</sup> after 6 days and steadily dropped to 0 mg L<sup>-1</sup> after 16 days, which is not statistically different from the temperature profile ( $p > 0.05$ ). In fact, these parameters correlated well ( $R^2 = 0.657$ ), indicating that developmental stages of composting bacteria could be influenced by temperature gradient of the co-composting process.

Although the total organic carbon of the soil was only 4%, the use of the aerobic co-composting remained promising for the biodegradation of PCDD/Fs. Actually, the first 14 days of aerobic co-composting brought about a high degradation rate of 283 ng-TEQ kg<sup>-1</sup> day<sup>-1</sup>. Following the high degradation of PCDD/Fs came the maturation stage, where PCDD/F degradation was shown to be lower than that in the thermophilic stage (Narihiro *et al.* 2010). In fact, in this study, TEQ values increased slightly although this might have been caused by variability of sampling.

Kinetic modelling was employed to assess the rate of PCDD/F degradation. In this study, we employed both first-order and second-order kinetic models to evaluate the degradation of PCDD/Fs (Table S5). The degradation of PCDD/F TEQs followed first-order kinetics with the rate constant ( $k$ ) of 0.0279 and correlation coefficient ( $R^2$ ) of 0.9047, which is slightly greater than the correlation coefficient of second-order kinetics (0.8605). Although the fact that PCDD/F degradation following the first-order kinetic model agrees well with another similar study by Narihiro *et al.* (2010), the complexity of the co-composting process with millions of organisms makes the process especially difficult to predict. The bioavailability of the deeply sorbed



PCDD/F compounds might advance/hinder the degradation progress. Therefore, any kinetic models may not be able to reliably characterize such wide variability (Siebielska and Sidelko 2015).

The TEQ value after the 42-day incubation decreased from 7,642 to 1,916 ng-TEQ kg<sup>-1</sup> dry weight. Despite such a significant reduction, the final TEQ was still higher than the standard limit of PCDD/Fs in soil (1,000 ng-TEQ kg<sup>-1</sup> dry weight) issued by the Taiwan EPA. However, it should be noted that in this study, the volume of the highly PCDD/F-contaminated field soil (20 kg) was considerably large compared to other similar biodegradation studies conducted on the field soil (Chen *et al.* 2013, Chen *et al.* 2016) (Table S6). Our co-composting experiment lasted only for 28 days, which is relatively short in comparison with other co-composting studies, e.g. 280 days for Chen *et al.* (2016) or 240 days for Covino *et al.* (2016). In fact, in another co-composting batch that we conducted for 49 days, the final PCDD/F TEQ was only 1,604 ng-TEQ kg<sup>-1</sup> dry weight (Lin *et al.* 2018). Another similar co-composting batch with lower initial PCDD/F TEQ (after mixing with compost materials) of 2,491 ng-TEQ kg<sup>-1</sup> dry weight was also undertaken and after 63-day incubation, the final TEQ value was only 751 ng-TEQ kg<sup>-1</sup> dry weight (Fig. S3). In this study, approximately 75% degradation of high PCDD/F TEQ soil is relatively positive compared to those studies of the same kind although there could be differences in experimental design (Table S6).

The MNA was stopped after 42 days (i.e., after the experiment commenced) because at this point, the TEQ value was low. Furthermore, the soil could easily and rapidly be treated by other physical or chemical methods. For example, our previously reported soil washing method with anaerobic compost tea solvent, did achieve over 60% PCDD/F removal for the first washing cycle and retained the soil's microbial activity (Hung *et al.* 2017). After being treated to below the standard limit, the soil could then be backfilled. Since the soil was enriched with bacterial

communities during the co-composting process, gardening/agricultural purposes could be performed, which is a salient advantage of co-composting bioremediation treatment compared to most current chemical and physical treatment methods.

The removal of PCDD/Fs was attributed to microbial degradation since the reactor was not designed for leachate discharge and the possibility of PCDD/Fs to be released into vapor from our experiment (temperature < 80°C) should be negligible (due to PCDD/Fs' low Henry's law constants and low water solubility/high log  $K_{ow}$ ) (Kulkarni *et al.* 2008, Alace 2016). Bacterial activity was most pronounced during the thermophilic period (up to day 14), during which the temperature increased rapidly. The temperature remained steady for 20 days before decreasing to the mesophilic stage. It was during this period of rapid rise in temperature that the emission of gaseous ammonia quickly reached its peak on day 6 and dropped to 0 mg L<sup>-1</sup> on day 14. Many studies have reported positive degradation organic contaminants during the thermophilic period: diesel oil (Lin *et al.* 2012), olive oil mill waste and municipal solid waste (Barje *et al.* 2013), polychlorinated biphenyls (PCBs) (Siebielska and Sidelko 2015), polycyclic aromatic hydrocarbons (PAHs) (Mattei *et al.* 2016), decabromodiphenyl ether (BDE-209) (Chang *et al.* 2016) and PCDD/Fs (Narihiro *et al.* 2010).

During the thermophilic period, the bacterial activity is higher than any other stage of the composting process (Ghaly *et al.* 2012). Higher temperatures are known to create favorable conditions for the activated energy breakdown of organic compounds (Barje *et al.* 2013). Then, during the maturation stage (from day 14 onward), bacterial activity slowed down, adversely affecting the catabolic ability of the co-composting process (Narihiro *et al.* 2010). Similar trends have also been observed in studies of other organic contaminants (Barje *et al.* 2013, Siebielska and Sidelko 2015, Chang *et al.* 2016, Mattei *et al.* 2016).

The fact that the number of OTUs at phylum level reduced to approximately 250 on day

10 prior to rising again to 700 on day 14 as the co-composting process moved toward the thermophilic stage implies that many bacterial species, especially the mesophiles which grow best between 20–45 °C, could be eliminated due to the increasing temperature. Our observation agrees with that of Zhang *et al.* (2011), who reported that when the temperature rose to 70 °C, the number of species within the compost samples dropped quickly.

From the bacterial composition, it is obvious that a high structural resistance was maintained between day 0 and day 10 due to the relative steadiness of the bacterial composition, which is interesting because co-composting is a highly changeable microbial-influenced process. The dynamics of the co-composting process were most pronounced at the thermophilic stage, a point in the process resembling an ecological bottleneck where the changes in magnitude of temperature should dramatically affect the microbial composition of the mesophilic stage (Covino *et al.* 2016). Changes in the abundance of a specific bacterium may be explained by changes in temperature, which could be pro/against the bacterium's favored temperature for reproduction and growth. The fact that there is no significant similarity in the bacterial communities of day 14 and other days indicates that bacterial composition was influenced by the decrease in temperature during the maturation stage so that to maintain an effective PCDD/F degradation process, temperature of the co-composting process should be well-controlled.

The fact that the co-composting process was dominated by certain phyla, including two gram-positive (*Firmicutes* and *Actinobacteria*) and two gram-negative (*Proteobacteria* and *Bacteroidetes*), was also observed by other similar PCDD/F-degradation studies (Yoshida *et al.* 2005, Kaiya *et al.* 2012). *Firmicutes*, which are predominant at the thermophilic stage in the co-composting process, have been reported to play a role in the dichlorination of PCDD/Fs in river sediment (Yoshida *et al.* 2005). These chemoheterotrophs have also been listed among the bacterial groups that are able to degrade polycyclic aromatic hydrocarbons (PAHs) (Ghosal *et al.*

2016).

*Bacillus* was the most thermophilic abundant phylotype (thermophile) at the genus level. NGS (454 pyrosequencing) analysis of bacterial communities during the co-composting degradation of PAH-contaminated wood revealed that Firmicutes (class *Bacilli*) were involved in the PAH degradation (Covino *et al.* 2016). Other two abundant thermophiles of *Firmicutes*, namely *Bacillaceae* and *Lactobacillus*, could also contribute to PCDD/F degradation. *Bacillaceae* have been detected in degradation of studies involving PAH and heavy oil-contaminated soils (Wu *et al.* 2008, Lladó *et al.* 2012). In the study by Abou-Arab *et al.* (2010), *Lactobacillus bulgaricus* helped achieve a high PAH degradation rate (92%) after 72-hour incubation. *Lactobacillus sakei* have also been found able to degrade PAHs and polychlorinated biphenyls (PCBs) in other recent studies (Polak *et al.* 2016, Bartkiene *et al.* 2017). However, their roles and ability to degrade PCDD/Fs have not been discussed in the literature. Since the high PCDD/F degradation rate in the first 14 days of the co-composting treatment should mostly be contributed by these thermophiles, their roles in the degradation of PCDD/Fs should therefore be examined in detail in future studies.

Firmicutes together with *Euryarchaeota* and *Proteobacteria* have been reported to be the major phyla (over 30% of sequence reads by pyrosequencing) in the methanogenic biodegradation of two-ringed PAHs (Berdugo-Clavijo *et al.* 2012). *Firmicutes* along with *Proteobacteria* and *Bacteroidetes* have been observed to be dominant phyla in the PCDD/F degradation study by Yoshida *et al.* (2005), which employed the denaturing gel gradient electrophoresis (DGGE) method for the analysis of bacterial composition. The result of that study was highly positive with an insignificant number of less-chlorinated congeners produced as intermediate or end products. PCDD/F degrading isolates, which are members of the phyla *Firmicutes*, *Actinobacteria* and *Proteobacteria*, were able to use naphthalene as a sole carbon and

energy source and have been found to reduce dibenzofuran through both metabolic and co-metabolic mechanisms (Kaiya *et al.* 2012). The ability of *Firmicutes* to degrade oil-contaminated soil has been reported by Yang *et al.* (2014), who investigated bacterial composition of *Firmicutes*, *Actinobacteria* and *Proteobacteria* in simulated contaminated deep layer and upper permafrost.

*Pseudoxanthomonas* sp. of the phylum *Proteobacteria* are well-known degraders of recalcitrant compounds, for instance PAH, BTEX, TNT, and many other organic contaminants (Nayak *et al.* 2011, Llado *et al.* 2015). In this study, the phylotype of *Pseudomonas* was detected at an abundance level of 10% on day 14, agreeing with observations of other similar studies (Lin *et al.* 2012, Chen *et al.* 2015, Covino *et al.* 2016). *Pseudomonas* spp. are widely recognized recalcitrant-pollutant-degrading bacteria able to be used by a wide range of organic contaminants as their sole carbon source (Loick *et al.* 2009). Also, in this study, the phylotype of *Sphingobacterium* (*Bacteroidetes*) was detected at an abundance level of 8%. *Sphingobacterium* sp. is reported to be an effective degrader of phenanthrene (Mohd-Kamil *et al.* 2014), flourene (Nam *et al.* 2015), PCBs (Leigh *et al.* 2006), insecticide (Cai *et al.* 2015), etc. *Actinobacteria* are popular colonizers in composting studies and nocardioform strains of this phylum such as *Mycobacterium* and *Rhodococcus* have been documented as playing a role in the degradation of organic contaminants (Antizar-Ladislao *et al.* 2008). *Actinomycete Thermomonospora* has also been successfully employed to enhance the degradation of creosote according to Ghaly *et al.* (2012) who did a composting study. With the presence of numerous organic compound degraders identified by NGS, the degradation of PCDD/Fs in highly contaminated field soil is justified. However, the specific roles of each bacterial strain at each stage of the co-composting process require further studies.

## 5. Conclusions

The present study demonstrates the feasibility of food waste co-composting degradation of highly PCDD/F-contaminated field soil. PCDD/F degradation followed first-order kinetics and the final TEQ after 42-day co-composting and follow-up MNA was 1,916 ng-TEQ kg<sup>-1</sup> dry weight (approximately 75% less than the initial TEQ), equal to the removal rate of 136.33 ng-TEQ kg<sup>-1</sup> day<sup>-1</sup>. Bacterial catabolic activity was analyzed using NGS. *Proteobacteria*, *Actinobacteria*, *Bacteroidetes* and *Firmicutes* proved to be the dominant phyla. At the genus level, the phylotype *Bacillus* (*Firmicutes*) was the most dominant species. However, further studies are needed to develop more detailed understanding of the specific roles of each bacterial strain. This especially refers to the thermophiles which contributed to high degradation rates within the first 14 days of the co-composting treatment.

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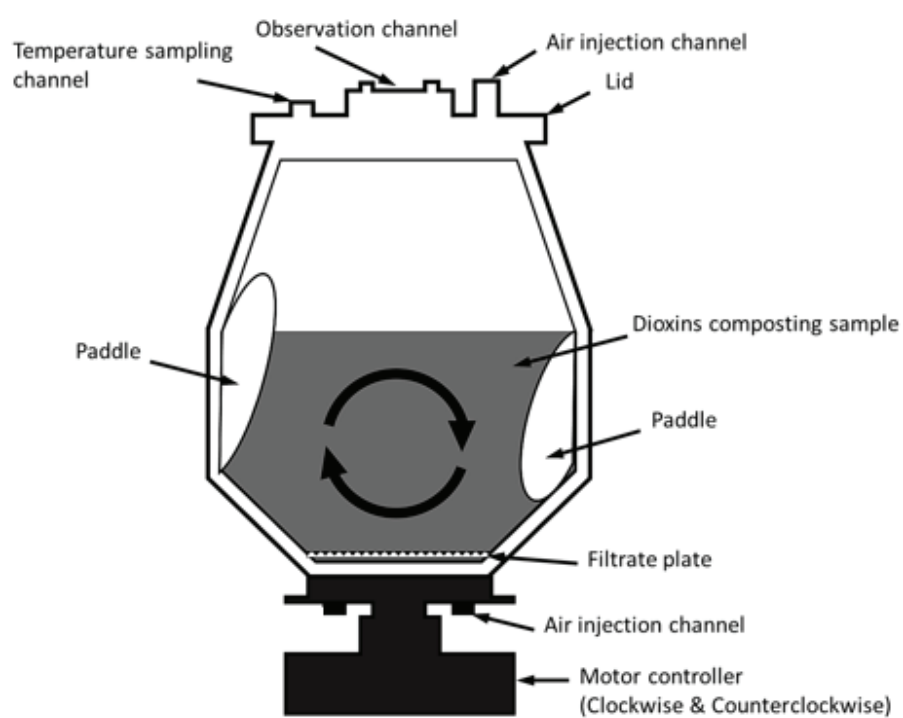
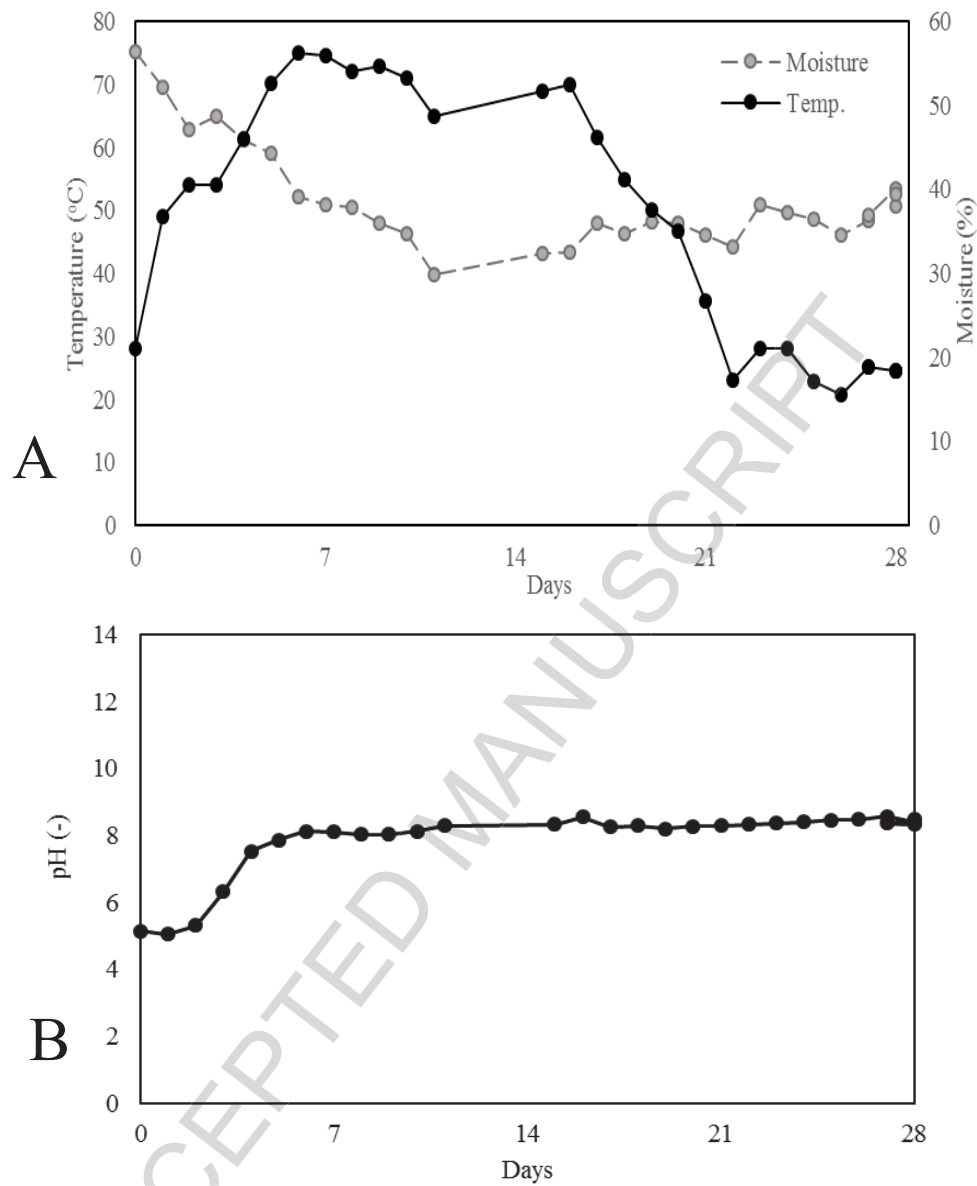


Fig. 1 Design of aerobic co-composting reactor for the degradation of dioxin-contaminated soil



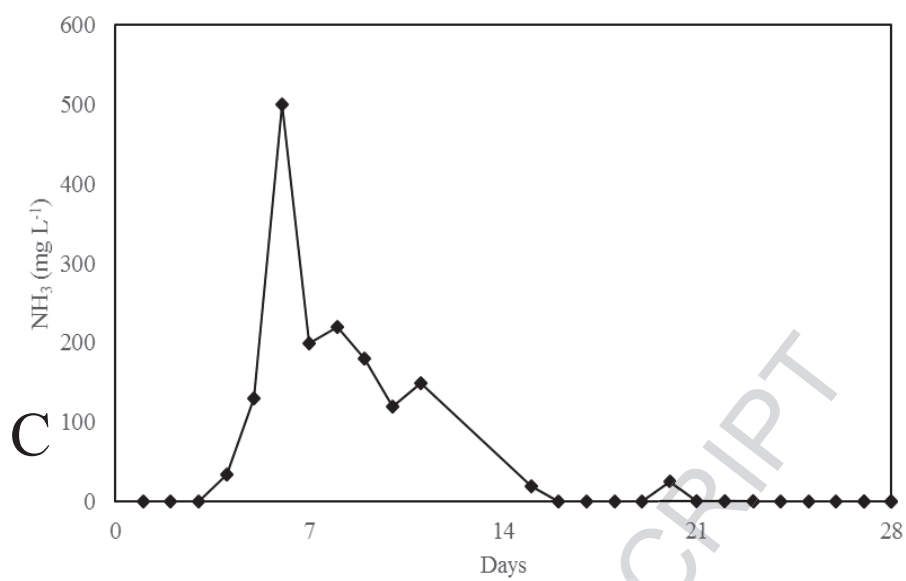


Fig. 2 Profiles of (A) temperature and moisture; (B) pH and (C) gaseous ammonia of the co-composting degradation of dioxin-contaminated soil

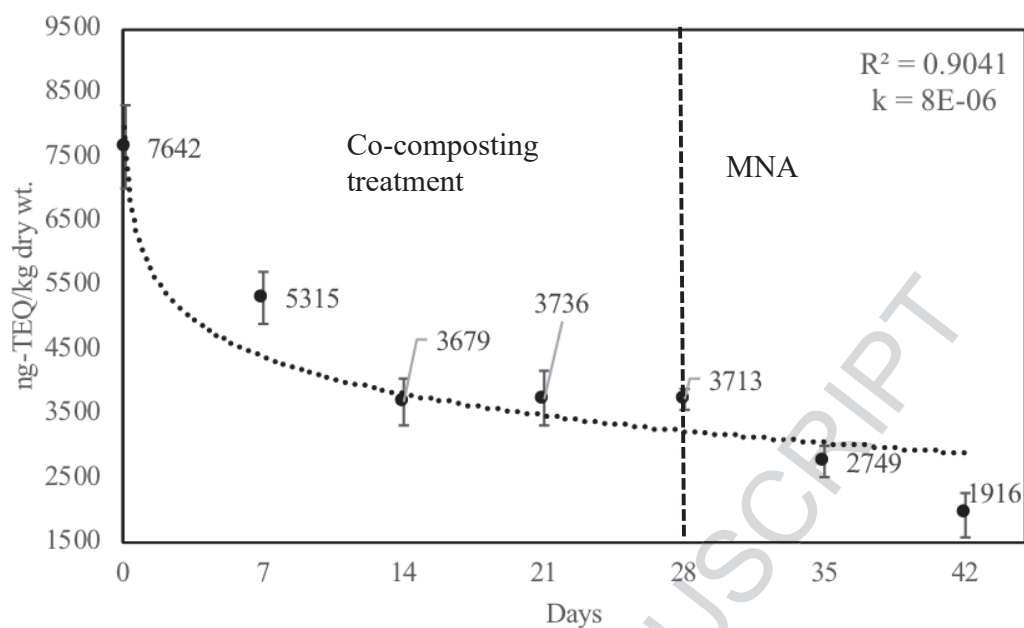


Fig. 3 Dioxin degradation of co-composted dioxin-contaminated soil [Figure was drawn based on second-order kinetics. Co-composting finished after 28 days when nutrient was totally used up and bacterial activity stopped functioning. Monitored natural attenuation (MNA) was followed up to 42 days.]

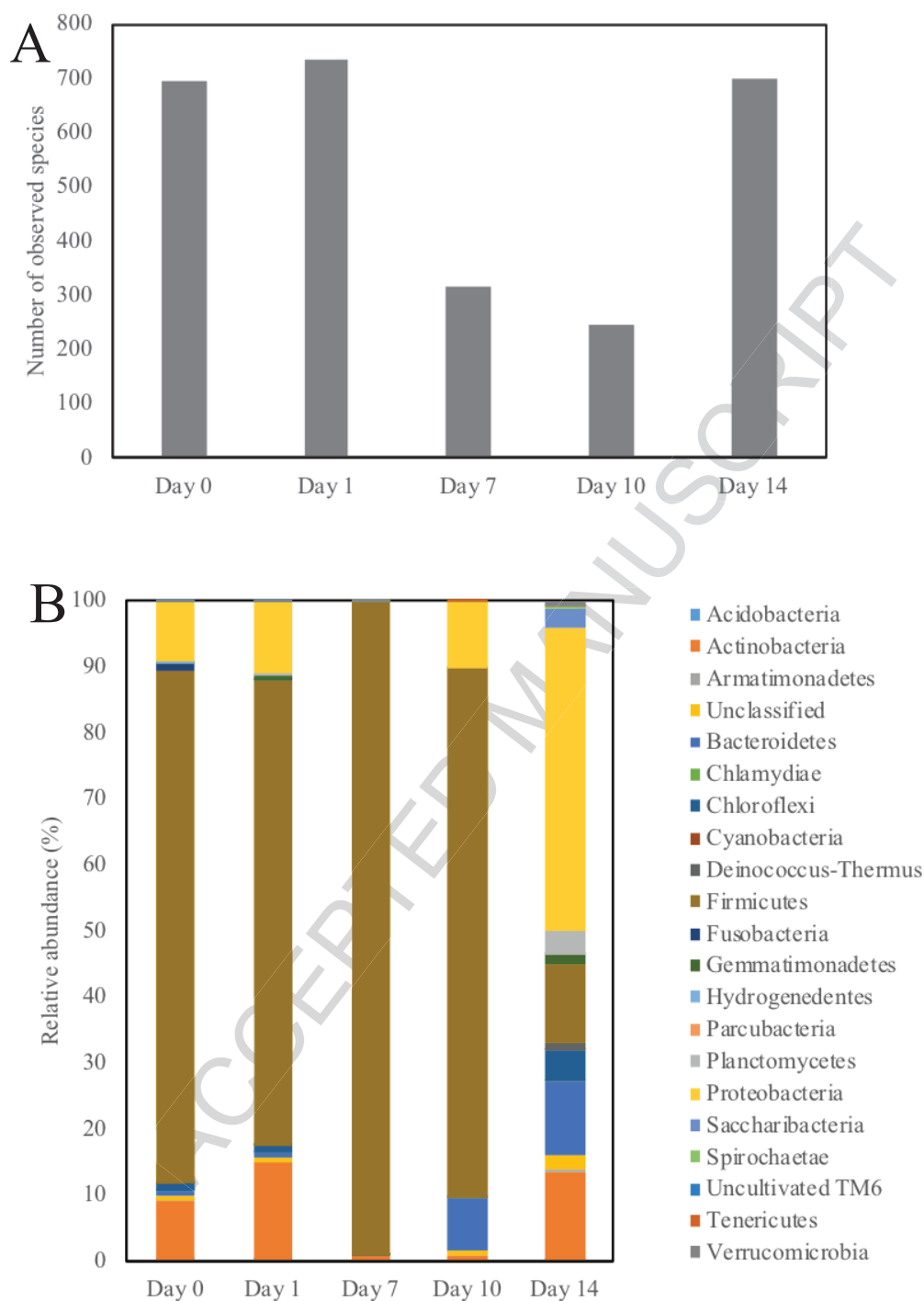


Fig. 4 (A) Changes in the number of observed species (B) Structure of bacterial composition of the co-composting degradation of dioxin-contaminated soil