

1 **Impact of Organic Amendments on the Development of Phenanthrene**

2 **Catabolism in Soil**

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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 <sup>14</sup>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 <sup>14</sup>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

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38 **1. Introduction**

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

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

57 Soils, sediments and other ubiquitous components such as microplastics can act as sinks  
58 for PAHs in the environment through sorption to soil mineral and organic fractions  
59 (Xiao *et al.*, 2014; Fries and Zarfl, 2012; Lee *et al.*, 2014). This influences the mobility,  
60 bioavailability and biodegradation and, as a result, may influence the persistence of  
61 these contaminants (Riding *et al.*, 2013). Biodegradation is a major mechanism for the  
62 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

113 had been amended with two organic wastes (SBG and SMC). Changes in catabolic  
114 activity were quantified by measuring the kinetics of  $^{14}\text{C}$ -phenanthrene mineralization to  
115  $^{14}\text{CO}_2$  and changes in microbial numbers over time.

116

## 117 **2. Materials and Methods**

### 118 *2.1 Soil and organic waste collection*

119 Soil (A horizon; 5–20 cm) was collected from a field belonging to Myerscough  
120 Agricultural College (Preston, UK). Soil samples were air-dried, homogenized by  
121 sieving through a 2mm mesh to remove plant debris, stones, and larger residue  
122 fragments and stored at  $4^{\circ}\text{C}$  in the dark until use. General soil properties are described  
123 in Table S1 (Couling et al., 2010). Fresh SBG and spent SMC were collected from  
124 Lancaster Brewery (Lancaster, UK) and Drinkwater's Mushrooms Ltd (Galgate, UK),  
125 respectively. General properties are described in Table S2. SMCs were pasteurized at  
126  $60^{\circ}\text{C}$  for 10-12 h prior to sampling. The materials used for SMC preparation were  
127 casing soil (peat), mushroom spawn, gypsum, water, and manure. Freshly collected  
128 organic wastes were homogenized by mixing and then stored in a sterile air-tight high-  
129 density polyethylene bags at a temperature of  $4^{\circ}\text{C}$ .

130

### 131 *2.2 Experimental set-up and amendment conditions*

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

136 and a control soil without amendment. The same water holding (60%) was maintained  
137 for all treatments throughout the study (water loss determined gravimetrically). The  
138 waste-soil mixtures, including the controls and blanks, were immediately transferred to  
139 amber glass bottles (to prevent photo-oxidation) and incubated in the dark at  $21 \pm 1^\circ\text{C}$   
140 over a period of up to 100 days and sampled at 1, 25, 50, 75 and 100 days soil-PAH  
141 contact time.

142

### 143 *2.3 Mineralization of $^{14}\text{C}$ -phenanthrene in soil-waste mixtures*

144 The impact of organic amendments on the catabolic potential of microbial community  
145 on  $^{14}\text{C}$ -phenanthrene mineralization to  $^{14}\text{CO}_2$  for each sampling time (1, 25, 50, 75 and  
146 100 days soil-PAH contact time) on the amended and/or aged soil was measured in  
147 triplicate using respirometry, which was carried out using modified 250 ml Schott  
148 bottles (Reid et al., 2001; Semple et al., 2006). At each time point,  $10 \pm 0.2$  g (dry  
149 weight) of the  $^{12}\text{C}$ -phenanthrene spiked soil and 30 ml of deionized water was added to  
150 a respirometer. A [ $^{14}\text{C}$ ] phenanthrene standard ( $100 \text{ Bq g}^{-1}$  soil) was then added to the  
151 respirometer and placed on a flat-bed orbital shaker at 100 rpm and incubated for 14  
152 days at  $21 \pm 1^\circ\text{C}$ . The  $^{14}\text{CO}_2$  was trapped in 1 M NaOH (1 ml) in a vial suspended from  
153 the lid of the respirometer and sampled bihourly for 1 d and then every 24 h for 14 days  
154 and measured by liquid scintillation analyzer (LSC, Canberra Packard Tri-  
155 Carb2250CA) using standard protocols for counting and automatic quench correction  
156 (Semple et al., 2006).

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

161 taken before <sup>14</sup>C- phenanthrene mineralization reached 5%), changes in the maximum  
162 rate of <sup>14</sup>CO<sub>2</sub> evolution (fastest rate of mineralization resulting from microbial  
163 degradation), and the cumulative extent of mineralization of <sup>14</sup>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<sub>2</sub> 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<sup>-1</sup> <sup>12</sup>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



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

198

### 199 **3. Results**

200 The development of phenanthrene catabolism was measured in a soil amended with  
201 1:10, 1:5, 1:2, 1:1 and 1:2 SBG:soil and SMC:soil ratios, respectively. Changes in  
202 kinetics of  $^{14}\text{C}$ -phenanthrene biodegradation were measured for 14 days after 1, 25, 50,  
203 75 and 100 days of soil-PAH contact time (Figures 1 and 2). The influence of each  
204 organic amendment was assessed by measuring changes in the lag phases, rates and  
205 extents of  $^{14}\text{C}$ -phenanthrene mineralization to  $^{14}\text{CO}_2$  (Tables 1 and 2). Changes in the  
206 total heterotrophic and phenanthrene degrading bacteria and fungi were also measured  
207 over the course of the incubation.

208

209 *3.1 Influence of spent brewery grains on mineralization of  $^{14}\text{C}$ -phenanthrene in soils*

210 The length of the lag phase (days) was measured in the SBG-amended soil incubations.  
211 Throughout the investigation, the lag phases were significantly shorter ( $p < 0.05$ ) in the  
212 soils amended with lower amounts of SBG (1:10 and 1:5). In particular, the shortest lag  
213 phases were observed for 1:5 SBG:soil ratio at 100 d of soil-PAH contact time, while  
214 the longest lag phases were observed in soils amended with 2:1 SBG:soil ratio ( $p <$   
215  $0.05$ ), which did not reach 5%. Soil incubations amended with SBG (1:10) were found  
216 to have significantly shorter lag phases ( $p < 0.05$ ) than 1:5 SBG:soil incubations after 1  
217 d and 25 d aging (Table 1 and Figure 1). There were no significant differences ( $p >$   
218  $0.05$ ) between the control and 1:2 SBG:soil incubations, after 1 d soil-PAH contact time  
219 and no measurable lag phases were observed for 1:1 and 2:1 SBG:soil incubations.  
220 Noticeably, soil-PAH contact time reduced the lag phases in all of the amended  
221 conditions and control incubations except 1:1 and 2:1 SBG:soil ratios; an effect which is  
222 reflected especially in the lag phases after 100 days ( $p < 0.05$ ), compared to previous  
223 time points. With respect to the amount of SBG added to the soil, lower organic waste  
224 additions resulted in shorter lag-phases (1:10>1:5>1:2>1:1 > 2:1), although the SBG:soil  
225 ratios (1:2, 1:1 and 2:1) showed significantly reduced lag phases compared with  
226 unamended control, at most time points during the incubation period.

227 The influence of increasing amounts of SBG on the fastest rates of  $^{14}\text{C}$ -phenanthrene  
228 mineralization was also measured at each time point over the 100 d incubation (Table 1  
229 and Figure 1). The fastest rates were observed for soils amended with 1:10 and 1:5  
230 waste:soil incubations, while the lowest were in the 2:1 SBG:soil incubations. The rates  
231 of  $^{14}\text{CO}_2$  mineralized in the 1:10 and 1:5 SBG:soil incubations did not significantly  
232 differ ( $p > 0.05$ ). After 100 d soil-PAH contact time, 1:1 and 2:1 SBG:soil incubations  
233 had no effect on rates of  $^{14}\text{C}$ -phenanthrene mineralized when compared to un-amended  
234 soil.

235

236 The influence of increasing amounts of SBG on the extents of  $^{14}\text{C}$ -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  $^{14}\text{C}$ -phenanthrene mineralized to  
240  $^{14}\text{CO}_2$  (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  $^{14}\text{CO}_2$   
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  $^{14}\text{CO}_2$  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  $^{14}\text{CO}_2$  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  $^{14}\text{CO}_2$  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

259 3.2 Enumeration of culturable bacterial and fungal heterotrophs and phenanthrene  
260 degraders in SBG-amended soils

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

274

275 Phenanthrene-degrading bacterial numbers did not statistically correlate with fastest rate  
276 of <sup>14</sup>C-phenanthrene mineralized in all amended soils throughout this study (1 d to 100  
277 d) (Figure S1), irrespective of the high numbers of phenanthrene-degraders observed in  
278 SBG amended soils. Correlations between phenanthrene degraders and total extents of  
279 <sup>14</sup>C-phenanthrene mineralization showed a significantly weak but negative correlation  
280 ( $r^2 = 0.40$ ,  $p = 0.02$ ) with bacterial numbers in 1:5 SBG:soil. Also, the extent of <sup>14</sup>C-  
281 phenanthrene mineralized for higher dose of SBG added to soils (1:2 and 2:1) showed  
282 strong negative and strong positive relationships ( $r^2 = 0.50$ ,  $p = 0.003$  and  $r^2 = 0.61$ ,  $p =$   
283  $0.001$ ) 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  $^{14}\text{C}$ -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

308 3.3 Influence of spent mushroom compost on mineralization of <sup>14</sup>C-phenanthrene in  
309 soils

310 In soils amended with SMC, the length of lag phases were also measured at each time  
311 point over the 100 d incubation. It was observed that the lag phases generally decreased  
312 in all of the treatments with increased soil-PAH contact time (Figure 2 and Table 2).  
313 After 1 day of soil incubation, the lag phase observed for control soils were generally not  
314 significantly different ( $p > 0.05$ ) from any of the SMC:soil conditions. However,  
315 significant reductions ( $p < 0.05$ ) in the lag phases, after 25 days soil-PAH contact time,  
316 were observed for all SMC:soil conditions as compared to 1 day contact time. In addition,  
317 at 25 d soil-PAH contact time, the SMC-amended soils except 2:1 showed significantly  
318 shorter lag phases compared with the unamended control condition. These observed  
319 reductions were not significantly different from the other contact points onwards (50 to  
320 100 days). Data showed that the 2:1 SMC:soil incubation displayed the longest lag phases  
321 ( $p > 0.05$ ) compared to the other conditions and control throughout the study period..

322

323 The impact of soil-PAH contact time and the amount of SMC on the fastest rate of <sup>14</sup>C-  
324 phenanthrene mineralization in soil were studied over 100 d. For all amendment  
325 conditions, increases in soil-PAH contact time resulted in faster rates within each SMC-  
326 soil treatment, ranging from 0.40% to a maximum of 2.0%. Similarly, the greatest  
327 change was observed from 1 d (0.40 – 0.60%) to 100 d (0.80 – 1.90%) soil-PAH contact  
328 time: the 1:5 SMC-soil amendment displayed the fastest rate of <sup>14</sup>CO<sub>2</sub> mineralized  
329 (2.37% <sup>14</sup>CO<sub>2</sub> h<sup>-1</sup>) after 100 d soil-PAH contact time. For control soils, the fastest rates  
330 of mineralization were not significantly lower ( $p > 0.05$ ) when compared to all SMC

331 amended soils except for 1:5 SMC:soil condition, which had significantly faster rates at  
332 1 and 25 d soil-PAH contact time (both  $p < 0.05$ ).

333

334 The influence of increasing amounts of SMC in soil on the extents of  $^{14}\text{C}$ -phenanthrene  
335 mineralization was also determined over a 100 d period. From this study, the lower  
336 SBG:soil ratios (1:10 and 1:5) exhibited significantly greater ( $p < 0.05$ ) extents of  $^{14}\text{C}$ -  
337 phenanthrene mineralized (50 and 100 d) compared to all other treatment (Table 2).

338 Following 1 d and 25 d of soil-PAH contact time, there were no significant differences  
339 in the overall extents in all SMC-amended soils ( $p > 0.05$ ) when compared to their non-  
340 equivalent amendment conditions and control soils. Higher extent of mineralization was  
341 observed for all amendment conditions, except for the largest dose rate (2:1) from 50 to  
342 100 d soil-PAH contact time. In particular, the lower doses (1:5 and 1:10) displayed  
343 higher extents of mineralization (50 to 100 d) in comparison with the other conditions.  
344 Furthermore, soils amended with SBG showed the highest extents of mineralization  
345 after 75 d but this was not statistically different from 50 d soil-PAH contact time.

346

#### 347 *3.4 Enumeration of culturable bacterial and fungal heterotrophs and phenanthrene* 348 *degraders in SMC-amended soils*

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

355 2:1 SMC-soil conditions displayed higher bacterial and fungal numbers (both  
356 heterotrophs and phenanthrene-degraders) before mineralization throughout the 100 d  
357 incubation ( $p < 0.05$ ). The growth of bacteria in amended soils were significantly  
358 influenced depending on the SMC dosage application. After 1 d aging, higher organic  
359 materials added to soils resulted in greater increases in the bacterial numbers in the  
360 following order: 2:1>1:1>1:2:1:5>1:10). Similarly, heterotrophs and phenanthrene-  
361 degraders showed higher CFUs in soil amended with higher dose of SMC in rest of the  
362 study periods (25 to 100 d).

363

364 Relationships between rates and extents of  $^{14}\text{C}$ -phenanthrene mineralized with  
365 phenanthrene-degrading bacterial numbers for all amended soils over the 100 incubation  
366 were explored (Table 4 and Figure S3). The 1:1 SMC-soil incubation showed a strong  
367 negative relationship for both fastest rates ( $r^2 = 0.76$ ,  $p = 0.000$ ) and overall extents ( $r^2 =$   
368  $0.69$ ,  $p = 0.000$ ) with phenanthrene-degrading bacteria, whilst a weak but significant  
369 positive correlation was found between extent and phenanthrene-degrading numbers in  
370 1:10 SMC:soil incubation ( $r^2 = 0.30$ ,  $p = 0.04$ ). In addition, both 1:5 and 2:1 SMC:soil  
371 incubations displayed weak positive ( $r^2 = 0.30$ ,  $p = 0.05$ ) and weak negative ( $r^2 = 0.31$ ,  $p$   
372  $= 0.03$ ) correlations between the rate of mineralization and phenanthrene-degraders,  
373 respectively.

374

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



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

387

#### 388 **4. Discussion**

##### 389 *4.1 Organic amendment ratios on $^{14}\text{C}$ -phenanthrene mineralization*

390 The addition of nitrogen-rich nutrients (biostimulation) and potentially viable microbes  
391 (bioaugmentation) through organic materials application to soils are two effective  
392 approaches for the bioremediation of PAH-contaminated soils (Wang et al., 2016). The  
393 results in the present study showed that contact time influenced the development of  
394 catabolic activity as defined by decreases in the length of the lag phases and increases in  
395 the rates and extents of mineralization of  $^{14}\text{C}$ -phenanthrene in amended soils. This  
396 agrees with findings reported from previous studies (Abioye et al., 2012; Adam et al.,  
397 2015), indicating an organic waste stimulatory effect on the biodegradation of  
398 phenanthrene. Generally, the addition of SBG and SMC stimulated phenanthrene  
399 catabolism in soils, especially with lower amendment ratios (1:5 and 1:10). Application  
400 of higher mix ratios (especially 2:1 organic material to soil) of both amendment types  
401 largely reduced the extent of mineralization. Das et al. (2011) also noted that due to the  
402 very high content of organic materials, microbes may metabolize the readily degradable  
403 substrate as carbon source, rather than the target PAH and this was further reflected in  
404 the microbial population after mineralization. The microcosm for the higher amendment

405 (2:1) could have limited oxygen transport for microbial activity, hence a reduced  
406 mineralization, due to the nature of water saturated bulky material formed after  
407 amendment. In addition, this could be linked to high sorptive capacity of organic  
408 materials for PAH and subsequent decrease in bioavailability of PAH for microbial  
409 degradation (Rhodes et al., 2008). More so, the degree of contaminant sorption and their  
410 rapidly/slowly desorbing fractions in amended soils are important factors that determine  
411 the extent of microbial sequestration and transformation (Rhodes et al., 2012). The  
412 decrease in the catabolic response by the higher amendment (2:1) agrees with previous  
413 studies on organic additives addition to PAHs contaminated soils (Namkoong et al.,  
414 2002; Semboung Lang et al., 2016).

415

416 In this study, soils amended with SBG and SMC (1:10 and 1:5) showed significantly  
417 shorter lag phases over time, while the lag phase of organic waste-soil mixture (2:1) was  
418 immeasurable compared to other amendment conditions. This clearly indicated that  
419 appropriate amounts of amendments could significantly influence microbial metabolism  
420 of the target contaminant (Schaefer and Juliane, 2007). In this study, the lower organic  
421 waste-soil mixtures indicated stimulated microbial action as a result of adaptation and  
422 bioavailability of PAH fraction as observed in the lag phase compared to the higher  
423 ones. Our results found that after 100 d soil incubation, both SGB and SMC showed a  
424 further reduction in lag phases with a substantive extent of  $^{14}\text{CO}_2$  mineralized indicating  
425 stimulatory effects of the supplements and acclimatization of the degrading microbial  
426 populations. Several previous studies have demonstrated that the extent of PAHs-  
427 association with soil matrices could potentially facilitate microbial adaptation and  
428 subsequently reduce the lag phase for microbial degradation (Oyelami et al., 2015;  
429 Okere et al., 2017). In this current study, the fastest rates of mineralization revealed a

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

445

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

478

## 479 **5. Conclusion**

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

490

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497

498

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669

670 **Figure legends**

671

672 **Figure 1.** Development of the catabolism of  $^{14}\text{C}$ -phenanthrene to  $^{14}\text{CO}_2$  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  $^{14}\text{C}$ -phenanthrene to  $^{14}\text{CO}_2$  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).

681 Table 1. Catabolic profile of <sup>14</sup>C-labelled phenanthrene in soil amended with different  
 682 proportions of **spent brewery grains** after 14 days respirometric assay. Values are  
 683 mean ± standard error (n = 3).

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

684

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

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

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

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695 Table 2. Catabolic profile of <sup>14</sup>C-labelled phenanthrene in soil amended with different  
696 proportions of **spent mushroom compost** after 14 days respirometric assay. Values are  
697 mean ± standard error (n = 3).

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



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

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

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

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709 Table 3. Autochthonous heterotrophic and phenanthrene-degrading microorganisms  
 710 present before 14 days mineralization of <sup>14</sup>C-phenanthrene in **spent brewery grains**  
 711 amended soil. Values are mean ± standard error (n = 3)

Contact time (days)	Amendment (%)	Bacteria CFU x 10 <sup>8</sup> g <sup>-1</sup> soil dw		Fungi CFU x 10 <sup>6</sup> g <sup>-1</sup> soil dw	
		Heterotrophs	PHE-degraders	Heterotrophs	PHE-degraders
<b>1</b>	0	a <sup>2</sup> 11.6 ± 0.49	a <sup>4</sup> 2.91 ± 0.03	a <sup>2</sup> 0.23 ± 0.00	a <sup>1</sup> 0.03 ± 0.00
	1:10	cd <sup>4</sup> 17.1 ± 0.38	c <sup>3</sup> 15.9 ± 0.34	c <sup>1</sup> 5.71 ± 0.54	c <sup>2</sup> 3.16 ± 0.45
	1:5	b <sup>4</sup> 13.8 ± 0.41	c <sup>3</sup> 13.8 ± 0.41	b <sup>1</sup> 4.44 ± 0.34	d <sup>2</sup> 3.72 ± 0.06
	1:2	a <sup>3</sup> 9.37 ± 0.48	c <sup>3</sup> 5.02 ± 0.45	d <sup>1,2</sup> 9.52 ± 0.32	e <sup>2</sup> 4.52 ± 0.65
	1:1	bc <sup>2</sup> 15.9 ± 0.18	ab <sup>3</sup> 2.91 ± 0.29	f <sup>1</sup> 11.2 ± 0.12	c <sup>2</sup> 3.11 ± 0.33
	2:1	de <sup>5</sup> 19.0 ± 1.04	ab <sup>3</sup> 2.45 ± 1.23	e <sup>5</sup> 10.2 ± 0.20	b <sup>1</sup> 2.33 ± 0.31
<b>25</b>	0	a <sup>2</sup> 0.40 ± 0.03	b <sup>5</sup> 4.05 ± 0.20	a <sup>2</sup> 0.30 ± 0.01	a <sup>1</sup> 0.02 ± 0.00
	1:10	f <sup>3</sup> 13.7 ± 0.17	e <sup>3</sup> 14.1 ± 0.34	b <sup>2,4</sup> 7.76 ± 0.80	b <sup>2</sup> 3.16 ± 0.40
	1:5	e <sup>3</sup> 11.9 ± 0.15	d <sup>3</sup> 12.8 ± 0.26	b <sup>2</sup> 8.23 ± 0.84	b <sup>5</sup> 5.74 ± 0.90
	1:2	b <sup>4</sup> 6.20 ± 0.19	c <sup>3</sup> 6.79 ± 0.17	c <sup>1</sup> 12.0 ± 0.77	c <sup>3</sup> 9.92 ± 0.70
	1:1	d <sup>1</sup> 10.4 ± 0.15	f <sup>4</sup> 16.0 ± 0.29	c <sup>2</sup> 14.0 ± 1.02	c <sup>3</sup> 9.07 ± 0.74
	2:1	c <sup>4</sup> 8.40 ± 0.08	a <sup>1</sup> 0.00 ± 0.00	a <sup>4</sup> 2.36 ± 0.10	b <sup>2</sup> 5.32 ± 0.34
<b>50</b>	0	a <sup>2</sup> 0.05 ± 0.04	a <sup>2</sup> 4.18 ± 0.19	a <sup>2</sup> 0.05 ± 0.00	a <sup>2</sup> 0.05 ± 0.00
	1:10	b <sup>5</sup> 19.0 ± 0.76	a <sup>2</sup> 0.41 ± 0.08	c <sup>2</sup> 7.22 ± 0.77	b <sup>2</sup> 0.24 ± 0.01
	1:5	b <sup>5</sup> 18.3 ± 0.68	b <sup>2</sup> 1.97 ± 0.22	b <sup>1</sup> 4.09 ± 0.40	c <sup>1</sup> 0.41 ± 0.02
	1:2	b <sup>5</sup> 22.2 ± 1.06	b <sup>2</sup> 1.67 ± 0.09	e <sup>1,2</sup> 14.0 ± 0.94	c <sup>1</sup> 0.41 ± 0.05
	1:1	b <sup>2</sup> 21.1 ± 4.96	b <sup>2</sup> 1.46 ± 0.18	d <sup>1</sup> 10.2 ± 0.70	d <sup>1</sup> 0.61 ± 0.03
	2:1	a <sup>1,2</sup> 0.14 ± 0.01	a <sup>3</sup> 0.45 ± 0.05	a <sup>3</sup> 0.99 ± 0.08	b <sup>1</sup> 0.26 ± 0.01
<b>75</b>	0	a <sup>2</sup> 0.09 ± 0.03	a <sup>1</sup> 0.02 ± 0.00	a <sup>4</sup> 1.09 ± 0.03	a <sup>2</sup> 0.05 ± 0.00
	1:10	b <sup>2</sup> 0.92 ± 0.02	b <sup>2</sup> 0.36 ± 0.01	b <sup>4</sup> 10.6 ± 0.30	a <sup>1</sup> 0.85 ± 0.04
	1:5	c <sup>2</sup> 2.01 ± 0.07	c <sup>2</sup> 0.77 ± 0.03	bc <sup>3</sup> 12.1 ± 0.37	bc <sup>3</sup> 4.73 ± 0.48
	1:2	d <sup>4</sup> 1.48 ± 0.04	d <sup>4</sup> 1.14 ± 0.05	bc <sup>1,2</sup> 14.7 ± 0.34	d <sup>3</sup> 7.64 ± 0.70
	1:1	e <sup>2</sup> 2.97 ± 0.09	c <sup>2</sup> 0.87 ± 0.07	c <sup>2</sup> 16.6 ± 0.41	cd <sup>3</sup> 6.37 ± 0.37
	2:1	a <sup>2,3</sup> 2.73 ± 0.03	a <sup>2</sup> 0.04 ± 0.00	a <sup>2</sup> 1.00 ± 0.92	b <sup>2</sup> 3.88 ± 0.18
<b>100</b>	0	a <sup>1</sup> 0.05 ± 0.00	a <sup>3</sup> 0.11 ± 0.00	a <sup>3</sup> 0.49 ± 0.02	a <sup>1</sup> 0.03 ± 0.00
	1:10	b <sup>1,2</sup> 0.92 ± 0.00	a <sup>1</sup> 0.15 ± 0.00	c <sup>5</sup> 10.3 ± 0.59	b <sup>2</sup> 3.76 ± 0.18
	1:5	d <sup>1</sup> 2.01 ± 0.11	b <sup>2</sup> 1.32 ± 0.04	f <sup>4</sup> 17.9 ± 0.18	c <sup>4</sup> 6.24 ± 0.18
	1:2	c <sup>1</sup> 1.48 ± 0.07	c <sup>1,2</sup> 1.45 ± 0.04	e <sup>2</sup> 16.5 ± 0.24	c <sup>3</sup> 6.88 ± 0.30
	1:1	e <sup>1</sup> 2.97 ± 0.16	a <sup>1</sup> 0.11 ± 0.00	d <sup>2</sup> 15.0 ± 0.15	c <sup>1</sup> 7.34 ± 0.53

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2:1	<sup>e3</sup> 2.73 ± 0.06	<sup>a2</sup> 0.07 ± 0.00	<sup>b2</sup> 1.98 ± 0.08	<sup>a1</sup> 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 <sup>14</sup>C-phenanthrene in **spent mushroom**

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

Contact time (days)	Amendment (%)	Bacteria CFU x 10 <sup>8</sup> g <sup>-1</sup> soil dw		Fungi CFU x 10 <sup>6</sup> g <sup>-1</sup> soil dw	
		Heterotrophs	PHE-degraders	Heterotrophs	PHE-degraders
<b>1</b>	0	a <sup>2</sup> 0.07 ± 0.00	a <sup>3</sup> 0.14 ± 0.01	c <sup>1</sup> 0.18 ± 0.01	c <sup>2</sup> 0.13 ± 0.00
	1:10	b <sup>1</sup> 0.42 ± 0.02	b <sup>1</sup> 0.14 ± 0.00	b <sup>1</sup> 0.16 ± 0.00	d <sup>1</sup> 0.16 ± 0.00
	1:5	c <sup>1</sup> 0.67 ± 0.02	c <sup>1</sup> 0.23 ± 0.04	b <sup>1</sup> 0.15 ± 0.00	c <sup>2</sup> 0.12 ± 0.00
	1:2	d <sup>1</sup> 0.98 ± 0.01	d <sup>1,2</sup> 1.51 ± 0.04	b <sup>1</sup> 0.15 ± 0.00	b <sup>2</sup> 0.10 ± 0.00
	1:1	e <sup>1</sup> 1.05 ± 0.01	e <sup>2</sup> 2.15 ± 0.00	a <sup>1</sup> 0.06 ± 0.00	c <sup>1</sup> 0.12 ± 0.00
	2:1	f <sup>1</sup> 1.46 ± 0.01	f <sup>4</sup> 2.60 ± 0.00	b <sup>2</sup> 0.51 ± 0.00	a <sup>1</sup> 0.02 ± 0.00
<b>25</b>	0	a <sup>1</sup> 0.03 ± 0.00	a <sup>3</sup> 0.16 ± 0.03	a <sup>2</sup> 0.32 ± 0.06	a <sup>3</sup> 0.29 ± 0.05
	1:10	a <sup>4</sup> 7.02 ± 0.31	b <sup>4</sup> 0.74 ± 0.05	c <sup>2</sup> 0.92 ± 0.10	b <sup>2</sup> 1.59 ± 0.31
	1:5	c <sup>2</sup> 14.3 ± 0.35	c <sup>5</sup> 1.36 ± 0.04	bc <sup>2</sup> 0.69 ± 0.02	b <sup>2</sup> 1.67 ± 0.16
	1:2	c <sup>4</sup> 13.9 ± 0.80	c <sup>3</sup> 1.42 ± 0.14	ab <sup>3</sup> 0.60 ± 0.08	b <sup>2</sup> 2.31 ± 0.40
	1:1	b <sup>2</sup> 8.58 ± 0.42	c <sup>2</sup> 1.36 ± 0.12	ab <sup>2</sup> 0.60 ± 0.08	b <sup>2</sup> 1.84 ± 0.14
	2:1	b <sup>3</sup> 7.07 ± 0.73	d <sup>3</sup> 2.37 ± 0.15	a <sup>1</sup> 0.27 ± 0.02	b <sup>2</sup> 1.60 ± 0.02
<b>50</b>	0	a <sup>3</sup> 0.21 ± 0.01	a <sup>1</sup> 0.01 ± 0.00	c <sup>1</sup> 0.16 ± 0.00	a <sup>1</sup> 0.04 ± 0.01
	1:10	a <sup>3</sup> 1.23 ± 0.04	b <sup>2</sup> 0.43 ± 0.02	c <sup>1</sup> 0.15 ± 0.00	a <sup>1</sup> 0.04 ± 0.00
	1:5	a <sup>1</sup> 3.24 ± 0.31	b <sup>2</sup> 0.44 ± 0.02	bc <sup>1</sup> 0.14 ± 0.00	a <sup>1</sup> 0.03 ± 0.00
	1:2	a <sup>3</sup> 4.84 ± 0.44	c <sup>1</sup> 0.71 ± 0.05	a <sup>1</sup> 0.09 ± 0.00	b <sup>1</sup> 0.09 ± 0.00
	1:1	b <sup>3</sup> 18.2 ± 1.23	c <sup>1</sup> 0.68 ± 0.02	b <sup>2</sup> 0.12 ± 0.00	b <sup>1</sup> 0.08 ± 0.00
	2:1	c <sup>4</sup> 36.4 ± 2.24	d <sup>2</sup> 0.88 ± 0.04	abc <sup>1</sup> 0.14 ± 0.00	a <sup>1</sup> 0.04 ± 0.00
<b>75</b>	0	a <sup>4</sup> 0.45 ± 0.08	a <sup>2</sup> 0.03 ± 0.00	a <sup>2</sup> 0.20 ± 0.00	a <sup>1</sup> 0.03 ± 0.00
	1:10	bc <sup>3</sup> 1.38 ± 0.21	b <sup>3</sup> 0.76 ± 0.02	a <sup>2</sup> 0.42 ± 0.02	ab <sup>1</sup> 0.14 ± 0.00
	1:5	b <sup>1</sup> 1.25 ± 0.28	b <sup>3</sup> 0.76 ± 0.02	a <sup>2</sup> 0.60 ± 0.76	ab <sup>1</sup> 0.60 ± 0.76
	1:2	b <sup>2</sup> 1.31 ± 0.19	d <sup>1</sup> 1.34 ± 0.02	a <sup>2</sup> 13.2 ± 0.76	ab <sup>1</sup> 1.35 ± 0.14
	1:1	c <sup>1</sup> 2.09 ± 0.41	c <sup>1</sup> 1.00 ± 0.09	a <sup>2</sup> 8.14 ± 0.40	ab <sup>1</sup> 1.29 ± 0.11
	2:1	d <sup>3</sup> 5.60 ± 0.27	de <sup>2</sup> 1.17 ± 0.06	b <sup>4</sup> 6.71 ± 0.70	c <sup>1</sup> 2.25 ± 0.14
<b>100</b>	0	a <sup>2</sup> 0.07 ± 0.00	a <sup>3</sup> 0.08 ± 0.00	a <sup>1</sup> 0.00 ± 0.00	a <sup>1</sup> 0.02 ± 0.00
	1:10	b <sup>2,3</sup> 0.71 ± 0.02	b <sup>2</sup> 0.40 ± 0.00	b <sup>2</sup> 0.24 ± 0.00	a <sup>1</sup> 0.03 ± 0.00
	1:5	c <sup>1</sup> 1.01 ± 0.03	d <sup>4</sup> 1.93 ± 0.04	b <sup>2</sup> 0.31 ± 0.02	b <sup>1</sup> 0.04 ± 0.00
	1:2	d <sup>2,3</sup> 1.82 ± 0.04	e <sup>1</sup> 1.32 ± 0.09	b <sup>2</sup> 0.23 ± 0.02	c <sup>1</sup> 0.05 ± 0.00
	1:1	d <sup>1</sup> 1.71 ± 0.05	c <sup>1</sup> 0.56 ± 0.05	a <sup>1</sup> 0.08 ± 0.00	c <sup>0</sup> 0.03 ± 0.00

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	2:1	<sup>e</sup> 2.3.05 ± 0.07	<sup>bc</sup> 1.0.43 ± 0.05	<sup>e</sup> 3.1.09 ± 0.05	<sup>a</sup> 1.0.03 ± 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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