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# 1 **Microbial community structure changes during** 2 **bioremediation of PAHs in an aged coal-tar contaminated soil** 3 **by in-vessel composting**

4  
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9

10 **Abstract.** The microbial community structure changes of an aged-coal-tar soil  
11 contaminated with polycyclic aromatic hydrocarbons (PAHs) were investigated during  
12 simulated bioremediation at the laboratory-scale using an in-vessel composting  
13 approach. The composting reactors were operated using a logistic 3-factor factorial  
14 design with three temperatures ( $T = 38^{\circ}\text{C}$ ,  $55^{\circ}\text{C}$  or  $70^{\circ}\text{C}$ ), four soil to green-waste  
15 amendment ratios (S:GW = 0.6:1, 0.7:1, 0.8:1 or 0.9:1 on a dry weight basis) and three  
16 moisture contents (MC = 40%, 60% or 80%). Relative changes in microbial populations  
17 were investigated by following the dynamics of phospholipid fatty acid (PLFA)  
18 signatures using a  $^{13}\text{C}$ -labelled palmitic acid internal standard and sensitive GC/MS  
19 analysis during in-vessel composting over 98 days. The results of this investigation  
20 indicated that fungal to bacterial PLFA ratios were significantly influenced by  
21 temperature ( $p < 0.05$ ), and Gram-positive to Gram-negative bacterial ratios were  
22 significantly influenced by temperature ( $p < 0.001$ ) and S:GW ratio ( $p < 0.01$ ) during  
23 in-vessel composting. Additionally, the Gram-positive to Gram-negative bacterial ratios  
24 were correlated to the extent of PAH losses ( $p < 0.005$ ) at  $70^{\circ}\text{C}$ .

25  
26 **Keywords:** Bioremediation; Coal-tar; Soil; Composting; polycyclic Aromatic  
27 hydrocarbons; Phospholipid fatty acids.  
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## 31 **1 Introduction**

32

33 Composting has been demonstrated to be effective in biodegrading PAHs (Potter *et al.*  
34 1999; Canet *et al.* 2001; Cajthaml *et al.* 2002; Antizar-Ladislao *et al.* 2006; Cai *et al.*  
35 2007), chlorophenols (Laine and Jorgensen 1997), polychlorinated biphenyls (PCBs)  
36 (Block 1998), explosives (Ro *et al.* 1998; Li *et al.* 2007) and petroleum hydrocarbons  
37 (Namkoong *et al.* 2002) at both the laboratory and field-scale. In contrast to  
38 conventional composting systems, the use of in-vessel systems for the bioremediation of  
39 contaminated soils provides operators with more control, enabling them to select  
40 suitable operating parameters (e.g., temperature, moisture content, mix ratios) to  
41 promote both microbial activity and contaminant degradation (Antizar-Ladislao *et al.*  
42 2004; Oleszczuk 2006), and also to ensure the use of high temperatures ( $>70^{\circ}\text{C}$ ) in order  
43 to meet regulatory requirements for pathogen control (EC 2003). Thus, in-vessel  
44 composting is presented as a sustainable bioremediation technology to treat  
45 contaminated soils amended with biodegradable municipal solid waste (i.e. green  
46 waste).

47

48 The implementation of in-vessel composting technology as a remediation strategy  
49 requires an understanding of the diversity and ecology of contaminant-degrading  
50 microorganisms. Thus, we have investigated changes within the microbial community  
51 structure in a system of in-vessel composting reactors by using a quantitative approach  
52 to phospholipid fatty acid (PLFA) analysis to detect and measure signature fatty acids  
53 and thereby describe major features of microbial communities by their fatty acid  
54 “fingerprint”. The fatty acyl chains within intact phospholipid molecules in microbial  
55 membranes are rapidly degraded once the cells die; thus, the PLFA extracted from  
56 media such as soil or compost represent the extant living community, both qualitatively  
57 and quantitatively (Carpenter-Boggs *et al.* 1998). PLFA analysis has been used to  
58 monitor fungi in PAH-contaminated soil (Andersson *et al.* 2000) and microbial  
59 community changes during conventional windrow-composting of a non-contaminated  
60 domestic household waste (Klamer and Bääth 1998; Bolta *et al.* 2003; Steger *et al.*  
61 2005), proving to correlate well with other microbial analysis. It has not been used for  
62 in-vessel composting systems.

63

64 The aim of the present work was to investigate the microbial community changes that  
65 occurred during in-vessel composting for the biotreatment of PAHs in an aged coal-tar  
66 contaminated soil, using a quantitative approach to PLFA analysis. Additionally, PLFA  
67 analysis was used to elucidate the influence of temperature, soil to green-waste  
68 amendment and moisture content on the dynamics of the microbial community  
69 structure. Finally, the Gram-positive to Gram-negative bacterial and fungal to bacterial  
70 microbial biomass ratios were evaluated as indicators of microbial community changes  
71 during the in-vessel composting biotreatment process.

72

## 73 **2 Materials and methods**

74

### 75 2.1 Experimental design

76

77 Eighteen experimental conditions were tested in triplicate using 360 laboratory-scale in-  
78 vessel composting reactors. The experimental design comprised three temperature levels  
79 ( $T = 38^{\circ}\text{C}$ ,  $55^{\circ}\text{C}$  or  $70^{\circ}\text{C}$ ) four soil to green-waste ratios ( $S:GW = 0.6:1$ ,  $0.7:1$ ,  $0.8:1$  or

80 0.9:1 on a dry weight basis) and three moisture contents (MC = 40%, 60% or 80%).  
81 Control reactors consisted of 1:0 S:GW ratio. To identify the optimal operational  
82 conditions for maximum PAH losses, we first investigated the influence of S:GW at  
83 three temperature levels and MC = 60%, and then determined the influence of MC at  
84 three temperature levels and the optimal S:GW ratio (Antizar-Ladislao *et al.* 2005a).  
85 The operational parameters investigated in the present work were selected to simulate  
86 the operation of a commercial-scale in-vessel system.

87

## 88 2.2 Contaminated soil

89

90 The coal-tar-contaminated soil was obtained from a manufactured-gas plant site  
91 commissioned in 1838 at Clitheroe, Lancashire, United Kingdom. Prior to  
92 experimentation, the coal-tar-contaminated soil was air-dried and homogenized by  
93 passing through first a 5 mm then a 2 mm sieve; the contaminated soil was stored in the  
94 laboratory at room temperature. The soil contained a post-dilution concentration of  
95 100.3 mg  $\Sigma$ 16 U.S.EPA PAH·kg<sup>-1</sup> soil, the soil organic content was 4.79±0.16%  
96 (w/dw), and the soil pH<sub>w</sub> was 7.3±0.1.

97

## 98 2.3 Green waste

99

100 For the composting studies, the post-diluted soil was conditioned with an artificial green  
101 waste, which was prepared by mixing foodstuff (carrots, cucumber, lettuce, onions,  
102 potatoes and tomatoes in equal amounts) (3% dw), sawdust (38% dw), leaves (17% dw),  
103 grass (27% dw) and wheat straw (14% dw). Foodstuff, sawdust, wheat straw and leaves  
104 were blended individually using a kitchen blender and the grass was cut with scissors.  
105 The composition of the green waste satisfied the nutrient requirement (C:N = 40-50) for  
106 composting according to the calculations using Cornell's system (Richard 1995).

107

## 108 2.4 Reactor design

109

110 A total of 360 glass composting reactors (200ml) were constructed (Antizar-Ladislao *et al.*  
111 *et al.* 2005a). These fully enclosed bench-scale reactors each held about 65 g total  
112 composting mixture. For each glass composting reactor, the composting mixture was  
113 thoroughly mixed in a glass beaker (500 ml), and then introduced into the reactor. Initial  
114 moisture content of the composting mixture was measured and double distilled water  
115 (DDW) was added when needed to reach the desirable moisture content. Composting  
116 moisture content was measured at intervals to ensure that it was maintained at the  
117 required level. The reactor units stood vertically with air flowing continuously to avoid  
118 oxygen content limitation and vented outdoors to avoid volatiles accumulation in the  
119 composting reactors. Air flow up through the composting mixture by means of a  
120 stainless steel air-delivery tube inserted into the bottom of the composting reactors was  
121 provided by 100% oil free diaphragm pumps (Model PXW-600-DIOV, VP1, Fisher  
122 Scientific). The air inlet was bubbled through a DDW reservoir to avoid excessive water  
123 evaporation during aeration. Composting reactors were placed in triplicate for each  
124 condition in temperature-controlled incubators at 38<sup>0</sup>C, 55<sup>0</sup>C or 70<sup>0</sup>C to simulate  
125 representative mesophilic and thermophilic microbiological stages during in-vessel  
126 composting processes (Walter *et al.* 1992; Antizar-Ladislao *et al.* 2004). Further details  
127 of reactor design can be found in Antizar-Ladislao *et al.* (2005b).

128

## 129 2.5 Sample analysis

130

131 Destructive sampling, in triplicate, for each experimental treatment was performed after  
132 0, 21, 56 and 98 days. The entire contents of each reactor were mixed thoroughly, and  
133 sub-samples collected for total organic carbon (TOM), MC, PAH and phospholipid fatty  
134 acid (PLFA) analyses. The TOM of composting mixtures was determined by ashing  
135 using a loss-on-ignition procedure (Faithful 2002). The residual moisture of the samples  
136 was determined to produce the results on a dry matter basis (110°C).

137

138 PAH extraction from compost mixtures and soil was by Accelerated Solvent Extraction  
139 (ASE<sup>TM</sup>) 200, with 22 ml stainless steel extraction cells that meet the requirements for  
140 the extraction of PAHs from solid waste as described in USEPA Method 3545. The  
141 extracts were purified on chromatographic columns packed with 1 g of activated-florisil  
142 (SiO<sub>2</sub>, 84.0%; MgO, 15.5%; Na<sub>2</sub>SO<sub>4</sub>, 0.5%; 60/100 mesh; 130°C; 12 h) and 2 g of  
143 Na<sub>2</sub>SO<sub>4</sub>. A Hewlett Packard 6890 series gas chromatograph (GC) with a 7673 series  
144 auto-sampler and a 5973 series mass selective detector (MS) was used for the analysis.  
145 Data acquisition and processing were achieved using a Hewlett Packard MS  
146 Chemstation (G1034C Version C.02.00). The GC-MS system was calibrated prior to the  
147 analysis of samples using seven calibration standards. The extraction efficiency of this  
148 method using two surrogate standards for the real samples, 1-fluoronaphthalene, 2-  
149 fluorobiphenyl varied between 70 and 98% primarily depending on the volatility of the  
150 compounds. Further details of PAH analysis can be found in Antizar-Ladislao *et al.*  
151 (2005a).

152

153 Sub-samples of compost (2 g) were spiked with 500 µg l<sup>-1</sup> <sup>13</sup>C-palmitic acid  
154 (hexadecanoic acid, internal standard), the total lipid was extracted using the Bligh and  
155 Dyer procedure, and the lipid acyl chains and internal standard fatty acid converted to  
156 FAME using MeOH/H<sub>2</sub>SO<sub>4</sub> (Kates 1985). The FAME were analysed using the same  
157 Hewlett Packard GC/MS system described above. The GC inlet was operated in pulsed  
158 (1.40 min, 40.0 psi) splitless mode at 260°C with helium as carrier gas. The injection  
159 volume was 1 µl and the inlet was purged at 50 ml·min<sup>-1</sup> 20 min after injection; inlet  
160 pressure was controlled by electronic pneumatics to maintain a constant column flow of  
161 1 ml·min<sup>-1</sup>. Separation was achieved using an HP-5MS column (19091S-433 30 m ×  
162 0.25 mm × 0.25 µm). The temperature program comprised 40°C for 3 min, 10°C·min<sup>-1</sup>  
163 to 150°C, 3°C·min<sup>-1</sup> to 230°C, and 30°C·min<sup>-1</sup> to 300°C that was maintained for 5 min to  
164 allow late eluting peaks to exit the column. The MS transfer line was held at 310°C, thus  
165 providing conductive heating of the MS source to about 230°C. The MS was operated in  
166 selective ion monitoring (SIM) mode, using m/z = 74 as the common fragment ion of  
167 FAME. To identify the fatty acids, the retention times were compared with those  
168 obtained for standard bacterial acid methyl esters (Cat. No. 47080-U, Supelco, UK).  
169 The amount of microbial signature acids was calculated using the <sup>13</sup>C-16:0 internal  
170 standard, which gives a characteristic fragment ion m/z = 75 that can be quantified  
171 separately from the bulk <sup>12</sup>C-FAME in the sample. The sum of the following fatty acids  
172 was used to represent total bacteria: i15:0, a15:0, i16:0, i17:0, cy17:0, 18:1ω7c and  
173 cy19:0 (Frostegard and Bääth 1996; Zelles 1999; Bolta *et al.* 2003). Gram-positive  
174 bacteria were represented by i15:0, a15:0 and i17:0 (Buyer *et al.* 1999) and Gram-  
175 negative bacteria by cy17:0, 18:1ω7c and cy19:0 (Klamer and Bääth 1998; Zelles  
176 1999). Thermophilic bacteria were represented by i15:0 and i17:0 (Carpenter-Boggs *et*  
177 *al.* 1998). Fungi were represented by 18:2ω6,9 (Frostegard and Bääth 1996).

## 178 2.6 Statistical analyses

179

180 The effect of different operational parameters during in-vessel composting of a coal-tar  
181 contaminated soil on the evaluated indicators was investigated using a two-way  
182 multivariable ANOVA analysis and *post hoc* Tukey test with StatistiXL Version 1.5.

183

## 184 **3 Results and Discussion**

185

### 186 3.1 Assessment of composting process

187

188 The TOM levels were ~62% at the start of composting and then decreased to ~40% after  
189 98 days treatment at 38<sup>0</sup>C resulting from the occurrence of mineralization. At 55-70<sup>0</sup>C  
190 the TOM decrease was less, indicating that lower mineralization occurred, possibly  
191 because higher temperatures constrained microbial growth (Antizar-Ladislao *et al.*  
192 2005b). Details have been published (Antizar-Ladislao *et al.* 2005a) on the  
193 biodegradation of PAHs in the aged coal-tar contaminated soil under simulated in-  
194 vessel conditions and the influence of temperature (T = 38<sup>0</sup>C, 55<sup>0</sup>C or 70<sup>0</sup>C), the  
195 contaminated soil to green waste (S:GW = 0.6:1, 0.7:1, 0.8:1 or 0.9:1 soil to green waste  
196 mixture ratio on a dry weight basis) and the moisture content (MC = 40%, 60% or  
197 80%). For the purposes of the present work, Table 1 summarizes the concentration of 16  
198 USEPA-listed PAHs following 98 days of continuous in-vessel composting treatment.  
199 Optimal operational conditions for degradation of PAHs in simulated in-vessel  
200 composting units occurred at T = 38<sup>0</sup>C, S:GW = 0.8:1 and MC = 60%, resulting in a  
201 76.7% removal of the total PAH (Antizar-Ladislao *et al.* 2005a). In previous studies we  
202 investigated the relative contributions of chemical and biological processes to the  
203 removal of PAHs (Antizar-Ladislao *et al.* 2005b). At the highest temperature  
204 investigated, most of the microorganisms would be rendered inactive (Antizar-Ladislo  
205 *et al.* 2004), and thus, the removal of PAHs would occur mainly due to volatilisation.  
206 This would indicate that the leading mechanism of removal at 38<sup>0</sup>C was biological,  
207 whereas at 70<sup>0</sup>C it was volatilisation, and most likely a combination of these two  
208 mechanisms at 55<sup>0</sup>C.

209

210 During the course of in-vessel composting, a difference in the microbial communities at  
211 the 18 different operational conditions was observed visually, indicating the obvious  
212 presence of fungal growth during the first three weeks of composting in the reactors  
213 incubated at 38<sup>0</sup>C. Table 2 summarizes the phospholipid fatty acids concentrations  
214 characteristic of Gram-positive and Gram-negative bacteria, and fungi in the  
215 composting mixture at 38<sup>0</sup>C during 98 days of continuous in-vessel composting. The  
216 concentration of PLFA biomarkers of Gram-positive and Gram-negative bacteria, and  
217 fungi generally decreased towards the end of 98 days continuous treatment. At moisture  
218 contents of 40% and 80% no major changes in the concentration of biomarkers of  
219 bacteria were observed throughout the treatment period.

220

### 221 3.2 Analysis of PLFA

222

223 In total, twenty three different microbial PLFA were identified during the composting  
224 process, although only eight were used as biomarkers in this study. The major shifts in  
225 the microbial community during the simulated in-vessel composting treatment could be  
226 ascertained using fungal to bacterial, and Gram-positive to Gram-negative bacterial

227 PLFA ratios. The initial PAH-contaminated soil contained small amounts of PLFA that  
228 were indicative of fungi, giving a low value of the fungal to bacterial ratio ( $\approx 0.03$ );  
229 there were approximately equal proportions of Gram-positive to Gram-negative bacteria  
230 on the basis of signature PLFA content. Figure 1 shows that when this PAH-  
231 contaminated soil was mixed with artificial green waste, the compost mixture (soil +  
232 green waste, S:GW = 0.6:1) contained PLFA indicative of fungi in a higher proportion  
233 than did the initial soil (fungal to bacterial PLFA ratio  $\approx 1.29$ ), with a dominance of  
234 Gram-positive bacteria (Gram-positive to Gram-negative ratio  $\approx 1.97$ ). This indicated  
235 that in the present study, the fatty acids attributed to fungal biomass (i.e., 18:2 $\omega$ 6,9)  
236 were originally present in the green waste, where the highest ratio was observed in the  
237 sawdust (fungal to bacterial PLFA ratio  $\approx 6.84$ ). Frostegard and Bääth (1996) found that  
238 the fungal to bacterial PLFA ratio varied from 0.02-0.04 in different agricultural soils  
239 that were low in organic matter to a ratio of 0.3-0.5 in forest soils that were dominated  
240 by fungal biomass. Bolta *et al.* (2003) found that the fungal to bacterial PLFA ratio in a  
241 composted household organic waste with shredded wood varied from 0.12 to 0.15.  
242 Andersson *et al.* (2000) reported a fungal to bacterial PLFA ratio of 0.5 in an aged  
243 PAH-contaminated soil mixed with birch wood. Fungal to bacterial PLFA ratios  
244 calculated in the present study for the initial PAH-contaminated soil and a mixture of  
245 the same soil with green waste correlate with the fungal to bacterial PLFA ratios  
246 reported in the literature. Thus, according to Frostegard and Bääth (1996) results, the  
247 initial composting mixture was probably dominated by fungal biomass.

248

### 249 3.3 Fungal to bacterial ratio changes during composting

250

251 Figure 2 shows the temporal profile of fungal to bacterial PLFA ratios for all  
252 experimental conditions investigated. Analysis of the relative abundance of the major  
253 microbial groups during composting revealed a high proportion of bacterial biomass  
254 over fungal biomass (fungal to bacterial PLFA ratio  $< 1$ ) for the first three weeks that  
255 was maintained to the end of the experiment after 98 days. The relative proportion of  
256 fungi in the in-vessel composting reactors was lowest at the highest in-vessel  
257 composting temperature investigated, namely 70<sup>0</sup>C ( $p < 0.05$ ). These results are  
258 comparable with the findings of Klamer and Bääth (1998) who reported a rapid  
259 decrease in the fungal to bacterial PLFA ratio from 0.37 to 0.007 during the heating  
260 phase reaching 69<sup>0</sup>C in the composting mixtures of straw materials. However,  
261 Carpenter-Boggs *et al.* (1998) reported that the PLFA markers for fungi (18:2 $\omega$ 6c,  
262 18:3 $\omega$ 6c) did not change significantly over 60 days following a conventional  
263 composting temperature profile that reached a maximum temperature of 60<sup>0</sup>C.

264

265 No significant influence of the S:GW ratio or MC on the fungal to bacterial PLFA ratio  
266 was observed in the composting reactors. Nevertheless, unexpectedly high values of  
267 fungal to bacterial PLFA ratio were observed in the treatments at 40% MC, which could  
268 be due to high values of fungal populations or low values of bacterial populations. In  
269 this study, high absolute values of fungi in the treatments at 40% MC were observed  
270 (Table 2). Low MC levels may facilitate high oxygen concentrations in the composting  
271 mixtures, leading to less stressed bacterial communities (Steger *et al.* 2005).  
272 Nevertheless, the very high value observed here (Fig. 2(b)) might mean that it is an  
273 anomaly due to some experimental error or artefact.

274

275 Fungal to bacterial PLFA ratios were within the range 0.02 to 0.56 after 98 days of  
276 composting treatment, showing that according to Frostegard and Bääth (1996), fungi  
277 probably are an important microbial group for in-vessel composting of a contaminated  
278 soil, and that drastic changes in microbial community structure occur during in-vessel  
279 composting at different operational conditions. Microorganisms degrading PAHs  
280 include various soil fungi, such as *Bjerkandera* sp., *Phanerochaete chrysosporium* and  
281 *Pleurotus ostreatus*, (Antizar-Ladislao *et al.* 2004). Nevertheless, it has been reported  
282 that most common effect of fungi on PAH degradation may be activation and  
283 solubilization of PAHs by non-specific fungal enzymes rather than complete  
284 mineralization (Johnsen *et al.* 2002). No correlation between fungal to bacterial ratios  
285 and PAH losses was found during the length of the in-vessel composting treatment in  
286 this study.

287

### 288 3.4 Gram-positive to Gram-negative bacterial ratio changes during composting

289

290 Figure 3 shows the temporal profile of Gram-positive to Gram-negative bacterial ratios  
291 at all experimental conditions under investigation. Temperature, S:GW ratio and MC all  
292 had a significant influence on the Gram-positive to Gram-negative bacterial ratios  
293 ( $p < 0.05$ ) during the in-vessel composting treatment. Thermophilic organic composting  
294 systems are largely comprised of bacilli and actinomycetes, and thus a higher relative  
295 proportion of Gram-positive bacterial PLFA would be expected at higher temperatures  
296 (Antizar-Ladislao *et al.* 2004). This was observed at 38<sup>0</sup>C and 55<sup>0</sup>C, but not at 70<sup>0</sup>C  
297 following 98 days of continuous in-vessel composting treatment ( $p < 0.001$ ), probably  
298 because 70<sup>0</sup>C is above the upper growth limits of such bacteria (Antizar-Ladislao *et al.*  
299 2004). The proportions of Gram-positive bacteria at 38<sup>0</sup>C and 55<sup>0</sup>C were similar, rising  
300 during the early stages of composting to a plateau after 21 days of treatment that was  
301 maintained to the end of the experiment after 98 days. A similar observation was made  
302 by Carpenter-Boggs *et al.* (1998) who used an initial temperature of 60<sup>0</sup>C decreasing to  
303 near 42<sup>0</sup>C and 22<sup>0</sup>C after an average 28 and 56 days, respectively, in a conventional  
304 composting treatment. A high ratio of Gram-positive (which include thermophiles) to  
305 Gram-negative bacteria, corresponded to the presence of a large amount of branched-  
306 chain fatty acids such as i15:0 and i17:0 that are common in species of *Bacillus*, a genus  
307 well known to be dominant in compost at high temperatures (Beffa *et al.* 1996).

308

309 In previous investigations we have observed that temperature is an important factor  
310 affecting in-vessel composting treatment of the same aged coal-tar contaminated soil  
311 investigated in the present study, with significantly ( $p < 0.01$ ) greater PAH losses due to  
312 biodegradation at 38<sup>0</sup>C than at 70<sup>0</sup>C (Antizar-Ladislao *et al.* 2005b; Antizar-Ladislao *et al.*  
313 2007). Correlations between Gram-positive to Gram-negative bacterial biomass ratio  
314 and PAH concentration in the in-vessel composting reactors following 21, 56 and 98  
315 days of continuous treatment at 38<sup>0</sup>C, 55<sup>0</sup>C or 70<sup>0</sup>C were sought. In general, there was a  
316 tendency (although not significant) where the Gram-positive to Gram-negative bacterial  
317 biomass ratio increased while PAH concentration in the composting mixtures decreased.  
318 This tendency indicated that Gram-positive bacteria were probably responsible for PAH  
319 degradation in this study. This is supported by previous studies which have reported that  
320 Gram-positive dominate the mineralization of PAHs in soil (Kästner *et al.* 1994). A  
321 different tendency was observed in the composting reactors treated at 70<sup>0</sup>C following 98  
322 days of treatment, which showed a slight decrease of Gram-positive to Gram-negative  
323 bacterial ratio at lower PAH concentrations in the composting reactors. These results



324 further indicated that the Gram-positive to Gram-negative bacterial ratio was  
325 significantly influenced by high temperatures (70<sup>0</sup>C) (p<0.001), at which was also  
326 significantly influenced by PAH concentrations (p<0.005). It has been suggested that  
327 Gram-positive nocardioform actinomycetes (*Mycobacterium*, *Rhodococcus* and  
328 *Gordonia*) may play an important role in the mineralization of PAHs (Kästner *et al.*  
329 1994, Larkin *et al.* 2005, Johnsen *et al.* 2002), and those which are also thermophiles  
330 will be probably encountered at higher temperatures in PAH contaminated soils.  
331 Additionally, the Gram-positive to Gram-negative bacterial ratio decreased from 21 to  
332 98 days of in-vessel composting, but only in those bioreactors having the larger  
333 populations (Fig. 2). These results are similar to the findings of Carpenter-Boggs *et al.*  
334 (1998) who reported a decrease of indicators of general bacteria (15:0 and 17:0) and  
335 aerobic bacteria (16:1ω7c) over time.

336

337 The effects of soil to green waste ratio and moisture content on the Gram-positive to  
338 Gram-negative bacterial ratio were also investigated. The relative proportion of PLFA  
339 indicative of Gram-negative bacteria increased with respect to Gram-positive bacteria at  
340 60% moisture content and a soil to green waste ratio of 0.8:1 to 0.9:1 (p<0.01). The  
341 Gram-positive to Gram-negative bacterial ratio changed significantly following the first  
342 21 days of in-vessel composting treatment (p<0.05) but thereafter no significant  
343 changes were observed when the moisture content varied from 40 to 80%. High  
344 moisture contents may lead to low oxygen concentration in the composting mixtures,  
345 particularly in large-scale systems, due the heterogeneous gas transport through the  
346 material in these systems which may eventually result in “local” anaerobic conditions  
347 and inefficient composting. Furthermore, it has been indicated that lower oxygen  
348 concentrations will result in a more stressed bacterial community (Steger *et al.* 2005),  
349 and possibly a lower capacity to metabolise PAHs in contaminated soils during  
350 composting. Thus, a high moisture content (i.e., >80%) is not recommended in practice.

351

#### 352 **4 Conclusions**

353

354 The present investigation of in-vessel composting has shown that it is possible to  
355 correlate changes in the major microbial groups with the bioremediation of an aged-  
356 coal-tar contaminated soil. Specifically, we have shown that the fungal to bacterial  
357 PLFA ratios were significantly influenced by temperature (p<0.05), and Gram-positive  
358 to Gram-negative bacterial ratios were significantly influenced by temperature  
359 (p<0.001) and S:GW ratio (p<0.01) during in-vessel composting. Additionally, the  
360 Gram-positive to Gram-negative bacterial ratios were correlated to the extent of PAH  
361 losses (p<0.005) at 70<sup>0</sup>C. This investigation has reported for the first time a quantitative  
362 approach to analyse microbial community changes in an in-vessel system, by using a  
363 <sup>13</sup>C-labelled fatty acid internal standard. The impact of in-vessel composting operational  
364 parameters (i.e., T, S:GW and MC) on the residential microbial community changes  
365 during the in-vessel composting process was demonstrated.

366

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468 **Table 1** PAH concentrations (mg PAH/kg dry soil,  $\pm$  standard deviation) and removal  
 469 (wt %) after 98 days of continuous in-vessel composting treatment (PAH concentration  
 470 at start:  $100.3 \pm 3.2$  mg  $\Sigma$ PAH /kg dry soil).  
 471

Bioreactor conditions MC/S:GW	Temperature					
	38 °C		55 °C		70 °C	
	$\Sigma$ PAH	Removal, wt %	$\Sigma$ PAH	Removal, wt %	$\Sigma$ PAH	Removal, wt %
60%/0.6:1	23.7 $\pm$ 1.2	76.4	48.0 $\pm$ 0.8	52.2	78.0 $\pm$ 1.4	22.3
60%/0.7:1	18.1 $\pm$ 4.1	82.0	32.4 $\pm$ 7.5	67.7	54.2 $\pm$ 6.7	46.0
60%/0.8:1	23.4 $\pm$ 3.1	76.7	39.6 $\pm$ 9.3	60.6	44.5 $\pm$ 9.4	55.7
60%/0.9:1	31.0 $\pm$ 0.1	69.1	37.7 $\pm$ 7.9	62.4	46.2 $\pm$ 2.2	54.0
80%/0.8:1	31.7 $\pm$ 7.9	68.4	49.0 $\pm$ 7.4	51.2	65.8 $\pm$ 10.4	34.4
40%/0.8:1	61.0 $\pm$ 10.8	39.2	78.3 $\pm$ 0.8	21.9	59.8 $\pm$ 8.1	40.4
Control, 0%/1.0:0	90.8 $\pm$ 0.6	9.5	83.4 $\pm$ 0.7	16.9	57.2 $\pm$ 1.1	43.0

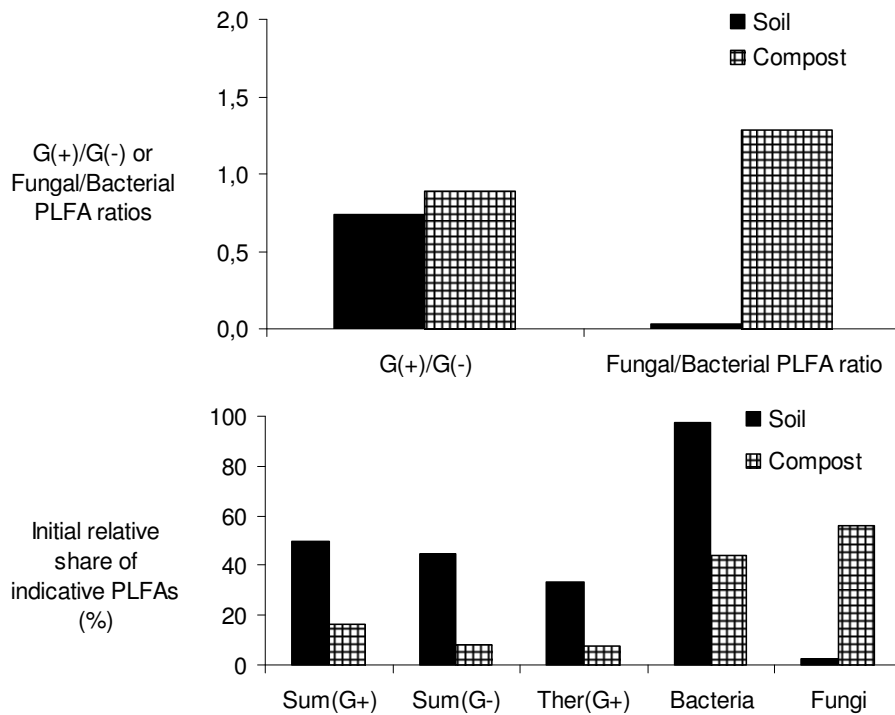
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474 **Table 2** Phospholipid fatty acids ( $\mu\text{g}\cdot\text{g}^{-1}$  dry weight soil,  $\pm$  standard deviation) characteristic of Gram-positive and Gram-negative bacteria,  
 475 and fungi in the composting mixture at  $38^{\circ}\text{C}$  during 98 days of continuous in-vessel composting.  
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Bioreactor conditions MC/S:GW	Gram-positive bacteria			Gram-negative bacteria			Fungi		
	21 days	56 days	96 days	21 days	56 days	96 days	21 days	56 days	96 days
60%/0.6:1	18.9 $\pm$ 1.3	27.3 $\pm$ 5.3	12.9 $\pm$ 1.7	15.9 $\pm$ 6.9	17.1 $\pm$ 1.6	8.0 $\pm$ 7.2	12.5 $\pm$ 3.3	18.2 $\pm$ 2.6	5.2 $\pm$ 0.2
60%/0.7:1	17.2 $\pm$ 2.7	17.5 $\pm$ 1.1	12.9 $\pm$ 2.9	11.0 $\pm$ 0.9	8.8 $\pm$ 1.5	8.1 $\pm$ 2.0	13.3 $\pm$ 2.9	12.8 $\pm$ 2.2	6.6 $\pm$ 1.2
60%/0.8:1	23.2 $\pm$ 6.3	13.1 $\pm$ 1.0	13.0 $\pm$ 1.0	14.8 $\pm$ 0.6	12.3 $\pm$ 7.9	9.4 $\pm$ 4.5	14.5 $\pm$ 1.2	12.4 $\pm$ 3.3	7.0 $\pm$ 3.9
60%/0.9:1	27.6 $\pm$ 4.1	40.2 $\pm$ 8.5	21.8 $\pm$ 9.4	19.6 $\pm$ 4.0	29.4 $\pm$ 5.7	17.3 $\pm$ 3.3	15.5 $\pm$ 2.8	26.3 $\pm$ 17.1	19.4 $\pm$ 15.3
80%/0.8:1	19.6 $\pm$ 7.8	31.0 $\pm$ 9.5	26.2 $\pm$ 10.7	18.1 $\pm$ 9.1	21.6 $\pm$ 9.0	20.9 $\pm$ 12.0	15.0 $\pm$ 0.6	13.2 $\pm$ 0.7	15.8 $\pm$ 9.1
40%/0.8:1	56.8 $\pm$ 6.9	52.4 $\pm$ 8.6	57.0 $\pm$ 0.1	61.4 $\pm$ 8.9	54.2 $\pm$ 7.6	62.6 $\pm$ 0.5	73.0 $\pm$ 0.5	44.0 $\pm$ 0.9	55.8 $\pm$ 5.4

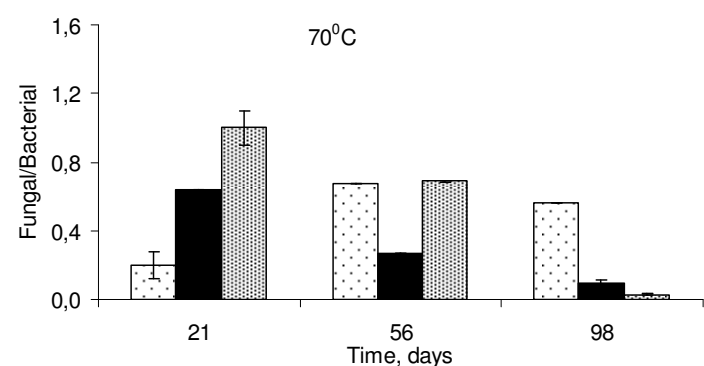
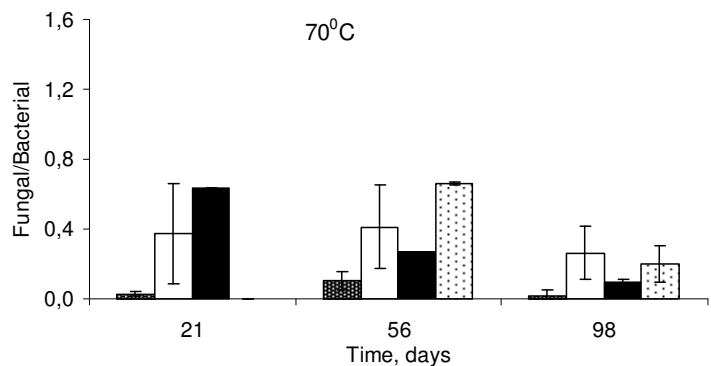
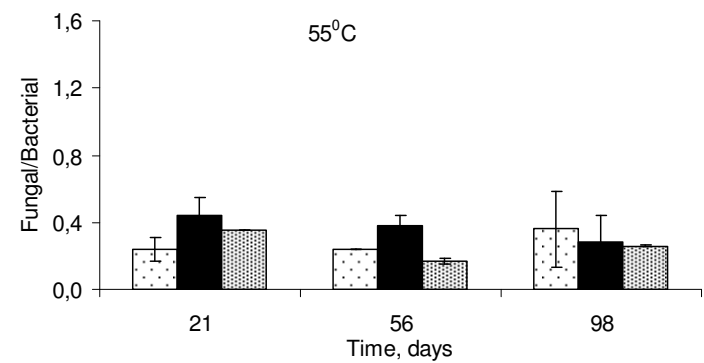
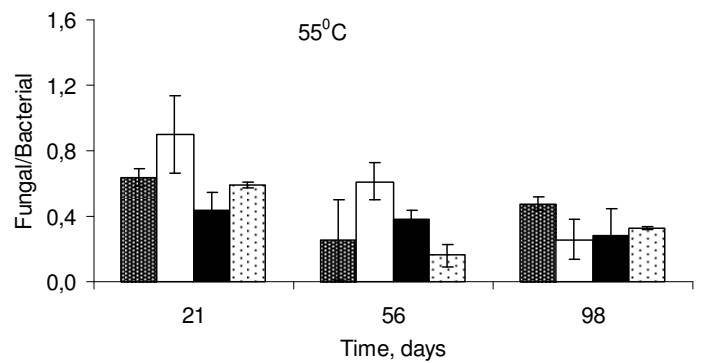
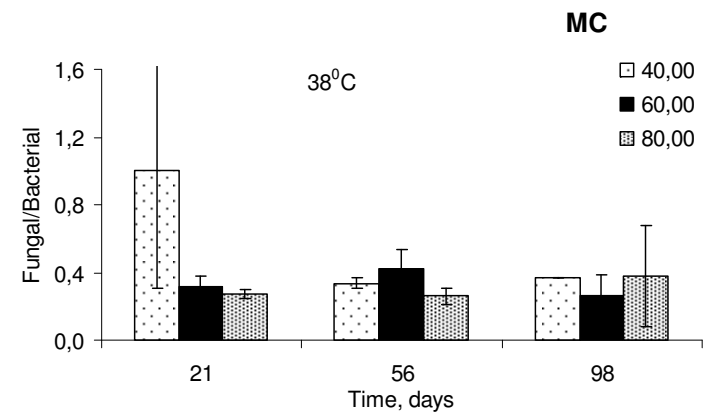
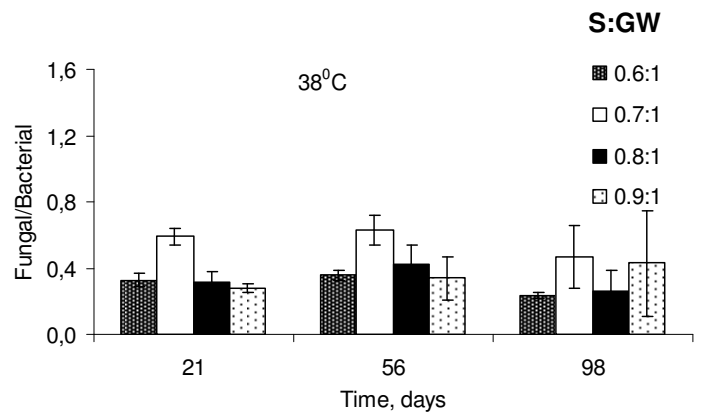
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 478 Abbreviations: MC, moisture content; S:GW, soil to green waste ratio.  
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**Fig. 1** Relative proportions of PLFA in the initial PAH-contaminated soil and compost mixture (soil+ green waste; S:GW, 0.6:1). Abbreviations: G(+), Gram-positive bacteria; G(-), Gram-negative bacteria; Ther, thermophiles.



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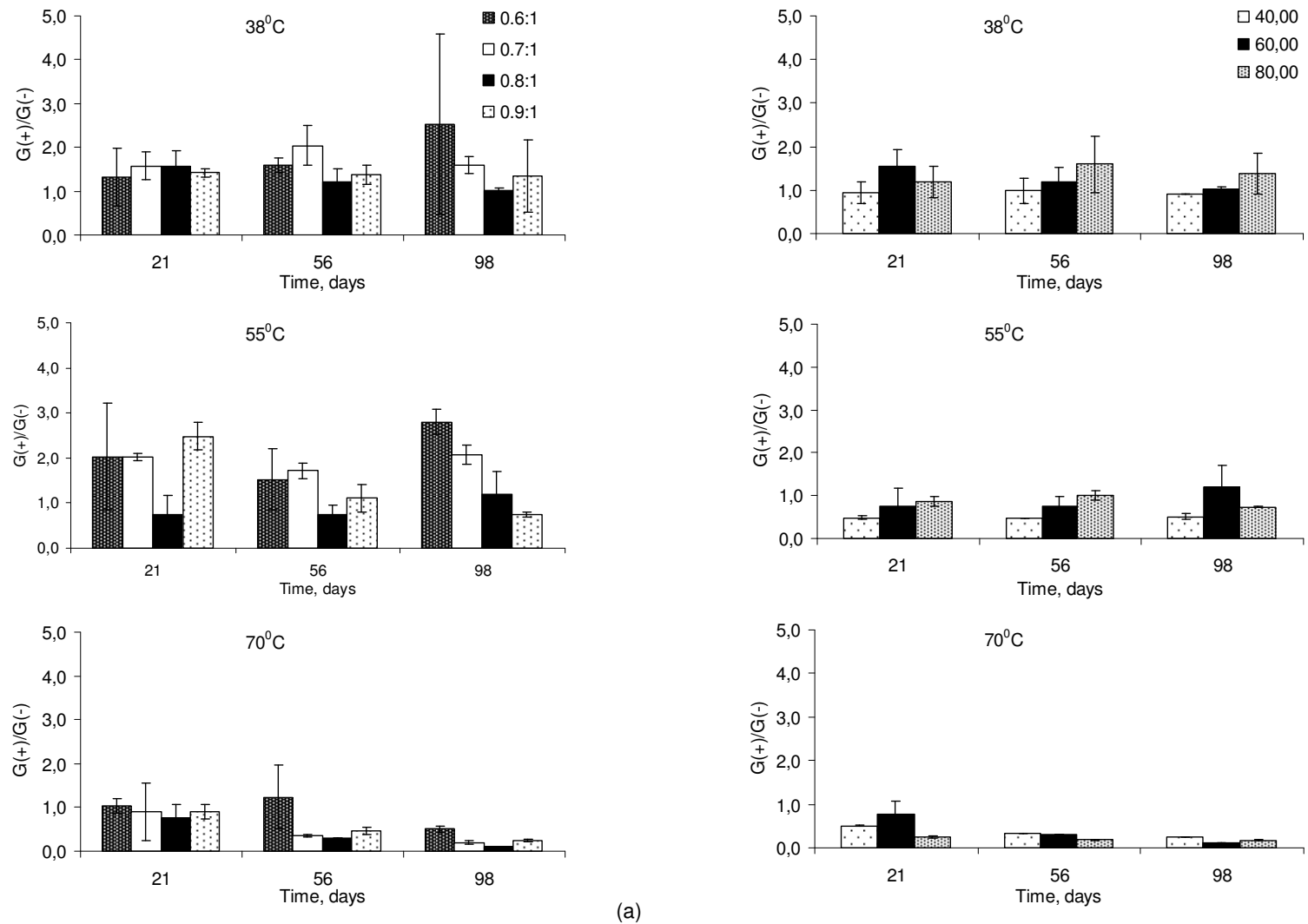
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**Fig. 2** Temporal profile of fungal to bacterial PLFA ratio. (a) Moisture content, MC = 60%. (b) Soil to green waste ratio, S:GW = 0.8:1.

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**Fig. 3** Temporal profile of Gram-positive and Gram-negative bacterial biomass ratio. (a) Moisture content, MC = 60%. (b) Soil to green waste ratio, S:GW = 0.8:1.

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