Impact of Organic Amendments on the Development of Phenanthrene Catabolism in Soil

Victor T. Omoni, Alfonso J. Lag-Brotons and Kirk T. Semple*

Lancaster Environment Centre, Lancaster University, Lancaster, LA1 4YQ, UK

* Corresponding Author: Email: k.semple@lancaster.ac.uk

21 **Abstract**

22 This study investigated the impact of spent brewery grains and spent mushroom
23 compost on the development of phenanthrene biodegradation in soil. Two aspects were
24 considered: (i) the influence of increasing waste-to-soil ratios (1:10, 1:5, 1:2, 1:1 & 2:1)
25 and (ii) the impact of soil-PAH contact time (1 – 100 d). Biodegradation was quantified
26 by measuring changes in the lag phase, the fastest rates and extents of mineralization of
27 ^{14}C-phenanthrene, as well as changes in the number of total heterotrophic and
28 phenanthrene degrading bacteria and fungi. The amendment of smaller amounts of the
29 wastes (1:10 & 1:5) resulted in greatest levels of biodegradation. Microbial numbers
30 increased in all of the amended soils but phenanthrene-degrading numbers in most
31 amended soils did not correlate with the rates and extents of ^{14}C-phenanthrene
32 mineralization. This investigation highlighted the value of waste organic materials as
33 nutrient sources to stimulate microbial degradation of contaminants in soil.

34

35 ***Keywords*** Spent brewery grains, spent mushroom compost**,** phenanthrene,
36 biodegradation, soil

37

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous contaminants of major concern in the environment, being potentially harmful to human health due to their carcinogenicity, teratogenicity and mutagenicity potential (European Commission DG Environment, 2013). They are produced primarily through three main processes: biogenic (or diagenetic), petrogenic and pyrogenic (Gennadiev et al., 2015). Both natural and anthropogenic sources can occur through petrogenic and pyrogenic processes.

Pyrogenic sources are formed from incomplete combustion of fossil fuels and biomass (petroleum, wood, coal and related products), which are the major routes of PAHs contamination in the environment compared to the natural (petrogenic) sources (Gennadiev et al., 2015). Thus, making these hydrophobic organic contaminants ubiquitous in the environment (Akpor et al., 2007), and recalcitrant, being composed by two or more fused aromatic rings (Ogbonnaya et al., 2017). Physico-chemical properties of PAHs such as molecular size, solid-liquid partition (Kd) and organic carbon-water partition coefficients (Koc) (Cerniglia, 1993), influence their biodegradability, persistence, and recalcitrance to microbial attack in the environment. In terms of mobility in the environment, properties such as hydrophobicity, lipophilicity, and low water solubility are also important (Semple *et al*., 2003).

Soils, sediments and other ubiquitous components such as microplastics can act as sinks for PAHs in the environment through sorption to soil mineral and organic fractions (Xiao *et al*., 2014; Fries and Zarfl, 2012; Lee et al., 2014). This influences the mobility, bioavailability and biodegradation and, as a result, may influence the persistence of these contaminants (Riding et al., 2013). Biodegradation is a major mechanism for the removal of organic contaminants in soils and is dependent on the interaction between

63 hydrocarbonoclastic microorganisms (hydrocarbon degraders) and their surrounding
64 environment; in particular, properties associated with the contaminants (concentration,
65 toxicity, mobility, bioavailability) and nutrient cycling (availability and degrading
66 enzyme presence) (Leahy and Colwell, 1990; Das and Chandran, 2011). Related to the
67 latter aspect, the efficiency of PAH biodegradation may be influenced by low nutrient
68 and organic carbon concentrations in contaminated soil (Zhang et al., 2012). In general
69 terms, the catabolic potential of microorganisms to detoxify and degrade hydrocarbons
70 depends largely on the amount of nutrients available for microbial metabolism
71 (Azubuike et al., 2016). Consequently, ensuring appropriate nutrient supply for
72 microorganism activity (catabolism) has been a recurrent strategy for the remediation of
73 hydrocarbon-polluted sites.

74 Nutrients provided by waste streams applied to land could offer a sustainable approach
75 to resolving the environmental problems arising from petroleum hydrocarbons
76 contaminated land. The use of organic waste materials (sugarcane bagasse, straw (pea
77 and rice), rice husks, food) have proven to speed up microbial growth and metabolism,
78 thus PAHs biodegradation in contaminated soil (Chiu et al., 2009; García-Delgado et
79 al., 2015). Due to the progressive change in waste streams types and nature throughout
80 time, there is a need to continue exploring their properties, especially those which are
81 relevant for biologically mediated remediation strategies (composition, cost-
82 effectiveness, safety, practicality, sustainability, shorter remediation time).

83 Today, a huge amount of wastes is generated from the food production industry in the
84 UK (post farm-gate waste is 9.5 million tonnes, with commercial and industrial business
85 producing 2.9 million tonnes). In Africa, especially in sub-Saharan Africa, waste
86 generation is estimated to be 62 million tonnes/year; and larger percentage (66-70%) of
87 the total waste generated is organic; however, most countries in Africa currently have

88 no estimated or documented value for these wastes. This has also contributed to massive
89 environmental pollution due to alternative use as well as improper disposal routes such
90 as landfilling, illegal dumping and burning. For example, in Africa soils are
91 vulnerable to degradation through environmental conditions (extremes of wetting and
92 drying, leaching, erosion, loss of organic matter) and anthropogenic impacts (fertiliser
93 and pesticide application, land use, deforestation, dumping of wastes and
94 pollution). However, the maintenance of soil health and fertility is crucial to
95 continued sustainable production of food, welfare of families and communities and
96 local and national economies. Some of these rich non-estimated green wastes include
97 spent mushroom compost (SMC) and spent brewery grains (SBG) which could
98 potentially be used for soil bioremediation (Mussatto, 2014). Focusing on the case of
99 SMC and SBG, about 200,000 tonnes of SMC are generated annually in UK (Finney et
100 al., 2009), while in Nigeria more than 750,000 tonnes of SBG and 3.4 million tonnes in
101 the EU (15% from UK) are produced annually, respectively (Aliyu and Bala, 2011;
102 Olawoye et al., 2017). These organic wastes can potentially improve soil structure,
103 increase soil fertility (directly and indirectly) and stimulate plant growth (Kästner and
104 Miltner, 2016, Sigmund *et al*., 2018). They also offer rich sources of enzymes,
105 including those involved in lignocellulosic decomposition (Phan and Sabaratnam,
106 2012), and represent a potential microbial inocula for biodegradation of recalcitrant
107 organic compounds (Leahy and Colwell, 1990). In contaminated soils, these effects
108 result in biodegradation, stimulation and stabilization of contaminated soil matrix, thus
109 promoting soil restoration (Kästner and Miltner, 2016).

110 To the best of the authors' knowledge, previous studies have not fully investigated the
111 impact of additions of SBG and SMC on biodegradation of PAHs in soil. In this study,
112 the development of phenanthrene (PHE) biodegradation was investigated in soil, which

had been amended with two organic wastes (SBG and SMC). Changes in catabolic activity were quantified by measuring the kinetics of ^{14}C-phenanthrene mineralization to $^{14}CO_2$ and changes in microbial numbers over time.

2. Materials and Methods

2.1 Soil and organic waste collection

Soil (A horizon; 5–20 cm) was collected from a field belonging to Myerscough Agricultural College (Preston, UK). Soil samples were air-dried, homogenized by sieving through a 2mm mesh to remove plant debris, stones, and larger residue fragments and stored at 4^0C in the dark until use. General soil properties are described in Table S1 (Couling et al., 2010). Fresh SBG and spent SMC were collected from Lancaster Brewery (Lancaster, UK) and Drinkwater's Mushrooms Ltd (Galgate, UK), respectively. General properties are described in Table S2. SMCs were pasteurized at 60^0C for 10-12 h prior to sampling. The materials used for SMC preparation were casing soil (peat), mushroom spawn, gypsum, water, and manure. Freshly collected organic wastes were homogenized by mixing and then stored in a sterile air-tight high-density polyethylene bags at a temperature of 4^0C.

2.2 Experimental set-up and amendment conditions

Soil (2.1 kg dry weight) was spiked with non-labelled phenanthrene (525 mg kg^{-1} dry weight) as described by Vázquez-Cuevas and Semple (2017). After blending and venting (to remove acetone), the soils were amended with different amounts of organic wastes (dry weight): 1:10, 1:5, 1:2, 1:1 and 2:1 organic waste-to-soil ratios (in triplicate)

and a control soil without amendment. The same water holding (60%) was maintained for all treatments throughout the study (water loss determined gravimetrically). The waste-soil mixtures, including the controls and blanks, were immediately transferred to amber glass bottles (to prevent photo-oxidation) and incubated in the dark at 21± 1°C over a period of up to 100 days and sampled at 1, 25, 50, 75 and 100 days soil-PAH contact time.

2.3 Mineralization of ^{14}C-phenanthrene in soil-waste mixtures

The impact of organic amendments on the catabolic potential of microbial community on ^{14}C-phenanthrene mineralization to $^{14}CO_2$ for each sampling time (1, 25, 50, 75 and 100 days soil-PAH contact time) on the amended and/or aged soil was measured in triplicate using respirometry, which was carried out using modified 250 ml Schott bottles (Reid et al., 2001; Semple et al., 2006). At each time point, 10 ± 0.2 g (dry weight) of the ^{12}C-phenanthrene spiked soil and 30 ml of deionized water was added to a respirometer. A [^{14}C] phenanthrene standard (100 Bq g^{-1} soil) was then added to the respirometer and placed on a flat-bed orbital shaker at 100 rpm and incubated for 14 days at 21 ± 1°C. The $^{14}CO_2$ was trapped in 1 M NaOH (I ml) in a vial suspended from the lid of the respirometer and sampled bihourly for 1 d and then every 24 h for 14 days and measured by liquid scintillation analyzer (LSC, Canberra Packard Tri-Carb2250CA) using standard protocols for counting and automatic quench correction (Semple et al., 2006).

Pristine soil samples spiked with both ^{12}C and ^{14}C-phenanthrene (without organic amendments), and ^{12}C- phenanthrene (without amendment and ^{14}C-labelled compound) were used as the control and analytical blank, respectively. The catabolic potential of the organic wastes was assessed by measuring the length of the lag phase (the time

161 taken before ^{14}C- phenanthrene mineralization reached 5%), changes in the maximum
162 rate of $^{14}CO_2$ evolution (fastest rate of mineralization resulting from microbial
163 degradation), and the cumulative extent of mineralization of ^{14}C- phenanthrene in the
164 soil samples (Macleod and Semple, 2006).

165

166 *2.4 Enumeration of heterotrophic and phenanthrene-degrading microorganisms*

167 Enumeration of total heterotrophic and phenanthrene-degrading microorganisms was
168 determined by dilution and spread plate method (Okere et al., 2012). For each of the
169 different microcosms, soil microbial numbers were determined before and after each
170 mineralization/respirometry assay. At each time point, 1 g (dry weight equivalent) was
171 taken before the start of the experiment (before CO_2 evolution) and 1 ml of soil slurry
172 after mineralization. Plate count (PCA) and Potato Dextrose (PDA) media were used for
173 enumeration of heterotrophic bacteria and fungi, respectively (in triplicate). For the
174 phenanthrene degraders, bacterial colonies were counted using minimal basal salt
175 (MBS) medium enriched with 50 mg l^{-1} ^{12}C- phenanthrene (carbon source) and
176 supplemented with an antifungal (fungizone) (Okere et al., 2012). Similar
177 microbiological culture medium supplemented with antibacterial (penicillin-
178 streptomycin-glutamate) was used for the enumeration of phenanthrene-degrading fungi
179 (Boochan et al., 2000).

180

181 *2.5 Data analysis*

182 Statistical analyses carried out included paired t-tests, ANOVA ($p < 0.05$) and Pearson's
183 correlation coefficient were performed using the Statistical Package for the Social
184 Sciences (IBM SPSS Version 23.0). Tukey's post-hoc and Games-Howell test were used
185 to determine significant differences in means of samples within and across groups

following the aging effect on ^{14}C-phenanthrene mineralization, lag phase, maximum rate, cumulative extent and microbial numbers at 95% confidence interval ($p < 0.05$) for organic waste-amended soils. Relationships between phenanthrene-degraders versus total extent and fastest rate of mineralization were analyzed using Pearson's product moment correlations. The Pearson's correlation coefficient (r) was ranked on a scale that range between +1 to -1. The value of r is either a perfect positive (+) or negative (-) correlation, when an increase in one variable led to an increase in the other variable (linear relationship) or an increase in one variable causing a decrease in the other variable, respectively. The strength of the relationship is either strong, weak or moderate between the two variables when the absolute values of their relationship approaches +1 or -1. The p-value shows the degree of association between the two variables. Data were plotted using SigmaPlot 10.0 software (Systat Software Inc., USA).

3. Results

The development of phenanthrene catabolism was measured in a soil amended with 1:10, 1:5, 1:2, 1:1 and 1:2 SBG:soil and SMC:soil ratios, respectively. Changes in kinetics of ^{14}C-phenathrene biodegradation were measured for 14 days after 1, 25, 50, 75 and 100 days of soil-PAH contact time (Figures 1 and 2). The influence of each organic amendment was assessed by measuring changes in the lag phases, rates and extents of ^{14}C-phenanthrene mineralization to $^{14}CO_2$ (Tables 1 and 2). Changes in the total heterotrophic and phenanthrene degrading bacteria and fungi were also measured over the course of the incubation.

3.1 Influence of spent brewery grains on mineralization of ^{14}C-phenanthrene in soils

The length of the lag phase (days) was measured in the SBG-amended soil incubations. Throughout the investigation, the lag phases were significantly shorter ($p < 0.05$) in the soils amended with lower amounts of SBG (1:10 and 1:5). In particular, the shortest lag phases were observed for 1:5 SBG:soil ratio at 100 d of soil-PAH contact time, while the longest lag phases were observed in soils amended with 2:1 SBG:soil ratio ($p < 0.05$), which did not reach 5%. Soil incubations amended with SBG (1:10) were found to have significantly shorter lag phases ($p < 0.05$) than 1:5 SBG:soil incubations after 1 d and 25 d aging (Table 1 and Figure 1). There were no significant differences ($p > 0.05$) between the control and 1:2 SBG:soil incubations, after 1 d soil-PAH contact time and no measurable lag phases were observed for 1:1 and 2:1 SBG:soil incubations. Noticeably, soil-PAH contact time reduced the lag phases in all of the amended conditions and control incubations except 1:1 and 2:1 SBG:soil ratios; an effect which is reflected especially in the lag phases after 100 days ($p < 0.05$), compared to previous time points. With respect to the amount of SBG added to the soil, lower organic waste additions resulted in shorter lag-phases (1:10>1:5>1:2>1:1 > 2:1),although the SBG:soil ratios (1:2, 1:1 and 2:1) showed significantly reduced lag phases compared with unamended control, at most time points during the incubation period.

The influence of increasing amounts of SBG on the fastest rates of ^{14}C-phenanthrene mineralization was also measured at each time point over the 100 d incubation (Table 1 and Figure 1). The fastest rates were observed for soils amended with 1:10 and 1:5 waste:soil incubations, while the lowest were in the 2:1 SBG:soil incubations. The rates of $^{14}CO_2$ mineralized in the 1:10 and 1:5 SBG:soil incubations did not significantly differ ($p > 0.05$). After 100 d soil-PAH contact time, 1:1 and 2:1 SBG:soil incubations had no effect on rates of ^{14}C-phenanthrene mineralized when compared to un-amended soil.

235

236 The influence of increasing amounts of SBG on the extents of ^{14}C-phenanthrene
237 mineralization was measured at each time point over the 100 d incubation. As with the
238 changes in lag phase and fastest rate measurements, the soil-PAH contact time and the
239 amount of SBG had an influence on the amount of ^{14}C-phenanthrene mineralized to
240 $^{14}CO_2$ (Figure 1 and Table 1). At 1 and 25 d soil:PAH contact time, treatments
241 containing organic materials were found with significantly higher ($p < 0.05$) extents of
242 mineralization in the following trend 1:10=1:5>1:2=1:1>2:1. The total extents of $^{14}CO_2$
243 mineralized were also significantly higher ($p < 0.05$) after 50 d contact time for 1:1 and
244 1:2 SGB to soil emndments compared to 25 d with nearly 120% and 278% increases,
245 respectively, in $^{14}CO_2$ mineralized in amended soils (Table 1). Furthermore, extents of
246 mineralization peaked at 50 d soil-PAH contact time in most amended soils. This
247 treatment period was found significantly higher ($p < 0.05$) in total extents of
248 mineralization compared to the other time points.

249 The 1:2 SBG:soil amendment showed the highest extent of mineralization (48.7%) after
250 50 d soil incubation, but this extent of mineralization to $^{14}CO_2$ was not sustained
251 following significant ($p < 0.05$) decreases by 50% and 41% after 75 d and 100 d soil
252 contact time, respectively. However, the lower amounts of SBG amended to soil (1:10
253 and 1:5) after 100 d incubation, showed significantly higher extents of mineralization by
254 nearly 19% and 13%, respectively, as compared to results observed at 75 d of soil-PAH
255 contact time. The $^{14}CO_2$ produced in 2:1 SBG-amended soil was significantly lower (p
256 < 0.05) than any other treatments and control soil at each sampling point during the
257 study.

258

3.2 Enumeration of culturable bacterial and fungal heterotrophs and phenanthrene degraders in SBG-amended soils

The colony forming units (CFUs g^{-1} soil dw) of heterotrophic and phenanthrene degrading bacteria were measured for all treatment conditions and control in SGB amended soils over a 100 d incubation (Table 3). CFUs were also measured at the end of the 14-d respirometric incubations and are presented in supplementary material (Table S3). The total heterotrophs and phenanthrene degraders in amended soils increased significantly ($p < 0.05$) based on the amounts of SBG applied to the soil (Table 3). Compared with the controls, significantly higher numbers ($p < 0.05$) of heterotrophic and phenanthrene-degrading CFUs were observed in the soils amended with SBG. In addition, the smaller amounts of SBG added to the soil (1:10 and 1:5) consistently showed higher CFUs (heterotrophs and phenanthrene-degraders) as compared to other amendment conditions. Furthermore, apart from the heterotrophs, phenanthrene degrading bacteria did not proliferate in the soils containing larger amounts of SBG (2:1); this was also observed in the control soil.

Phenanthrene-degrading bacterial numbers did not statistically correlate with fastest rate of ^{14}C-phenanthrene mineralized in all amended soils throughout this study (1 d to 100 d) (Figure S1), irrespective of the high numbers of phenanthrene-degraders observed in SBG amended soils. Correlations between phenanthrene degraders and total extents of ^{14}C-phenanthrene mineralization showed a significantly weak but negative correlation ($r^2 = 0.40$, $p = 0.02$) with bacterial numbers in 1:5 SBG:soil. Also, the extent of ^{14}C-phenanthrene mineralized for higher dose of SBG added to soils (1:2 and 2:1) showed strong negative and strong positive relationships ($r^2 = 0.50$, $p = 0.003$ and $r^2 = 0.61$, $p = 0.001$) with phenanthrene-degrading bacterial numbers in soils, respectively.

284

285 The addition of organic amendment at different ratios resulted in a significant increase
286 ($p < 0.05$) in both heterotrophic and phenanthrene-degrading fungal numbers (Table 3).
287 Overall, both fungal numbers were significantly higher than control before
288 mineralization and after amendment of soils with SBG, although 2:1 SBG application to
289 soil showed a lower heterotrophic fungal number compared to other treatments.
290 Noticeably, 1:2 and 1:1 SBG:soil conditions showed significantly higher fungal
291 numbers (heterotrophs and phenanthrene-degraders) over time (1 d to 100 d) compared
292 to other amendments during the investigation. Similar trends were observed for the 1:5
293 SBG:soil incubations, but did not increase consistently throughout the 100 d incubation.
294 Additionally, all SBG:soil conditions (except 2:1) showed high fungal proliferation
295 from 75 d to 100 d soil-PAH contact time. After 100 d soil-PAH contact time, the 1:5
296 SBG:soil incubations displayed the highest CFUs for heterotrophic fungi; while the
297 highest CFUs for phenanthrene-degrading fungi were observed in the 1:2 and 1:1
298 SBG:soil incubations after 25 d soil-PAH contact time.

299

300 As with bacterial numbers, the phenanthrene-degrading fungal numbers for most
301 SBG:soil treatment ratios have no positive relationships with the rates and extents of
302 ^{14}C-phenanthrene mineralization during the study period (Figure S2), except for 1:5 and
303 1:2 SBG:soil incubations in which the overall extents of mineralization significantly
304 correlated with phenanthrene-degrading fungal numbers. However, weak positive (r^2 =
305 0.30, $p = 0.05$) and strong negative ($r^2 = 0.55$, $p = 0.003$) correlations were found in the
306 1:5 and 1:2 SBG:soil conditions, respectively.

307

3.3 Influence of spent mushroom compost on mineralization of ^{14}C-phenanthrene in soils

In soils amended with SMC, the length of lag phases were also measured at each time point over the 100 d incubation. It was observed that the lag phases generally decreased in all of the treatments with increased soil-PAH contact time (Figure 2 and Table 2). After 1 day of soil incubation, the lag phase observed for control soils were generally not significantly different ($p > 0.05$) from any of the SMC:soil conditions. However, significant reductions ($p < 0.05$) in the lag phases, after 25 days soil-PAH contact time, were observed for all SMC:soil conditions as compared to 1 day contact time. In addition, at 25 d soil-PAH contact time, the SMC-amended soils except 2:1 showed significantly shorter lag phases compared with the unamended control condition. These observed reductions were not significantly different from the other contact points onwards (50 to 100 days). Data showed that the 2:1 SMC:soil incubation displayed the longest lag phases ($p > 0.05$) compared to the other conditions and control throughout the study period..

The impact of soil-PAH contact time and the amount of SMC on the fastest rate of ^{14}C-phenanthrene mineralization in soil were studied over 100 d. For all amendment conditions, increases in soil-PAH contact time resulted in faster rates within each SMC-soil treatment, ranging from 0.40% to a maximum of 2.0%. Similarly, the greatest change was observed from 1 d (0.40 – 0.60%) to 100 d (0.80 – 1.90%) soil-PAH contact time: the 1:5 SMC-soil amendment displayed the fastest rate of $^{14}CO_2$ mineralized (2.37% $^{14}CO_2$ h^{-1}) after 100 d soil-PAH contact time. For control soils, the fastest rates of mineralization were not significantly lower ($p > 0.05$) when compared to all SMC

amended soils except for 1:5 SMC:soil condition, which had significantly faster rates at 1 and 25 d soil-PAH contact time (both $p < 0.05$).

The influence of increasing amounts of SMC in soil on the extents of ^{14}C-phenanthrene mineralization was also determined over a 100 d period. From this study, the lower SBG:soil ratios (1:10 and 1:5) exhibited significantly greater ($p < 0.05$) extents of ^{14}C-phenanthrene mineralized (50 and 100 d) compared to all other treatment (Table 2). Following 1 d and 25 d of soil-PAH contact time, there were no significant differences in the overall extents in all SMC-amended soils ($p > 0.05$) when compared to their non-equivalent amendment conditions and control soils. Higher extent of mineralization was observed for all amendment conditions, except for the largest dose rate (2:1) from 50 to 100 d soil-PAH contact time. In particular, the lower doses (1:5 and 1:10) displayed higher extents of mineralization (50 to 100 d) in comparison with the other conditions. Furthermore, soils amended with SBG showed the highest extents of mineralization after 75 d but this was not statistically different from 50 d soil-PAH contact time.

3.4 Enumeration of culturable bacterial and fungal heterotrophs and phenanthrene degraders in SMC-amended soils

Bacterial numbers (both heterotrophs and phenanthrene-degraders) in the SMC amended soils were significantly higher ($p < 0.05$) than control soil before mineralization throughout the course of the study. In comparison to the bacterial numbers after mineralization, most of the amended conditions were not higher than the non-amended soil except for 1d and 50 d for heterotrophic bacteria (Table 4). CFUs after mineralization is presented in the supplementary material (Table S4). The 1:1 and

2:1 SMC-soil conditions displayed higher bacterial and fungal numbers (both heterotrophs and phenanthrene-degraders) before mineralization throughout the 100 d incubation ($p < 0.05$). The growth of bacteria in amended soils were significantly influenced depending on the SMC dosage application. After 1 d aging, higher organic materials added to soils resulted in greater increases in the bacterial numbers in the following order: 2:1>1:1>1:2:1:5>1:10). Similarly, heterotrophs and phenanthrene-degraders showed higher CFUs in soil amended with higher dose of SMC in rest of the study periods (25 to 100 d).

Relationships between rates and extents of ^{14}C-phenanthrene mineralized with phenanthrene-degrading bacterial numbers for all amended soils over the 100 incubation were explored (Table 4 and Figure S3). The 1:1 SMC-soil incubation showed a strong negative relationship for both fastest rates ($r^2 = 0.76$, $p = 0.000$) and overall extents ($r^2 = 0.69$, $p = 0.000$) with phenanthrene-degrading bacteria, whilst a weak but significant positive correlation was found between extent and phenanthrene-degrading numbers in 1:10 SMC:soil incubation ($r^2 = 0.30$, $p = 0.04$). In addition, both 1:5 and 2:1 SMC:soil incubations displayed weak positive ($r^2 = 0.30$, $p = 0.05$) and weak negative ($r^2 = 0.31$, $p = 0.03$) correlations between the rate of mineralization and phenanthrene-degraders, respectively.

Fungal numbers for SMC amended soils did not show significant differences from control, apart from 1:5 and 1:2 amended soils before (Table 4) and after mineralization (Table S6). In most cases, the control soil showed a significant heterotrophic and phenanthrene-degrading numbers ($p < 0.05$) than all treatment soils, especially at 50 d and 25d, respectively. After 1 d incubation, the CFUs (heterotrophic and phenanthrene-

degrading fungi) from unamended soil were not significantly different ($p > 0.05$) from the rest of the treatments except for 1:5 SMC amended to soils. In contrast to 75 d aging, all amended soils showed significantly higher fungal numbers ($p < 0.05$) compared to 1 d aging. However, results from this study revealed there were no significant relationships ($r^2 < 0.2$; $p > 0.05$) found between rates and extents of ^{14}C-phenanthrene mineralization with phenanthrene-degrading fungal numbers in virtually all amended soils throughout the study.

4. Discussion

4.1 Organic amendment ratios on ^{14}C-phenanthrene mineralization

The addition of nitrogen-rich nutrients (biostimulation) and potentially viable microbes (bioaugmentation) through organic materials application to soils are two effective approaches for the bioremediation of PAH-contaminated soils (Wang et al., 2016). The results in the present study showed that contact time influenced the development of catabolic activity as defined by decreases in the length of the lag phases and increases in the rates and extents of mineralization of ^{14}C-phenanthrene in amended soils. This agrees with findings reported from previous studies **(**Abioye et al., 2012; Adam et al., 2015), indicating an organic waste stimulatory effect on the biodegradation of phenanthrene. Generally, the addition of SBG and SMC stimulated phenanthrene catabolism in soils, especially with lower amendment ratios (1:5 and 1:10). Application of higher mix ratios (especially 2:1 organic material to soil) of both amendment types largely reduced the extent of mineralization. Das et al. (2011) also noted that due to the very high content of organic materials, microbes may metabolize the readily degradable substrate as carbon source, rather than the target PAH and this was further reflected in the microbial population after mineralization. The microcosm for the higher amendment

(2:1) could have limited oxygen transport for microbial activity, hence a reduced mineralization, due to the nature of water saturated bulky material formed after amendment. In addition, this could be linked to high sorptive capacity of organic materials for PAH and subsequent decrease in bioavailability of PAH for microbial degradation (Rhodes et al., 2008). More so, the degree of contaminant sorption and their rapidly/slowly desorbing fractions in amended soils are important factors that determine the extent of microbial sequestration and transformation (Rhodes et al., 2012). The decrease in the catabolic response by the higher amendment (2:1) agrees with previous studies on organic additives addition to PAHs contaminated soils (Namkoong et al., 2002; Semboung Lang et al., 2016).

In this study, soils amended with SBG and SMC (1:10 and 1:5) showed significantly shorter lag phases over time, while the lag phase of organic waste-soil mixture (2:1) was immeasurable compared to other amendment conditions. This clearly indicated that appropriate amounts of amendments could significantly influence microbial metabolism of the target contaminant (Schaefer and Juliane, 2007). In this study, the lower organic waste-soil mixtures indicated stimulated microbial action as a result of adaptation and bioavailability of PAH fraction as observed in the lag phase compared to the higher ones. Our results found that after 100 d soil incubation, both SGB and SMC showed a further reduction in lag phases with a substantive extent of $^{14}CO_2$ mineralized indicating stimulatory effects of the supplements and acclimatization of the degrading microbial populations. Several previous studies have demonstrated that the extent of PAHs-association with soil matrices could potentially facilitate microbial adaptation and subsequently reduce the lag phase for microbial degradation (Oyelami et al., 2015; Okere et al., 2017). In this current study, the fastest rates of mineralization revealed a

similar pattern as observed for lag phases with increasing soil-PAH contact period. However, the fastest rates of mineralization remained relatively constant in the SBG-amended soils compared to the SMC-amended soils. Therefore, this may be attributed to the higher available nutrient, and low total organic carbon (TOC) initially present in SMC slurry system. Soil fertility and species richness have been reported as driving force for ^{14}C-phenanthrene degradation (Oyelami et al., 2012). Okere et al. (2017) suggested, however, that a higher TOC in soils may decrease the bioavailability of ^{14}C-phenanthrene to PAH-degraders, hence a reduction on the rate of mineralization. Higher rates of ^{14}C-phenanthrene mineralization in amended soils (1:10 and 1:5) of both SMC and SBG in this study indicated the potential influence of low organic amendments resulting from optimal waste ratios for microbial community uptake and degradation (Namkoong et al., 2002). Abioye et al. (2012) and Sigmund et al. (2018) also demonstrated that the addition of smaller amounts of organic amendments to soil (1:10) largely increased degradation rates, despite the sequestration of the chemicals in the soil.

The extents of ^{14}C-phenanthrene mineralization in waste-amended soils depended on the amount of organic material added to the soil. Extents of ^{14}C-phenanthrene mineralization were greater at most time points for both SBG- and SMC-amended soils as the application rates decreased (2:1 < 1:1 < 1:2 < 1:5 < 1:10). In addition, we also found that the lower mix ratios (1:10 and 1:5) consistently displayed higher extents of mineralization than the other amendment conditions in almost all time points. This may be attributed to the potential of the organic wastes to reduce or speed up the desorption process of phenanthrene and/or the likely effect of aging on the bioavailability of PAHs

454 in soil matrices (Semple et al., 2007). It is important to emphasize that sorption of
455 contaminants to soil is a slow and reversible process (Hatzinger and Alexander, 1995).

456

457 *4.2 Influence of microbial population on ^{14}C-phenanthrene catabolism in soils*

458 The data from this study showed that the addition of exogenous organic materials to soil
459 increase the microbial populations with soil microbial response (both heterotrophs and
460 degraders). The potential of organic amendments to stimulate microbial numbers in soil
461 have been reported in previous studies (Semple et al., 2001; Agarry and Latinwo, 2015),
462 as well as their catabolic response in PAH-contaminated soil (Zhang et al., 2012;
463 Sigmund et al., 2018). The present study suggested that the rich ingredients provided by
464 both amendment types increased microbial proliferation and activity. However, the
465 numbers of phenanthrene-degraders were generally low compared to their counterparts
466 (heterotrophs) in amended soils and this may have affected mineralization end-points in
467 this study. Thus, organic supplements could stimulate the microbial populations
468 (heterotrophs and PAH-degraders) but the low response of PAHs-degrading
469 microorganisms could reduce PAH catabolism in soil (Carmichael and Pfaender, 1997).
470 The results from ^{14}C-phenanthrene mineralization (both rates and extents) reported here
471 were not influenced by the number of phenanthrene-degraders, except the mix ratio of
472 1:5 for SBG and SMC-amended soils. Also, the CFUs (phenanthrene-degrading fungi
473 and bacteria) present in the 1:1 and 2:1 waste-soil conditions; however, had an influence
474 on the rates and extents of mineralization. Hydrocarbonoclastic microorganisms are the
475 key bio-actors on the biodegradation of pollutants in the environments, but their
476 synergistic role with non-PAH degraders may play an important role in PAH
477 metabolization in contaminated soils (Leahy, 1990).

5. Conclusion

The present study indicates that organic amendment (SBG & SMC) addition to soil can influence the ^{14}C-mineralization of phenanthrene over time. Mineralization of phenanthrene varies for different amendment ratios to soil as well as the length of soil incubation. We found that the lower mix ratios (1:5 and 1:10) of both amendment types can provide the most optimal conditions and could effectively enhance ^{14}C-phenanthrene mineralization and microbial numbers over time compared to other mix ratios. Aging the soil significantly reduced the lag phases and increased rates and extents of mineralization in phenanthrene-contaminated soil. These two organic wastes should be considered as nutrient supplements during bioremediation of contaminated soil.

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669

670 **Figure legends**

671

672 **Figure 1**. Development of the catabolism of ^{14}C-phenanthrene to $^{14}CO_2$ in soils

673 amended with spent brewery grains: control (unamended)(□), 1:10 (■), 1:5 (△), 1:2 (

674 ▼), 1:1 (○) and 2:1 (●) after 1, 25, 50, 75, and 100d soil-phenanthrene contact time.

675 Values of standard error of mean (SEM) are triplicate samples (n = 3).

676

677 **Figure 2**. Development of the catabolism of ^{14}C-phenanthrene to $^{14}CO_2$ in soils

678 amended with spent mushroom compost: control (unamended)(□), 1:10 (■), 1:5 (△),

679 1:2 (▼), 1:1 (○) and 2:1 (●) after 1, 25, 50, 75, and 100d soil-phenanthrene contact

680 time. Values of standard error of mean (SEM) are triplicate samples (n = 3).

Table 1. Catabolic profile of ^{14}C-labelled phenanthrene in soil amended with different proportions of **spent brewery grains** after 14 days respirometric assay. Values are mean ± standard error (n = 3).

Contact time (days)	**Amendment (%)**	**Lag phase ($^{14}CO_2$ ≥5%) d^{-1}**	**Fastest rate (% $^{14}CO_2$ h^{-1})**	**Cumulative Extent (%)**
1	0	^{a1}9.04 ± 0.08	^{a1}0.20 ± 0.04	^{b1}11.13 ± 1.06
	1:10	^{c1}5.06 ± 0.01	^{a1}0.36 ± 0.03	^{c1}25.99 ± 1.23
	1:5	^{b1}6.91 ± 0.17	^{a1}0.23 ± 0.04	^{c1}24.54 ± 1.73
	1:2	^{a1}9.47 ± 0.01	^{a1}0.25 ± 0.00	^{b1}14.53 ± 0.61
	1:1	N/L*	^{a1}0.02 ± 0.00	^{a1}0.39 ± 0.06
	2:1	N/L*	^{a1}0.23 ± 0.08	^{a1}0.76 ± 0.21
25	0	^{b2}2.34 ± 0.14	^{b1}0.13 ± 0.00	^{b2}16.55 ± 0.32
	1:10	^{d3}0.34 ± 0.00	^{d3}1.50 ± 0.02	^{c1}30.18 ± 2.13
	1:5	^{c2}0.40 ± 0.01	^{d2}1.34 ± 0.08	^{c1}28.60 ± 2.42
	1:2	^{a1}10.16 ± 0.23	^{ac1}0.27 ± 0.05	^{b1}12.86 ± 2.22
	1:1	^{a1}11.05 ± 0.35	^{c2}0.22 ± 0.00	^{b2}16.70 ± 2.56
	2:1	N/L*	^{a1}0.04 ± 0.00	^{a3,4}0.43 ± 0.02
50	0	^{c4}0.69 ± 0.01	^{c3}0.70 ± 0.04	^{b4}27.74 ± 1.27
	1:10	^{d2}0.50 ± 0.01	^{cd2}0.96 ± 0.06	^{c1}39.84 ± 2.08
	1:5	^{d1,3}0.48 ± 0.03	^{d2}1.10 ± 0.02	^{c2}44.25 ± 3.08
	1:2	^{b3}2.05 ± 0.02	^{c3}0.62 ± 0.01	^{c3}48.66 ± 1.32
	1:1	^{a2}3.56 ± 0.11	^{b4}0.35 ± 0.00	^{b3}36.66 ± 2.26
	2:1	N/L*	^{a1}0.01 ± 0.00	^{a2,4}0.28 ± 0.02
75	0	^{c3}1.16 ± 0.01	^{b2}0.32 ± 0.01	^{b3}23.71 ± 0.03
	1:10	^{d2}0.49 ± 0.02	^{d2}0.85 ± 0.06	^{bc1}26.18 ± 0.91
	1:5	^{d2}0.42 ± 0.00	^{cd3}2.11 ± 0.04	^{c1}30.30 ± 0.95
	1:2	^{a2}4.14 ± 0.03	^{b2}0.35 ± 0.04	^{b2}25.31 ± 2.00
	1:1	^{b2}1.55 ± 0.05	^{b3}0.27 ± 0.01	^{bc4}27.28 ± 0.29
	2:1	N/L*	^{a1}0.01 ± 0.00	^{a1,2,4}0.10 ± 0.04
100	0	^{a4}1.01 ± 0.03	^{b2}0.33 ± 0.01	^{b2}19.54 ± 0.46
	1:10	^{b3}0.33 ± 0.03	^{d3}2.18 ± 0.13	^{c2}32.26 ± 0.93
	1:5	^{b3}0.31 ± 0.00	^{d4}1.79 ± 0.09	^{c1,2}34.68 ± 2.75
	1:2	^{ab4}0.69 ± 0.14	^{c3}0.78 ± 0.05	^{c2}28.96 ± 1.10
	1:1	^{ab2}1.78 ± 0.20	^{a3}0.27 ± 0.01	^{c4}28.56 ± 0.71
		N/L*	^{a1}0.02 ± 0.01	^{a1,4}0.00 ± 0.02

	2:1

N/L* No lag phase (Mineralization did not reach or exceed 5%)

* Same letters indicate no statistical differences ($p > 0.05$) in amendment levels within each aging time while different letters indicate significant differences ($p < 0.05$) across amendment levels within each aging time

* Same numbers indicate no statistical differences ($p < 0.05$) in aged amended soils across the four sampling points while different numbers indicate significant differences in aged amended soils across the four sampling points (1d to 100d).

Table 2. Catabolic profile of ^{14}C-labelled phenanthrene in soil amended with different proportions of **spent mushroom compost** after 14 days respirometric assay. Values are mean ± standard error (n = 3).

Contact time (days)	**Amendment (%)**	**Lag phase ($^{14}CO_2$ ≥5%) d^{-1}**	**Fastest rate (% $^{14}CO_2$ h^{-1})**	**Cumulative Extent (%)**
1	0	^{a1}3.71 ± 0.26	^{a1}0.34 ± 0.00	^{a1}21.22 ± 2.13
	1:10	^{a1}3.25 ± 0.06	^{ab1}0.57 ± 0.00	^{a1}21.61 ± 1.39
	1:5	^{a1}3.50 ± 0.35	^{b1}0.58 ± 0.00	^{a1}23.97 ± 0.49
	1:2	^{a1}3.43 ± 0.06	^{ab1}0.41 ± 0.00	^{a1}23.48 ± 1.32
	1:1	^{a1}3.93 ± 0.21	^{ab1}0.39 ± 0.00	^{a1}19.54 ± 1.48
	2:1	^{a2}4.09 ± 0.05	^{ab1}0.38 ± 0.00	^{a1}25.37 ± 4.57
25	0	^{a2} 0.45 ± 0.02	^{a1}0.66 ± 0.05	^{a1}26.46 ± 0.50
	1:10	^{c3} 0.23 ± 0.02	^{b2}1.94 ± 0.17	^{a1}26.81 ± 0.91
	1:5	^{c3,4}0.24 ± 0.01	^{b2}1.72 ± 0.04	^{a1,2}25.07 ± 1.31
	1:2	^{c2} 0.27 ± 0.02	^{b2}1.64 ± 0.04	^{a1}33.08 ± 3.97
	1:1	^{b2} 0.35 ± 0.01	^{a2}1.14 ± 0.11	^{a2}25.75 ± 1.52
	2:1	^{a4} 0.52 ± 0.00	^{a2}0.86 ± 0.00	^{a1}25.66 ± 0.23
50	0	^{b3} 0.21 ± 0.01	^{bc2} 1.75 ± 0.14	^{ab1} 27.44 ± 0.47
	1:10	^{b3} 0.22 ± 0.01	^{c2} 2.02 ± 0.09	^{c1}31.79 ± 0.47
	1:5	^{b4} 0.21 ± 0.01	^{c3}2.06 ± 0.10	^{bc2,3}28.94 ± 0.83
	1:2	^{b2}0.26 ± 0.02	^{bc2}1.67 ± 0.18	^{ab1}27.02 ± 1.37
	1:1	^{b2,3}0.30 ± 0.01	^{b2,3}1.36 ± 0.03	^{bc2}28.69 ± 0.49
	2:1	^{a1} 6.08 ± 0.11	^{a1} 0.36 ± 0.04	^{a1}24.70 ± 0.37
75	0	^{a3}0.25 ± 0.02	^{b2}1.68 ± 0.10	^{a1}29.36 ± 1.55
	1:10	^{b2}0.37 ± 0.02	^{b2}1.62 ± 0.05	^{a2}34.99 ± 0.29
	1:5	^{a2}0.37 ± 0.01	^{b2}1.74 ± 0.03	^{a3}33.14 ± 1.26
	1:2	^{a2}0.30 ± 0.04	^{b2}1.86 ± 0.20	^{a1}32.87 ± 2.89
	1:1	^{a2}0.33 ± 0.00	^{b3}1.61 ± 0.08	^{a2}30.55 ± 0.54
	2:1	^{a3}3.41 ± 1.61	^{a1}0.51 ± 0.12	^{a1}32.43 ± 5.19
100	0	^{b2}0.22 ± 0.01	^{bc3}1.83 ± 0.11	^{ab1}28.96 ± 0.62
	1:10	^{b2,3}0.26 ± 0.01	^{c2}2.03 ± 0.14	^{c1}32.97 ± 0.08
	1:5	^{b4}0.23 ± 0.00	^{b4}2.37 ± 0.04	^{bc3}31.47 ± 0.57
	1:2	^{b2}0.36 ± 0.19	^{b2}2.28 ± 0.25	^{bc1}30.36 ± 1.68
	1:1	^{b3}0.23 ± 0.01	^{b4}1.99 ± 0.05	^{abc2}29.30 ± 0.53
	2:1	^{a3}1.91 ± 0.02	^{ac1}0.42 ± 0.00	^{a1}26.09 ± 0.67

* N/L indicates no lag phase (mineralization did not reach or exceed 5%)

* Same letters indicate no statistical differences ($p > 0.05$) in amendment levels within each aging time while different letters indicate significant differences ($p < 0.05$) across amendment levels within each aging time

* Same numbers indicate no statistical differences ($p > 0.05$) in aged amended soils across the four sampling points while different numbers indicate significant differences ($p < 0.05$) in aged amended soils across the four sampling points (1d to 100d).

709 Table 3. Autochthonous heterotrophic and phenanthrene-degrading microorganisms

710 present before 14 days mineralization of ^{14}C-phenanthrene in **spent brewery grains**

711 amended soil. Values are mean ± standard error (n = 3)

		Bacteria CFU x 10^8 g^{-1} soil dw		**Fungi** CFU x 10^6 g^{-1} soil dw	
Contact time (days)	Amendment (%)	Heterotrophs	PHE-degraders	Heterotrophs	PHE-degraders
1	0	^{a2}11.6 ± 0.49	^{a4}2.91 ± 0.03	^{a2}0.23 ± 0.00	^{a1}0.03 ± 0.00
	1:10	^{cd4}17.1 ± 0.38	^{c3}15.9 ± 0.34	^{c1}5.71 ± 0.54	^{c2}3.16 ± 0.45
	1:5	^{b4}13.8 ± 0.41	^{c3}13.8 ± 0.41	^{b1}4.44 ± 0.34	^{d2}3.72 ± 0.06
	1:2	^{a3}9.37 ± 0.48	^{c3}5.02 ± 0.45	^{d1,2}9.52 ± 0.32	^{e2}4.52 ± 0.65
	1:1	^{bc2}15.9 ± 0.18	^{ab3}2.91 ± 0.29	^{f1}11.2 ± 0.12	^{c2}3.11 ± 0.33
	2:1	^{de5}19.0 ± 1.04	^{ab3}2.45 ± 1.23	^{e5}10.2 ± 0.20	^{b1}2.33 ± 0.31
25	0	^{a2}0.40 ± 0.03	^{b5}4.05 ± 0.20	^{a2}0.30 ± 0.01	^{a1}0.02 ± 0.00
	1:10	^{f3}13.7 ± 0.17	^{e3}14.1 ± 0.34	^{b2,4}7.76 ± 0.80	^{b2}3.16 ± 0.40
	1:5	^{e3}11.9 ± 0.15	^{d3}12.8 ± 0.26	^{b2}8.23 ± 0.84	^{b5}5.74 ± 0.90
	1:2	^{b4}6.20 ± 0.19	^{c3}6.79 ± 0.17	^{c1}12.0 ± 0.77	^{c3}9.92 ± 0.70
	1:1	^{d1}10.4 ± 0.15	^{f4}16.0 ± 0.29	^{c2}14.0 ± 1.02	^{c3}9.07 ± 0.74
	2:1	^{c4}8.40 ± 0.08	^{a1}0.00 ± 0.00	^{a4}2.36 ± 0.10	^{b2}5.32 ± 0.34
50	0	^{a2}0.05 ± 0.04	^{a2}4.18 ± 0.19	^{a2}0.05 ± 0.00	^{a2}0.05 ± 0.00
	1:10	^{b5}19.0 ± 0.76	^{a2}0.41 ± 0.08	^{c2}7.22 ± 0.77	^{b2}0.24 ± 0.01
	1:5	^{b5}18.3 ± 0.68	^{b2}1.97 ± 0.22	^{b1}4.09 ± 0.40	^{c1}0.41 ± 0.02
	1:2	^{b5}22.2 ±1.06	^{b2}1.67 ± 0.09	^{e1,2}14.0 ± 0.94	^{c1}0.41 ± 0.05
	1:1	^{b2}21.1 ± 4.96	^{b2}1.46 ± 0.18	^{d1}10.2 ± 0.70	^{d1}0.61 ± 0.03
	2:1	^{a1,2}0.14 ± 0.01	^{a3}0.45 ± 0.05	^{a3}0.99 ± 0.08	^{b1}0.26 ± 0.01
75	0	^{a2}0.09 ± 0.03	^{a1}0.02 ± 0.00	^{a4}1.09 ± 0.03	^{a2}0.05 ± 0.00
	1:10	^{b2}0.92 ± 0.02	^{b2}0.36 ± 0.01	^{b4}10.6 ± 0.30	^{a1}0.85 ± 0.04
	1:5	^{c2}2.01 ± 0.07	^{c2}0.77 ± 0.03	^{bc3}12.1 ± 0.37	^{bc3}4.73 ± 0.48
	1:2	^{d4}1.48 ±0.04	^{d4}1.14 ± 0.05	^{bc1,2}14.7 ± 0.34	^{d3}7.64 ± 0.70
	1:1	^{e2}2.97 ± 0.09	^{c2}0.87 ± 0.07	^{c2}16.6 ± 0.41	^{cd3}6.37 ± 0.37
	2:1	^{a2,3}2.73 ± 0.03	^{a2}0.04 ± 0.00	^{a2}1.00 ± 0.92	^{b2}3.88 ± 0.18
100	0	^{a1}0.05 ± 0.00	^{a3}0.11 ± 0.00	^{a3}0.49 ± 0.02	^{a1}0.03 ± 0.00
	1:10	^{b1,2}0.92 ± 0.00	^{a1}0.15 ± 0.00	^{c5}10.3 ± 0.59	^{b2}3.76 ± 0.18
	1:5	^{d1}2.01 ± 0.11	^{b2}1.32 ± 0.04	^{f4}17.9 ± 0.18	^{c4}6.24 ± 0.18
	1:2	^{c1}1.48 ± 0.07	^{c1,2}1.45 ± 0.04	^{e2}16.5 ± 0.24	^{c3}6.88 ± 0.30
	1:1	^{e1}2.97 ± 0.16	^{a1}0.11 ± 0.00	^{d2}15.0 ± 0.15	^{c1}7.34 ± 0.53

	2:1	[e3]2.73 ± 0.06	[a2]0.07 ± 0.00	[b2]1.98 ± 0.08	[a1]0.21 ± 0.01

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714 * Same letters indicate no statistical differences ($p > 0.05$) in amendment levels within

715 each aging time while different letters indicate significant differences ($p < 0.05$) across

716 amendment levels within each aging time

717 * Same numbers indicate no statistical differences ($p > 0.05$) in aged amended soils

718 across the four sampling points while different numbers indicate significant differences

719 ($p < 0.05$) in aged amended soils across the four sampling points (1d to 100d).

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736 Table 4. Autochthonous heterotrophic and phenanthrene-degrading microorganisms

737 present before 14 days mineralization of ^{14}C-phenanthrene in **spent mushroom**

738 **compost** amended soil. Values are mean ± standard error (n = 3)

		Bacteria CFU x 10^8 g^{-1} soil dw		**Fungi** CFU x 10^6 g^{-1} soil dw	
Contact time (days)	Amendment (%)	Heterotrophs	PHE-degraders	Heterotrophs	PHE-degraders
1	0	^{a2}0.07 ± 0.00	^{a3}0.14 ± 0.01	^{c1}0.18 ± 0.01	^{c2}0.13 ± 0.00
	1:10	^{b1}0.42 ± 0.02	^{b1}0.14 ± 0.00	^{b1}0.16 ± 0.00	^{d1}0.16 ± 0.00
	1:5	^{c1}0.67 ± 0.02	^{c1}0.23 ± 0.04	^{b1}0.15 ± 0.00	^{c2}0.12 ± 0.00
	1:2	^{d1}0.98 ± 0.01	^{d1,2}1.51 ± 0.04	^{b1}0.15 ± 0.00	^{b2}0.10 ± 0.00
	1:1	^{e1}1.05 ± 0.01	^{e2}2.15 ± 0.00	^{a1}0.06 ± 0.00	^{c1}0.12 ± 0.00
	2:1	^{f1}1.46 ± 0.01	^{f4}2.60 ± 0.00	^{b2}0.51 ± 0.00	^{a1}0.02 ± 0.00
25	0	^{a1}0.03 ± 0.00	^{a3}0.16 ± 0.03	^{a2}0.32 ± 0.06	^{a3}0.29 ± 0.05
	1:10	^{a4}7.02 ± 0.31	^{b4}0.74 ± 0.05	^{c2}0.92 ± 0.10	^{b2}1.59 ± 0.31
	1:5	^{c2}14.3 ± 0.35	^{c5}1.36 ± 0.04	^{bc2}0.69 ± 0.02	^{b2}1.67 ± 0.16
	1:2	^{c4}13.9 ± 0.80	^{c3}1.42 ± 0.14	^{ab3}0.60 ± 0.08	^{b2}2.31 ± 0.40
	1:1	^{b2}8.58 ± 0.42	^{c2}1.36 ± 0.12	^{ab2}0.60 ± 0.08	^{b2}1.84 ± 0.14
	2:1	^{b3}7.07 ± 0.73	^{d3}2.37 ± 0.15	^{a1}0.27 ± 0.02	^{b2}1.60 ± 0.02
50	0	^{a3}0.21 ± 0.01	^{a1}0.01 ± 0.00	^{c1}0.16 ± 0.00	^{a1}0.04 ± 0.01
	1:10	^{a3}1.23 ± 0.04	^{b2}0.43 ± 0.02	^{c1}0.15 ± 0.00	^{a1}0.04 ± 0.00
	1:5	^{a1}3.24 ± 0.31	^{b2}0.44 ± 0.02	^{bc1}0.14 ± 0.00	^{a1}0.03 ± 0.00
	1:2	^{a3}4.84 ± 0.44	^{c1}0.71 ± 0.05	^{a1}0.09 ± 0.00	^{b1}0.09 ± 0.00
	1:1	^{b3}18.2 ± 1.23	^{c1}0.68 ± 0.02	^{b2}0.12 ± 0.00	^{b1}0.08 ± 0.00
	2:1	^{c4}36.4 ± 2.24	^{d2}0.88 ± 0.04	^{abc1}0.14 ± 0.00	^{a1}0.04 ± 0.00
75	0	^{a4}0.45 ± 0.08	^{a2}0.03 ± 0.00	^{a2}0.20 ± 0.00	^{a1}0.03 ± 0.00
	1:10	^{bc3}1.38 ± 0.21	^{b3}0.76 ± 0.02	^{a2}0.42 ± 0.02	^{ab1}0.14 ± 0.00
	1:5	^{b1}1.25 ± 0.28	^{b3}0.76 ± 0.02	^{a2}0.60 ± 0.76	^{ab1}0.60 ± 0.76
	1:2	^{b2}1.31 ± 0.19	^{d1}1.34 ± 0.02	^{a2}13.2 ± 0.76	^{ab1}1.35 ± 0.14
	1:1	^{c1}2.09 ± 0.41	^{c1}1.00 ± 0.09	^{a2}8.14 ± 0.40	^{ab1}1.29 ± 0.11
	2:1	^{d3}5.60 ± 0.27	^{de2}1.17 ± 0.06	^{b4}6.71 ± 0.70	^{c1}2.25 ± 0.14
100	0	^{a2}0.07 ± 0.00	^{a3}0.08 ± 0.00	^{a1}0.00 ± 0.00	^{a1}0.02 ± 0.00
	1:10	^{b2,3}0.71 ± 0.02	^{b2}0.40 ± 0.00	^{b2}0.24 ± 0.00	^{a1}0.03 ± 0.00
	1:5	^{c1}1.01 ± 0.03	^{d4}1.93 ± 0.04	^{b2}0.31 ± 0.02	^{b1}0.04 ± 0.00
	1:2	^{d2,3}1.82 ± 0.04	^{e1}1.32 ± 0.09	^{b2}0.23 ± 0.02	^{c1}0.05 ± 0.00
	1:1	^{d1}1.71 ± 0.05	^{c1}0.56 ± 0.05	^{a1}0.08 ± 0.00	^{c}0.03 ± 0.00

	2:1	^{e2}3.05 ± 0.07	^{bc1}0.43 ± 0.05	^{c3}1.09 ± 0.05	^{a1}0.03 ± 0.00

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741 * Same letters indicate no statistical differences ($p > 0.05$) in amendment levels within

742 each aging time while different letters indicate significant differences ($p < 0.05$) across

743 amendment levels within each aging time

744 * Same numbers indicate no statistical differences ($p > 0.05$) in aged amended soils

745 across the four sampling points while different numbers indicate significant differences

746 ($p < 0.05$) in aged amended soils across the four sampling points (1d to 100d).

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