RESEARCH ARTICLE

Remediation of polycyclic aromatic hydrocarbon (PAH) contaminated soil through composting with fresh organic wastes

**Yuan Zhang · Yong-Guan Zhu · Sabine Houot ·
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Abstract
Introduction Composting may enhance bioremediation of PAH-contaminated soils by providing organic substrates that stimulate the growth of potential microbial degraders. However, the influence of added organic matter (OM) together with the microbial activities on the dissipation of PAHs has not yet been fully assessed.
Materials and methods An in-vessel composting-bioremediation experiment of a contaminated soil amended with fresh wastes was carried out. Four different experimental conditions were tested in triplicate during 60 days using laboratory-scale reactors: treatment S (100% soil), W (100% wastes), SW (soil/waste mixture), and SWB (soil/waste mixture with inoculation of degrading microorganisms).
Results and discussion A dry mass loss of $35\pm5\%$ was observed in treatments with organic wastes during composting in all the treatments except treatment S. The dissipation of the 16 USEPA-listed PAHs was largely enhanced from no significant change to $50.5\pm14.8\%$ (for SW)/$63.7\pm10.0\%$ (for SWB). More obvious dissipation was observed when fresh wastes were added at the beginning of composting to the contaminated soil, without significant difference between the inoculated and non-inoculated treatments. Phospholipid fatty acid (PLFA) profiling showed that fungi and G-bacteria dominated at the beginning of experiment and were probably involved in PAH dissipation. Subsequently, greater relative abundances of G+bacteria were observed as PAH dissipation slowed down.
Conclusions The results suggest that improving the composting process with optimal organic compositions may be a feasible remediation strategy in PAH-contaminated soils through stimulation of active microbial populations.

Keywords Composting · Contaminated soil · PAHs · Bioremediation · Microbial communities · PLFAs

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1 Introduction

Polycyclic aromatic hydrocarbons (PAHs) are one of the main contaminant classes in soils of former coal gasification sites, tar oil distillation plants, or wood-preserving industries (Wischmann and Steinhart 1997). Such highly polluted soils lead to ecotoxicological risks and have to be cleaned up. Various remediation, and in particular bioremediation procedures, are available (Alexander 1999; Wilson and Jones 1993). Since PAHs are hydrophobic compounds with low solubility in water, they have a great tendency to bind to organic matter or soil, limiting their availability to microorganisms (Volkering et al. 1992; Pignatello and Xing 1996; Carmichael et al. 1997; Zhang et al. 1998). The addition of organic materials can enhance biodegradation by improving soil texture and oxygen transfer and by providing energy sources to rapidly establish a

large microbial population (Englert et al. 1993). Several studies have shown that the degradation of PAHs in contaminated soils is significantly higher after addition of compost (Kästner et al. 1994; Wischmann and Steinhart 1997). Kästner and Mahro (1996) found that adding compost enhanced the removal of soil-associated PAHs and suggested that the presence of microorganisms capable of degrading natural humic substances were responsible for the co-metabolic degradation of the PAHs. Moreover, microbial activity has been shown to form bound residues in soil–compost system (Kästner et al. 1999). PAH dissipation has been frequently observed during composting of various contaminated substrates including sewage sludge (Cai et al. 2007) and organic wastes (Brändli et al. 2005). The composting of contaminated soils has also been demonstrated to enhance the biodegradation of PAHs using both conventional windrow composting systems (Amir et al. 2005; Atagana 2004) and in-vessel composting systems (Antizar-Ladislao et al. 2006; Sasek et al. 2003). In-vessel composting techniques are easier to operate than conventional composting systems as temperature, moisture content, ventilation, etc. can all be controlled. Composting after addition of fresh green wastes would appear to be more efficient than the addition of mature compost for decreasing PAH concentrations in contaminated soil (Antizar-Ladislao et al. 2006).

In the field of bioremediation, most studies have applied final composting products, and these studies have established the fate of PAHs (Cai et al. 2007) and the effect of fungal–bacterial culture on PAH degradation (Boonchan et al. 2000). However, few investigations have focused on the effect of fresh organic matter addition and microbial inoculation on PAH dissipation during composting of contaminated soil. Only a few reports have described the degradation of contaminants in soil after addition of complex organic materials such as composts (Sims et al. 1990). Research on biodegradation has demonstrated the potential of white-rot fungi to degrade PAH (Canet et al. 1999), and using the PLFA as a biomarker, it has been found that the Gram-negative bacteria are more sensitive to PAHs than Gram-positive bacteria or fungi (Yang et al. 2007).

Therefore, our objective was to study how the addition of fresh waste mixtures might enhance PAH dissipation in a contaminated soil during composting in an in-vessel composting system and to identify the relationship between PAH dissipation and the evolution of organic matter characteristics, PAH retention, and changes in microbial community structure.

2 Materials and methods

2.1 Experimental design

Four experimental conditions were tested in triplicate during 60 days using laboratory-scale reactors. Treatments S with 100% contaminated soil and W with 100% waste mixtures were considered as control treatments. In treatment SW, the contaminated soil was blended with the mixture of wastes (see below) with an optimal ratio of 0.8:1 (dry wt. soil/dry wt. wastes) (Antizar-Ladislao et al. 2005). The treatment SWB was similar to SW with the addition of two PAH-degrading microorganisms (*Bacillus* sp. and *Fusarium* sp.).

Treatments S (control), SW, and SWB aimed at studying the degradation of PAHs in the soil during the composting process. The three treatments were conducted with the same amounts of soil. The treatment W was used to study the decomposition of waste organic matter (OM) during composting.

2.2 Contaminated soil

The contaminated soil was collected from a site located in Beijing (39.85°N, 116.54°E) which was highly polluted by the coal industry. The soil was sieved to 2 mm and then air-dried before analysis. The soil was a loamy-sand with 12.4% clay, 33.2% silt, and 54.4% sand. The initial total concentration of 16 USEPA PAHs ($\sum_{PAHs}$) was 69.7 ± 2.2 mg kg^{-1} soil (on dry wt. basis). The initial carbon content of contaminated soil was $1.5 \pm 0.3\%$ (grams per 100 g dry wt.).

2.3 Mixture of wastes

The mixture of wastes included leaves, branches, and bio-wastes consisting of green vegetables such as cabbage, spinach, and celery (60%, 28%, and 12% of total mixture on dry matter basis, respectively). All waste materials were crushed to 2 mm before mixing. The initial C/N ratio of a composting mixture should range between 25 and 40 to provide suitable composting conditions. Therefore, the initial proportions in the waste mixture were calculated to get an initial C/N ratio of 29.3 in the initial mixture.

The initial concentration of $\sum$ 16 USEPA listed PAHs in the mixture of wastes was 5.0 ± 0.2 mg kg^{-1} dry wt. The initial content of total carbon content in the mixture of wastes was $42.8 \pm 0.2\%$ (g 100 g^{-1} dry wt.$^{-1}$).

2.4 Initial soil/waste mixtures

The mixture of wastes was added to the contaminated soil with a ratio of 0.8:1 (dry wt. soil/dry wt. wastes) as used in Antizar-Ladislao et al. (2005). The TC content in the initial mixture was $24.5 \pm 0.7\%$. The C/N ratio of the initial soil/waste mixture was 25.6. The initial total concentration of 16 USEPA PAHs ($\sum_{PAHs}$) was 75.95 ± 2.4 mg kg^{-1} dry wt. of soil (33.75 mg kg^{-1} dry wt. of mixture). The $\sum_{PAHs}$ concentration

was higher than that in contaminated soil because PAHs were also detected in the mixture of fresh wastes.

2.5 Specific added microorganisms

Bacillus sp., a Gram-positive rod-shaped bacterium, and *Fusarium* sp., a filamentous fungus widely distributed in soil and in association with plants, were isolated from the contaminated soil and cultured at 30°C and 175 rpm in basal salt–glucose medium supplemented with pyrene (50 mg L^{-1}) before application to the soil–waste mixture of the SWB treatment. The population density was determined before inoculation using the dilution and spread plate method. The initial concentration of the two microbial populations (*Bacillus* sp. and *Fusarium* sp.) were 1×10^8 and 4.8×10^8 (colony forming units, CFU/ml), respectively.

Bacillus sp. was chosen because it represents a genus of bacteria that can survive during the thermophilic phase of composting. The *Fusarium* sp. offers other advantages: (1) most filamentous fungi are adapted to contaminated environments allowing the inoculum to survive and (2) *Fusarium* sp., preferentially degrades the high-molecular-weight PAHs with 5- and 6-rings (Olivier et al. 2004).

2.6 Bioreactors

Beakers with a volume of 1 L (diameter of 106 mm and a height of 150 mm) were used (see details in Fig. 1) and placed in water-bathes to control the temperature. Each treatment was run in triplicate in three separate beakers. During composting, the optimal aeration during the active phrase varied from 3 Lh^{-1} kg^{-1} dry wt. (Lau et al. 1992; Sadaka and Taweel 2003) to 150 Lh^{-1} kg^{-1} dry wt. (Mathur et al. 1993). The used aeration rate decreased during composting from 12–25 Lh^{-1} kg^{-1} dry wt. at the beginning to 3–6 Lh^{-1} kg^{-1} dry wt. at the end as proposed by Francou et al. (2008).

2.7 Temperature and moisture

The simulation of composting at a small scale is not obvious since the mass of biodegradable organic matter put into the reactors is not large enough to ensure a thermal inertia leading to thermophilic conditions and to maintain microbial activity over a long period. Bioreactors were placed in temperature-controlled water-bathes to simulate representative thermal stages during in-vessel composting processes. The variation temperature profile for the time periods days 0–14 at 38°C, days 15–35 at 55°C, days 36–42 at 70°C, and days 43–60 at 38°C was chosen according to Antizar-Ladislao et al. (2005). These conditions simulated the operation of a commercial in-vessel operating system by including the minimum thermophilic temperature of 70°C required for pathogen control (Antizar et al. 2007). The optimal moisture content during composting generally varies between 50% and 80% of total wet mass (Willson 1989; Richard et al. 2002). The moisture content (100× mass of water/mass of wet mixture) in treatments W, SW, and SWB were maintained to 50% by adding water into the bioreactors after each sampling event. In the control treatment S, a moisture content of 50% made the contaminated soil too wet to be in aerobic condition, and a moisture content of 15% in treatment S was chosen within the maximum water content of soil. Li et al. (2006) also found that submerged conditions can inhibit the degradation of B[a] P in soil.

2.8 Sample collection

With the exception of the beginning and the end of composting (day 0 and 60), sampling occurred immediately prior to each change of temperature at days 14, 35, and 42. At every sampling date, the beakers were emptied, mixed thoroughly, and sub-sampled in triplicate. The sub-samples with about 20 g (wet weight) per sub-

Fig. 1 Design of bioreactor (volume of 1 L)
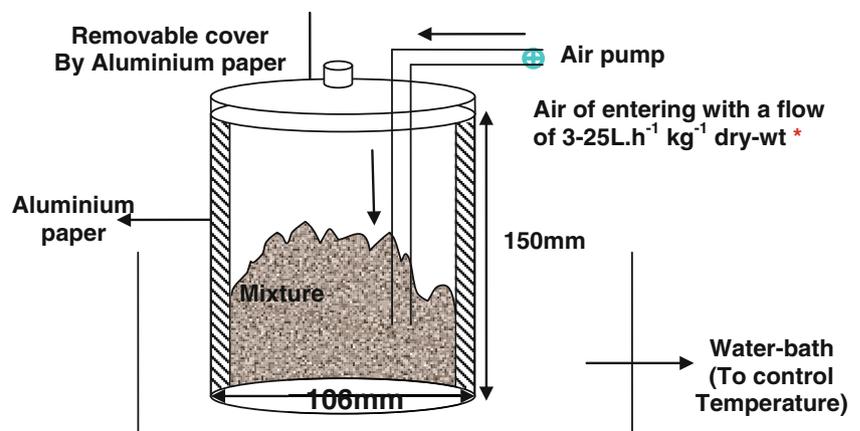
sample were frozen at −80°C for the analysis of total organic matter (TOM), PAHs, phospholipid fatty acid (PLFA), and adsorption coefficient (Kd) of FLT and PHE on soil and on soil/waste mixture.

2.9 Organic matter analysis

The samples were dried in an oven at 105°C to obtain the dry weight. The evolution of biochemical fractions was measured using the Van Soest method (Van Soest and Wine 1967), but just for the treatment W because of the limits of the Van Soest method. Successive extractions of soluble in hot water (H_2O), soluble substances in neutral detergent (SOL), hemicellulose-like (HEM), cellulose-like (CEL) and lignin and cutin-like (LIC) fractions were carried out as described in the French XPU 44–162 standard (AFNOR 2005).

2.10 PAHs analysis

The PAHs were extracted from 4 g samples which were dried by lyophilization and then weighed in triplicate into the pressure tubes used for accelerated solvent extractor (Dionex ASE-300). The solvent was a dichloromethane/acetone (1:1) mixture. The sample and solvent were heated to 100°C and 10.35 MPa pressure using the standard ASE program. The extracts were purified on chromatographic columns packed with 12 cm SiO_2 (100/200 mesh; conditioned at 180°C during 12 h before use), 6 cm Al_2O_3 (100/200 mesh; 250°C; 12 h), and 1 cm Na_2SO_4 (450°C; 4 h). In order to remove hydrophobic impurities, the columns were previously washed with 10 ml hexane, then 5 ml of extracts were eluted, and the columns were left to dry for 1 min. The PAHs were then eluted with 70 ml solvent (hexane: dichloromethane, 7:3 v/v). The extracts were concentrated to 1 mL by flux of N_2.

PAHs were analyzed using a GC-MS Chemstation (6890 N/5975 C, Agilent, USA) using a HP-5MS column (19091S-433, 30 m×0.25 mm×0.25 μm). The injection volume was 1 μL. The temperature program was 50°C for 2 min, 20°C min^{-1} to 200°C, which was maintained for 2 min, 4.5°C min^{-1} to 240°C which was maintained for 2 min and then 2.5°C min^{-1} to 290°C. The MS was operated in selective ion monitoring (SIM) mode according to chemical ionization.

The 16 PAHs were grouped into three groups, according to the number of rings: small PAHs with 2- and 3-ring (naphthalene, acenaphthylene, acenaphthene, fluorine, anthracene); medium PAHs with 4-ring (fluoranthene, pyrene, benzo[a]anthracene, chrysene), and large PAHs with 5- and 6-ring (benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, ideno[1,2,3-c,d]pyrene, dipbenzo[a,h]anthracene, benzo[g,h,i]perylene).

2.11 PAH sorption

The sorption of PAHs was measured on fresh samples of the feedstock materials, the initial mixtures, the homogenized soil/waste mixture at each sampling event, and the initial contaminated soil. Two PAHs were used: fluoranthene (FLT) and phenanthrene (PHE). Average sample weight of soil/waste mixture was 1.0 g (soil)/0.06 g (wastes)±0.003 g dry wt., 1.0 g dry wt. for waste, and 3.0±0.003 g dry wt. for the soil samples. Each measurement was done in triplicate. The equivalent PAH concentration in soil/waste mixture samples (expressed in milligram per kilogram dry wt.) was 1.95 and 1.98 for FLT and PHE. Volumes of 9, 15, or 15 ml of ^{14}C-PAH solution (methanol and alkaline solvents) prepared for the soil/waste mixture, wastes or soil sorption experiments, respectively, were added to the samples in glass corex tubes which were then shaken for 24 h at 20°C and then centrifuged for 10 min at 1,644 g. The final concentration of ^{14}C-PAH in the supernatants was determined by liquid scintillation counting (Tri-Carb 2100 TR, Perkin Elmer Ins., Courtabeuf, France) using Ultima Gold XR (Packard) as scintillation cocktail. The amount of ^{14}C-PAH sorbed on the soil/waste mixture samples or soil sample (Q_s in milligram per kilogram dry wt.) was calculated from the difference in concentration between the initial PAH solution and the corresponding centrifuged supernatant after 24 h of equilibration. The sorption coefficients Kd (in liters per kilogram dry wt.) were determined as $Kd = Q_s Q_w^{-1}$ where Q_w (in milligrams per liter) was the PAH concentration in supernatant solution after equilibration.
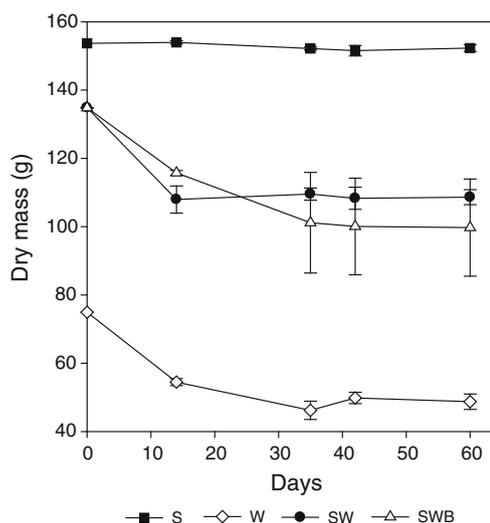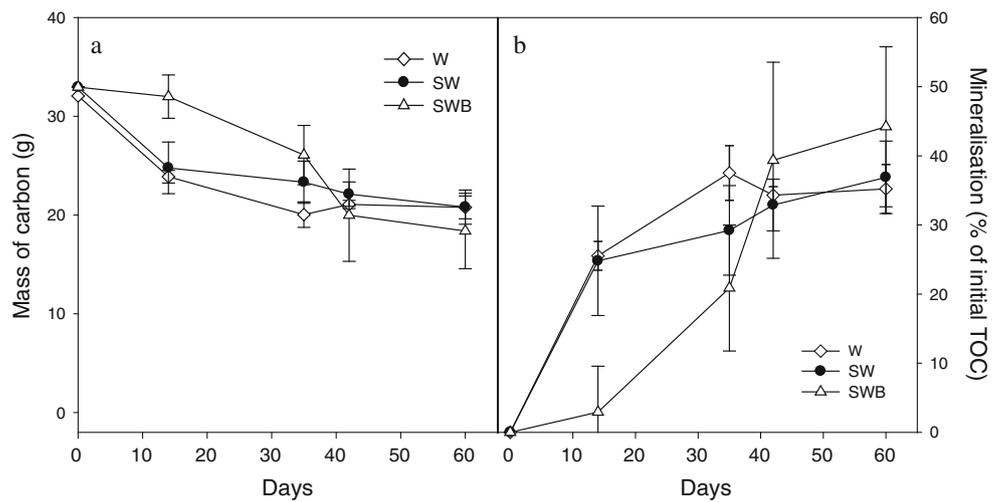

Fig. 2 Evolution of dry mass during composting for the different treatments (S, W, SW, and SWB)

Fig. 3 Loss of carbon mass (Fig. 3a) and percentage of total initial organic carbon mineralization calculated according to the loss of carbon mass (Fig. 3b)


2.12 PLFA analysis

The lipids were extracted and stored under an atmosphere of N_2. Soil and composting material lipids were extracted on 4 g samples in 30 ml of chloroform/methanol/buffer (1:2:0.8 $v/v/v$) by shaking for at least 2 h (biofilm) or overnight (soil and composting materials) at 21°C. The chloroform phase was reduced by evaporation. Fatty acid methyl esters were quantified and identified by mass spectral comparison using a MS Chemstation (G1034C Version C.02.00) fitted with a HP-5MS column (19091S-433, 30 m×0.25 mm×0.25 μm). The injection volume was 1 μL. The initial oven temperature was set at 120°C for 5 min and then increased at a rate of 20°C min^{-1} to 180°C

followed by a 4°C min^{-1} increase to 240°C. The MS was operated in selective ion monitoring (SIM) mode. Fatty acid nomenclature used was that described by Frostegård et al. (1993). Mono-unsaturated and cyclopropyl fatty acids were deemed to be Gram-negative bacterial (G−) biomarkers (Zelles 1999), iso- and anteiso-fatty acids Gram-positive bacteria (G+) biomarkers (Zelles et al. 1995), C18:2(9,12), a fungal biomarker (Frostegård et al. 1993), and carboxylic acids with a methyl function on the carbon chain were treated as biomarkers of actinobacteria (Zelles and Bai 1994). The ratio of cyc-C17:0 to C16:1(7) *cis*, the *trans*-to-*cis* ratio, and the *i:a* ratio were calculated as stress indicators (Navarrete et al. 2000). The ratio of fungal-PLFA to bacterial-PLFAs (as specified above) was

Fig. 4 Changes of the biochemical fractions during composting in the W treatment (100% wastes) using the Van Soest method
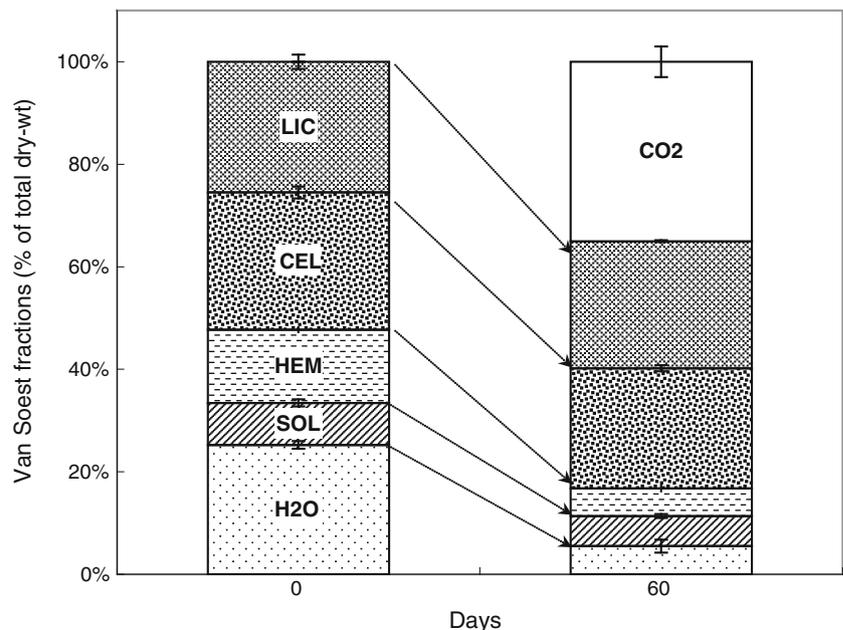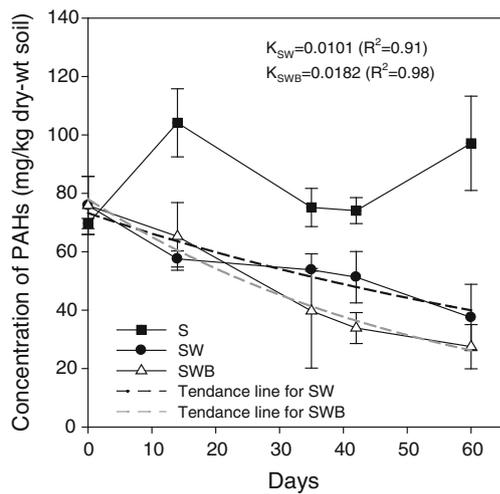
Fig. 5 Evolution of total PAHs during composting (mean±standard deviation) in the different treatments (S, W, SW, and SWB); first-order adjusted kinetics of dissipation in the SW and SWB treatments

used as an estimate of the relative importance of the bacterial and fungal energy channels (Parekh and Bardgett 2002). The fungal to bacterial PLFA and G+/G− bacterial ratios are considered as indicators of microbial community changes during in-vessel composting (Antizar-Ladislao et al. 2007).

2.13 Statistical analysis

Statistical procedures used to describe and evaluate the results were appropriate for the data of our study. The differences between treatments were tested by one-way analysis of variance using SAS (Version 8.1). The paired groups were evaluated using Burtlett's test. The comparison

method Fisher's LSD with a significance level of 0.05 was used to test the significance of pairwise differences.

3 Results and discussion

3.1 Dry mass

The soil dry mass in treatment S remained constant during composting while dry mass losses were observed in the treatments W, SW, and SWB (Fig. 2). This occurred mainly during the first 14 days. A mean dry mass loss of 34.9± 4.6%, 19.4±2.0%, and 26.0±14.3% was found in treatments W, SW, and SWB, respectively, after 2 months of composting. The dry mass loss in treatment W (34.9±4.6%) was close to that previously observed during the composting of other organic wastes (Sánchez-Monedero et al. 1999).

The mineralization of total carbon organic (TOC) into CO_2 explained TOC loss which was calculated according to the dry mass loss during composting (Fig. 3a). The carbon loss represented 35.2±3.2%, 36.9±5.2%, and 44.2±11.6% for treatments W, SW, and SWB, respectively. The mineralization of TOC was calculated according to the carbon loss (see details in Fig. 3b). Significant differences were found between treatment W and SW/SWB ($P<0.05$), while no significant differences could be found between treatments ($P>0.05$). These results indicated that the influence of added degrading bacteria on the decomposition of organic matters was not noticeable.

3.2 Organic matter analysis

The evolution of Van Soest fractions of OM during the incubation is portrayed for the W treatment in Fig. 4. The
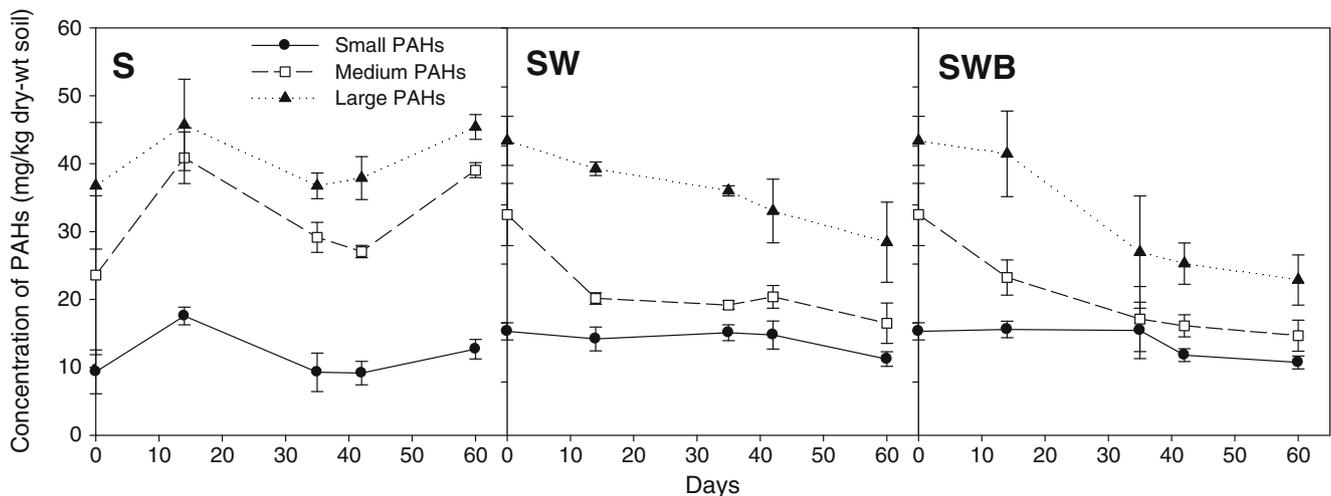
Fig. 6 Evolution of small (2- and 3-rings), medium (4-rings), and large (5- and 6-rings) PAHs concentrations in soil during composting

Table 1 Small, medium, and large PAHs removal percentage for treatment SW and SWB

Treatment	PAHs removal (%)			
	Small PAHs	Medium PAHs	Large PAHs	Total 16 USEPA PAHs
SW	55±14	64.9±12.0	42±17	50.5±14.8
SWB	61.3±12.7	72.3±9.3	57.7±10.4	63.7±10.0
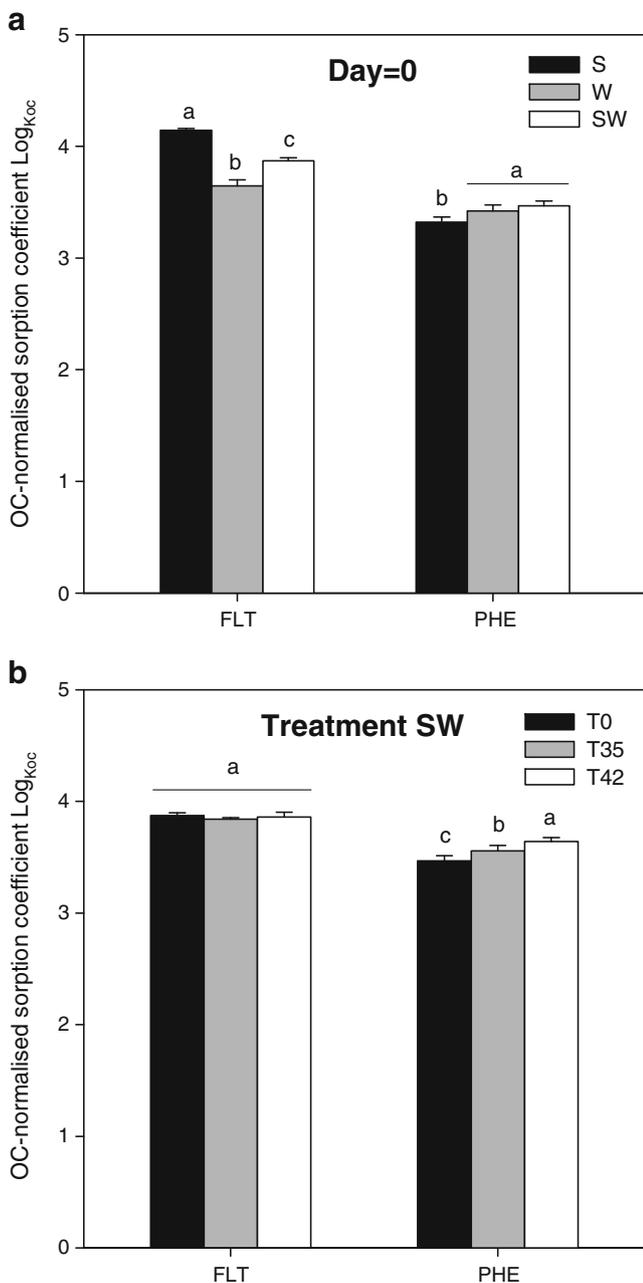
Fig. 7 a Organic carbon-normalized sorption coefficient of FLT and PHE in initial soil, waste, and soil–waste mixtures; **b** evolution of organic carbon-normalized sorption coefficient during composting in the soil–waste mixture (SW treatment). *Different letters* (*a*, *b*, and *c*) *within rows* are significantly different ($P<0.05$) according to one way-ANOVA analysis
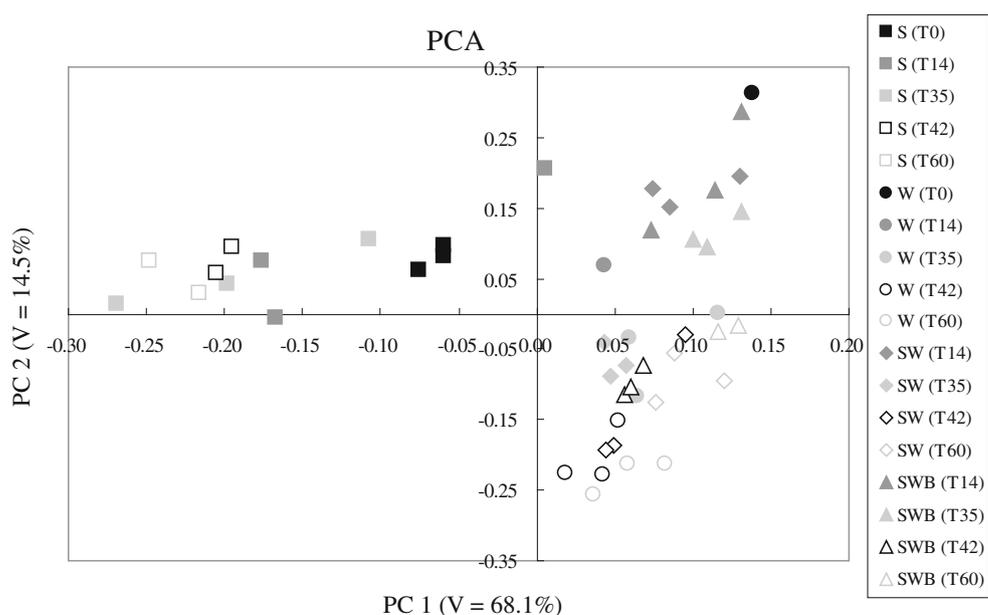
mineralization fraction CO_2 was calculated by using the dry mass balance, since the sum of all the organic fractions (SOL, H_2O, HEM, CEL, LIC, and CO_2) represented 100% of the initial total dry mass (see details in Fig. 4). The proportion of HEM and H_2O fractions decreased while LIC, CEL, and SOL fractions remained constant. These results have generally been observed in other composting studies (Doublet et al. 2010; Francou et al. 2008) with the exception of the CEL fraction that generally decreases during composting. However, its degradation varies with the substrate of origin because of its spatial interaction with lignin (Doublet et al. 2010). The compost made from leafs may contribute to limit CEL degradation. The SOL fraction remained constant most likely because this pool was replenished by the decomposition of other organic matter pools at the same rate at which it was being degraded. The LIC fraction was always the most recalcitrant compartment for microbial degradation, and it changed least during the composting (Francou et al. 2008; Doublet et al. 2010). The H_2O fraction, which represented the easily degradable compartment, contributed most to the dry mass loss (about 19.7%). We assumed that the degradation of OM mixed with soil in SW and SWB treatments would follow a similar degradation pattern because at the end of the experiments, we found approximately the same amount of carbon lost (see Fig. 3).

3.3 PAHs

3.3.1 Degradation of PAHs

No significant degradation of PAHs was observed for treatment S during composting (Fig. 5), and the slight variations of concentrations observed were attributed to experimental variation of moisture content during the composting treatment that would potentially affect PAH concentrations (Antizar-Ladislao et al. 2005). The soil microflora was not able to degrade PAHs as observed by Kästner et al. (1999). On the other hand, total 16 USEPA PAH concentration decreased by 50.5±14.8% and 63.7±10.0% in treatments SW and SWB, respectively (Fig. 5), without significant difference between these two treatments ($P=0.564$). The results indicated that degrading bacteria had no obvious effect on the PAH dissipation. This

Fig. 8 Principal component analysis of PLFA data. PC1 and PC2 represents different dates of composting course. Principal component 1 (PC1) explained 68.1% of the variation, while PC2 explained 14.5%. (*Filled square*) Experimental data of treatment S; (*filled circle*) data of treatment W; (*filled diamond*): data of treatment SW; (*filled triangle*): data of treatment SWB. *Different colors* represented data with different date. *Black*=day 0; *deep gray*=day 14; *gray*=day 35; *black circle*=day 42, and *gray circle*=day 60

### 3.3.2 Evolution of PAH sorption during composting

In initial materials (S, SW, or W), the organic carbon-normalized sorption coefficient (expressed in Log K_{OC}) ranged from 3.4 to 4.1 (liters per kilogram dry wt.) for both PAHs, which confirmed the strong sorption properties of these pollutants (Fig. 7a). These values were similar to the value (4.6–4.8) found by He et al. (1995) in soil. Significant differences in Log K_{OC} were observed for FLT between initial materials of S and the other initial materials of SW and W ($P<0.05$). We found a value of 4.1 for S treatment and 3.5–3.7 for W and SW treatments. Farenhorst (2006) found also that the K_{OC} of pesticide was higher in soil with humified organic matter compared to soil mixed with fresh organic matter. No significant difference could be found in PHE between treatments.

During composting of SW, no difference has been observed for Log K_{OC} of FLT, while for PHE, a slight increase of Log K_{OC} was noted over the time but without significant differences (Fig. 7b). These values of Log K_{OC} for SW were in agreement with the range from 4.4 to 4.9 found by Zhang et al. (2010) who investigated the K_{OC} values between PHE and biochar-amended soils. These results showed that the organic matter decomposition was not long enough to lead to noticeable changes of reactivity with these PAHs.

3.4 PLFA profiling

The implementation of composting technology as a remediation method requires an understanding of the diversity and ecology of contaminant-degrading micro-

conclusion is supported by the research of Canet et al. (2001) who suggested that in soil amended by OM system, the use of the autochthonous microflora, with no introduction of foreign microorganisms, offered the greatest potential for PAH degradation. Other evidence has shown that in the experiments with soil from a contaminated site (Mahro et al. 1994), PAH degradation was most effectively stimulated after addition of compost in comparison to the addition of fertilizer or degrading bacteria.

First order kinetic equations were fitted to the kinetics of dissipation of PAHs, and the first order rate constants were 0.0102 day^{-1} and 0.0176 day^{-1} for treatments SW and SWB, respectively (Fig. 5), similar to the value (0.0160 day^{-1}, R^2=0.93) reported in Antizar-Ladislao et al. (2007).

The amount and rate of PAHs dissipation varied according to their molecular weights (Fig. 6). Larger dissipation rates were observed for all PAHs in the SWB treatment compared to the SW treatment, but differences were not significant ($P>0.05$). Detailed results of PAHs dissipation are shown in Table 1. The highest dissipation was observed for medium PAHs in both treatments. This was particularly evident for four-ring PAHs, which are normally less reactive and biodegradable than lighter PAHs (Joner et al. 2004). Generally, large PAHs were more persistent than small and medium PAHs. Since large PAHs have higher octanol–water partition coefficients and lower water solubility than medium and small PAHs (Antizar-Ladislao et al. 2005), thus, bioavailability (Carriere and Mesania 1995; Potter et al. 1999; Lee et al. 2001) and toxicity (Sverdrup et al. 2002) may have limited their biodegradation, resulting in their persistence.

organisms. It is for this reason that changes within the microbial community structure were investigated by PLFA profiling to detect the major changes in microbial community structure during composting. The first two ordination axes, representing 83% of the total variability in the data, are presented in Fig. 8, where principal component (PC1) characterized the stages of composting. Contaminated soil samples were significantly ($P<0.05$) separated from the waste samples and the soil and waste mixture samples along the first ordination axis. Inspection of the loadings
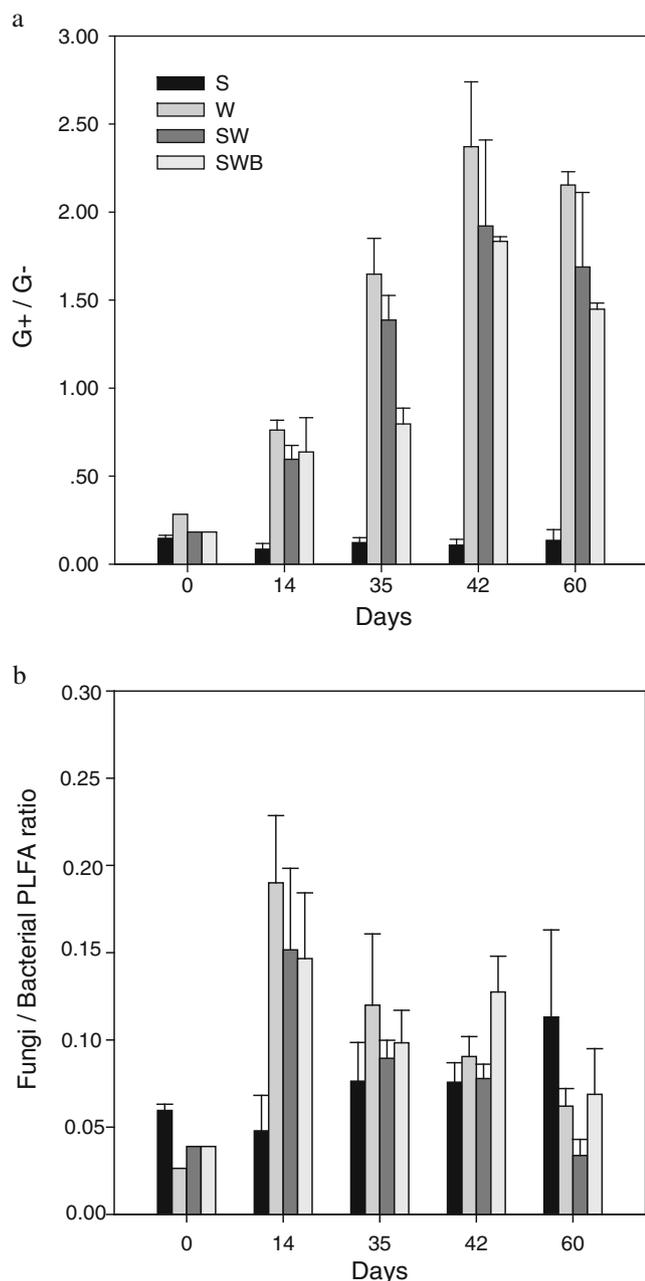
a

revealed that the difference between soil and other samples was primarily due to a higher relative abundance of fatty acids indicative of actinomycetes in the soil samples and higher relative abundance G+ bacterial biomarkers in the other samples. No significant difference was observed between the waste samples and the soil–waste mixtures, suggesting that the waste microbial communities dominated in the soil–waste mixture. Microbial community structure also varied significantly ($P<0.05$) with time in all treatments. Variation as a function of time was more pronounced in the contaminated soil than in the waste or soil–waste mixture, and it was apparent on the first ordination axis. It was due to an increase in the abundance of actinobacteria. The second ordination axis showed the variation as a function of time in the other treatments. The lipid profiles of these samples were initially dominated by G− bacterial and fungal biomarkers. As the incubation progressed, these treatments saw a distinct increase in the abundance of G+ bacteria (see Fig. 9a). It has been suggested that the G+ bacteria like actinomycete may play an important role in the mineralization of PAHs (Kästner et al. 1994; Larkin et al. 2005), and these are also thermophiles, meaning they are likely to be encountered at the higher temperatures (above 38°C) used during the variable temperature profile. Thus, a higher relative proportion of G+ was expected (Antizar-Ladislao et al. 2008).

According to Fig. 9b, the fungal to bacterial PLFA ratio was observed to be in the range of 0.03 to 0.19 which was within the range of 0.01–0.48 found by Antizar-Ladislao et al. (2007). The fungal to bacterial PLFA ratio in treatments SW and SWB displayed a large degree of variation compared to treatment S. The same trends with time were observed for both of these treatments with an increase of the ratio during the first 14 days and a steady decline thereafter. These results corroborate the results of Antizar-Ladislao et al. (2007), which indicated that an increase in temperature in the bioreactors from 38°C to 70°C reduced the fungal to bacterial PLFA ratio to low values.

4 Conclusion

In this study, the carbon loss was observed in all treatments W (100% of wastes), SW (soil:wastes=0.8:1 on dry wt.) and SWB (soil/wastes mixtures with a ratio of 0.8:1 and added by degrading microorganisms). The organic matter degradation occurred due to the decomposition of H_2O (soluble organic matter in hot water) and HEM (hemicellulose-like) fractions during the composting. The soluble fraction H_2O contributed most to the total organic matter loss. The composting of contaminated soil amended with fresh waste mixtures can reduce PAH concentrations within a 40th and 70th percentile range by a dissipation rate

b

Fig. 9 G+/G− (a) and fungal/bacterial PLFA ratio (b) in different treatments during composting process

varying from 0.0101 to 0.0182 day^{-1} for treatments SW and SWB after 60 days of continuous treatment following a variable temperature profile. No significant influence of degrading bacteria could be found on the dissipation of PAHs. The observation of microbial activity showed that these samples were initially dominated by G− bacterial and fungal biomarkers. Fungal biomarkers were particularly abundant during the first 14 days of composting when the PAHs dissipated most obviously. As was demonstrated by Canet et al. (1999), white-rot fungi have the potential to degrade PAH. These microorganisms naturally decompose lignin and cellulose inside wood fibers using a non-specific enzymatic complex, which also enables them to degrade a wide range of contaminants if they are introduced to soils. As the incubation progressed, these treatments showed a distinct increase in the abundance of G+ bacteria. Thus, using fresh green wastes as composting amendment could achieve a higher dissipation of PAHs than in non-amended soil due the organic nutrients provided for microbial communities. However, the active microbial communities in each composting phase have not yet been quantified and qualified in this study, and more research in this direction might be beneficial. The bioremediation in contaminated soil by composting showed a complex system. Therefore, in future studies, we will focus on modeling these connected processes in order to answer the following questions: how the PAHs are affected by the composting process with and without foreign degraders; which are the dominant microbial populations responsible for the PAH dissipation during the composting.

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