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Microbial community structure changes during bioremediation of PAHs in an aged coal-tar contaminated soil by in-vessel composting

Blanca Antizar-Ladislao*, Katerina Spanova, Angus J. Beck and Nicholas J. Russell

Imperial College London, Wye campus, Wye, Ashford, TN25 5AH, United Kingdom

Abstract. The microbial community structure changes of an aged-coal-tar soil contaminated with polycyclic aromatic hydrocarbons (PAHs) were investigated during simulated bioremediation at the laboratory-scale using an in-vessel composting approach. The composting reactors were operated using a logistic 3-factor factorial design with three temperatures (T = 38^0C, 55^0C or 70^0C), four soil to green-waste amendment ratios (S:GW = 0.6:1, 0.7:1, 0.8:1 or 0.9:1 on a dry weight basis) and three moisture contents (MC = 40%, 60% or 80%). Relative changes in microbial populations were investigated by following the dynamics of phospholipid fatty acid (PLFA) signatures using a ^{13}C-labelled palmitic acid internal standard and sensitive GC/MS analysis during in–vessel composting over 98 days. The results of this investigation indicated that fungal to bacterial PLFA ratios were significantly influenced by temperature ($p<0.05$), and Gram-positive to Gram-negative bacterial ratios were significantly influenced by temperature ($p<0.001$) and S:GW ratio ($p<0.01$) during in-vessel composting. Additionally, the Gram-positive to Gram-negative bacterial ratios were correlated to the extent of PAH losses ($p<0.005$) at 70^0C.

Keywords: Bioremediation; Coal-tar; Soil; Composting; polycyclic Aromatic hydrocarbons; Phospholipid fatty acids.

* Corresponding author. Present address Department of Water and Environment Science and Technology, University of Cantabria, 39316 Torrelavega, Spain. Tel: +34(0) 942 846 542, Fax: +34(0) 942 846 541. E-mail addresses: b_antizar@hotmail.com (B. Antizar-Ladislao) a.beck@imperial.ac.uk (A.J. Beck), nicholas.russell@imperial.ac.uk (N.J. Russell).

1 Introduction

Composting has been demonstrated to be effective in biodegrading PAHs (Potter *et al*. 1999; Canet *et al*. 2001; Cajthaml *et al*. 2002; Antizar-Ladislao *et al.* 2006; Cai *et al.* 2007), chlorophenols (Laine and Jorgensen 1997), polychlorinated biphenyls (PCBs) (Block 1998), explosives (Ro *et al.* 1998; Li *et al.* 2007) and petroleum hydrocarbons (Namkoong *et al.* 2002) at both the laboratory and field-scale. In contrast to conventional composting systems, the use of in-vessel systems for the bioremediation of contaminated soils provides operators with more control, enabling them to select suitable operating parameters (e.g., temperature, moisture content, mix ratios) to promote both microbial activity and contaminant degradation (Antizar-Ladislao *et al.* 2004; Oleszczuk 2006), and also to ensure the use of high temperatures (>70^{0}C) in order to meet regulatory requirements for pathogen control (EC 2003). Thus, in-vessel composting is presented as a sustainable bioremediation technology to treat contaminated soils amended with biodegradable municipal solid waste (i.e. green waste).

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

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

2 Materials and methods

2.1 Experimental design

Eighteen experimental conditions were tested in triplicate using 360 laboratory-scale in-vessel composting reactors. The experimental design comprised three temperature levels (T = 38^{0}C, 55^{0}C or 70^{0}C) four soil to green-waste ratios (S:GW = 0.6:1, 0.7:1, 0.8:1 or

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

2.2 Contaminated soil

The coal-tar-contaminated soil was obtained from a manufactured-gas plant site commissioned in 1838 at Clitheroe, Lancashire, United Kingdom. Prior to experimentation, the coal-tar-contaminated soil was air-dried and homogenized by passing through first a 5 mm then a 2 mm sieve; the contaminated soil was stored in the laboratory at room temperature. The soil contained a post-dilution concentration of 100.3 mg Σ16 U.S.EPA PAH·kg^{-1} soil, the soil organic content was 4.79±0.16% (w/dw), and the soil pH_w was 7.3±0.1.

2.3 Green waste

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

2.4 Reactor design

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

2.5 Sample analysis

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

PAH extraction from compost mixtures and soil was by Accelerated Solvent Extraction (ASE^{TM}) 200, with 22 ml stainless steel extraction cells that meet the requirements for the extraction of PAHs from solid waste as described in USEPA Method 3545. The extracts were purified on chromatographic columns packed with 1 g of activated-florisil (SiO_2, 84.0%; MgO, 15.5%; Na_2SO_4, 0.5%; 60/100 mesh; 130^0C; 12 h) and 2 g of Na_2SO_4. A Hewlett Packard 6890 series gas chromatograph (GC) with a 7673 series auto-sampler and a 5973 series mass selective detector (MS) was used for the analysis. Data acquisition and processing were achieved using a Hewlett Packard MS Chemstation (G1034C Version C.02.00). The GC-MS system was calibrated prior to the analysis of samples using seven calibration standards. The extraction efficiency of this method using two surrogate standards for the real samples, 1-fluoronaphthalene, 2-fluorobiphenyl varied between 70 and 98% primarily depending on the volatility of the compounds. Further details of PAH analysis can be found in Antizar-Ladislao *et al.* (2005a).

Sub-samples of compost (2 g) were spiked with 500 µg l^{-1} ^{13}C-palmitic acid (hexadecanoic acid, internal standard), the total lipid was extracted using the Bligh and Dyer procedure, and the lipid acyl chains and internal standard fatty acid converted to FAME using MeOH/H_2SO_4 (Kates 1985). The FAME were analysed using the same Hewlett Packard GC/MS system described above. The GC inlet was operated in pulsed (1.40 min, 40.0 psi) splitless mode at 260^0C with helium as carrier gas. The injection volume was 1 µl and the inlet was purged at 50 ml·min^{-1} 20 min after injection; inlet pressure was controlled by electronic pneumatics to maintain a constant column flow of 1 ml·min^{-1}. Separation was achieved using an HP-5MS column (19091S-433 30 m × 0.25 mm × 0.25 µm). The temperature program comprised 40^0C for 3 min, 10^0C·min^{-1} to 150^0C, 3^0C·min^{-1} to 230^0C, and 30^0C·min^{-1} to 300^0C that was maintained for 5 min to allow late eluting peaks to exit the column. The MS transfer line was held at 310^0C, thus providing conductive heating of the MS source to about 230^0C. The MS was operated in selective ion monitoring (SIM) mode, using m/z = 74 as the common fragment ion of FAME. To identify the fatty acids, the retention times were compared with those obtained for standard bacterial acid methyl esters (Cat. No. 47080-U, Supelco, UK). The amount of microbial signature acids was calculated using the ^{13}C-16:0 internal standard, which gives a characteristic fragment ion m/z = 75 that can be quantified separately from the bulk ^{12}C-FAME in the sample. The sum of the following fatty acids was used to represent total bacteria: i15:0, a15:0, i16:0, i17:0, cy17:0, 18:1ω7c and cy19:0 (Frostegard and Bääth 1996; Zelles 1999; Bolta *et al.* 2003). Gram-positive bacteria were represented by i15:0, a15:0 and i17:0 (Buyer *et al.* 1999) and Gram-negative bacteria by cy17:0, 18:1ω7c and cy19:0 (Klamer and Bääth 1998; Zelles 1999). Thermophilic bacteria were represented by i15:0 and i17:0 (Carpenter-Boggs *et al.* 1998). Fungi were represented by 18:2ω6,9 (Frostegard and Bääth 1996).

2.6 Statistical analyses

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

3 Results and Discussion

3.1 Assessment of composting process

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

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

3.2 Analysis of PLFA

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

PLFA ratios. The initial PAH-contaminated soil contained small amounts of PLFA that were indicative of fungi, giving a low value of the fungal to bacterial ratio (≈ 0.03); there were approximately equal proportions of Gram-positive to Gram-negative bacteria on the basis of signature PLFA content. Figure 1 shows that when this PAH-contaminated soil was mixed with artificial green waste, the compost mixture (soil + green waste, S:GW = 0.6:1) contained PLFA indicative of fungi in a higher proportion than did the initial soil (fungal to bacterial PLFA ratio ≈ 1.29), with a dominance of Gram-positive bacteria (Gram-positive to Gram-negative ratio ≈ 1.97). This indicated that in the present study, the fatty acids attributed to fungal biomass (i.e., 18:2ω6,9) were originally present in the green waste, where the highest ratio was observed in the sawdust (fungal to bacterial PLFA ratio ≈ 6.84). Frostegard and Bääth (1996) found that the fungal to bacterial PLFA ratio varied from 0.02-0.04 in different agricultural soils that were low in organic matter to a ratio of 0.3-0.5 in forest soils that were dominated by fungal biomass. Bolta *et al*. (2003) found that the fungal to bacterial PLFA ratio in a composted household organic waste with shredded wood varied from 0.12 to 0.15. Andersson *et al*. (2000) reported a fungal to bacterial PLFA ratio of 0.5 in an aged PAH-contaminated soil mixed with birch wood. Fungal to bacterial PLFA ratios calculated in the present study for the initial PAH-contaminated soil and a mixture of the same soil with green waste correlate with the fungal to bacterial PLFA ratios reported in the literature. Thus, according to Frostegard and Bääth (1996) results, the initial composting mixture was probably dominated by fungal biomass.

3.3 Fungal to bacterial ratio changes during composting

Figure 2 shows the temporal profile of fungal to bacterial PLFA ratios for all experimental conditions investigated. Analysis of the relative abundance of the major microbial groups during composting revealed a high proportion of bacterial biomass over fungal biomass (fungal to bacterial PLFA ratio < 1) for the first three weeks that was maintained to the end of the experiment after 98 days. The relative proportion of fungi in the in-vessel composting reactors was lowest at the highest in-vessel composting temperature investigated, namely 70^0C ($p<0.05$). These results are comparable with the findings of Klamer and Bääth (1998) who reported a rapid decrease in the fungal to bacterial PLFA ratio from 0.37 to 0.007 during the heating phase reaching 69^0C in the composting mixtures of straw materials. However, Carpenter-Boggs *et al.* (1998) reported that the PLFA markers for fungi (18:2ω6c, 18:3ω6c) did not change significantly over 60 days following a conventional composting temperature profile that reached a maximum temperature of 60ºC.

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

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

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

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

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

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

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

4 Conclusions

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

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

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

472
473

474 **Table 2** Phospholipid fatty acids ($\mu g \cdot g^{-1}$ dry weight soil, ± standard deviation) characteristic of Gram-positive and Gram-negative bacteria,
475 and fungi in the composting mixture at 38^0C during 98 days of continuous in-vessel composting.
476

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±1.3	27.3±5.3	12.9±1.7	15.9±6.9	17.1±1.6	8.0±7.2	12.5±3.3	18.2±2.6	5.2±0.2
60%/0.7:1	17.2±2.7	17.5±1.1	12.9±2.9	11.0±0.9	8.8±1.5	8.1±2.0	13.3±2.9	12.8±2.2	6.6±1.2
60%/0.8:1	23.2±6.3	13.1±1.0	13.0±1.0	14.8±0.6	12.3±7.9	9.4±4.5	14.5±1.2	12.4±3.3	7.0±3.9
60%/0.9:1	27.6±4.1	40.2±8.5	21.8±9.4	19.6±4.0	29.4±5.7	17.3±3.3	15.5±2.8	26.3±17.1	19.4±15.3
80%/0.8:1	19.6±7.8	31.0±9.5	26.2±10.7	18.1±9.1	21.6±9.0	20.9±12.0	15.0±0.6	13.2±0.7	15.8±9.1
40%/0.8:1	56.8±6.9	52.4±8.6	57.0±0.1	61.4±8.9	54.2±7.6	62.6±0.5	73.0±0.5	44.0±0.9	55.8±5.4

477
478 Abbreviations: MC, moisture content; S:GW, soil to green waste ratio.
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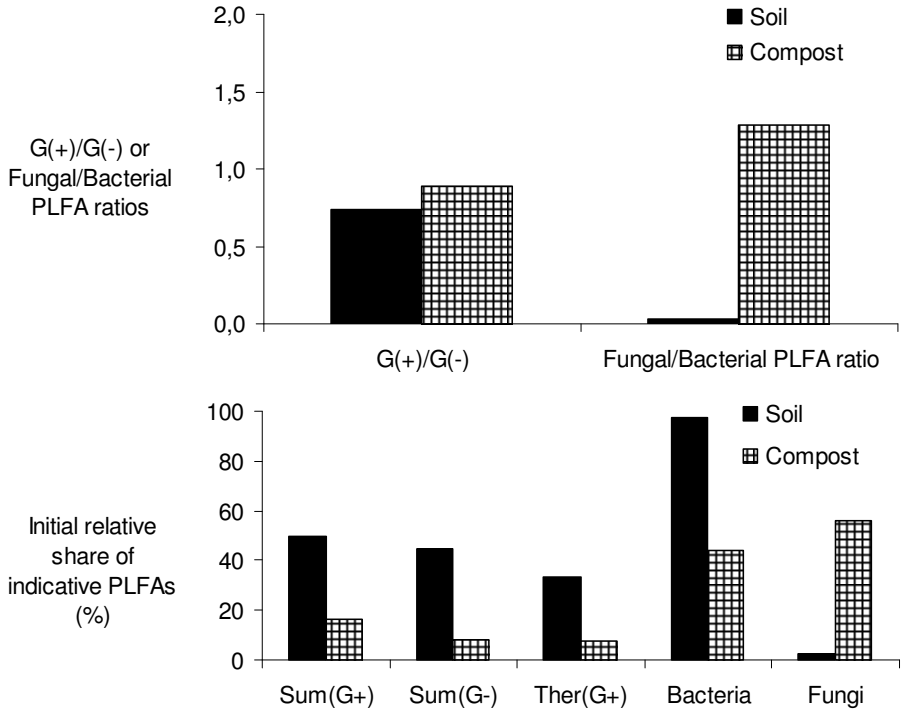
480



481
482

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

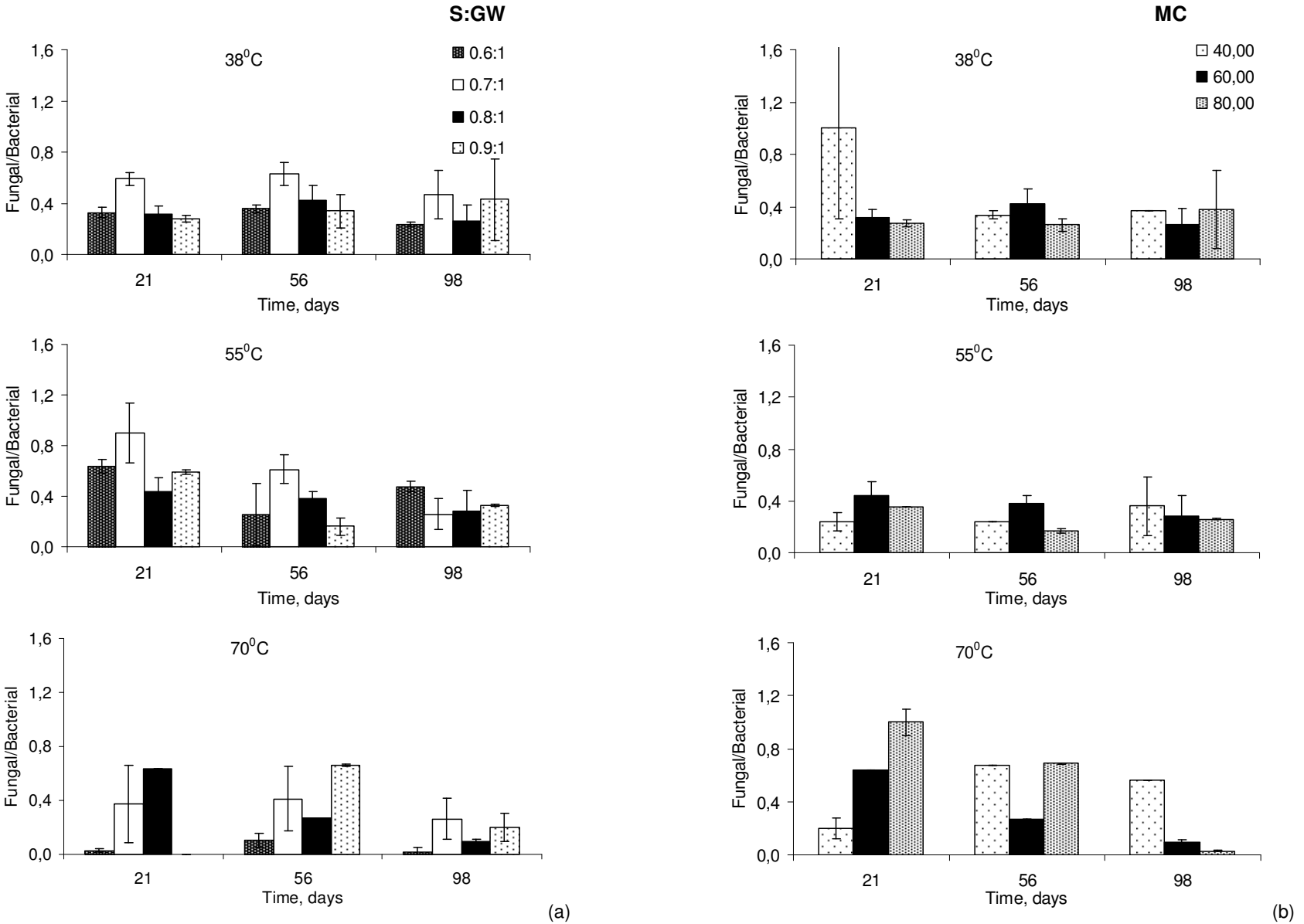


Fig. 2 Temporal profile of fungal to bacterial PLFA ratio. (a) Moisture content, MC = 60%. (b) Soil to green waste ratio, S:GW = 0.8:1.

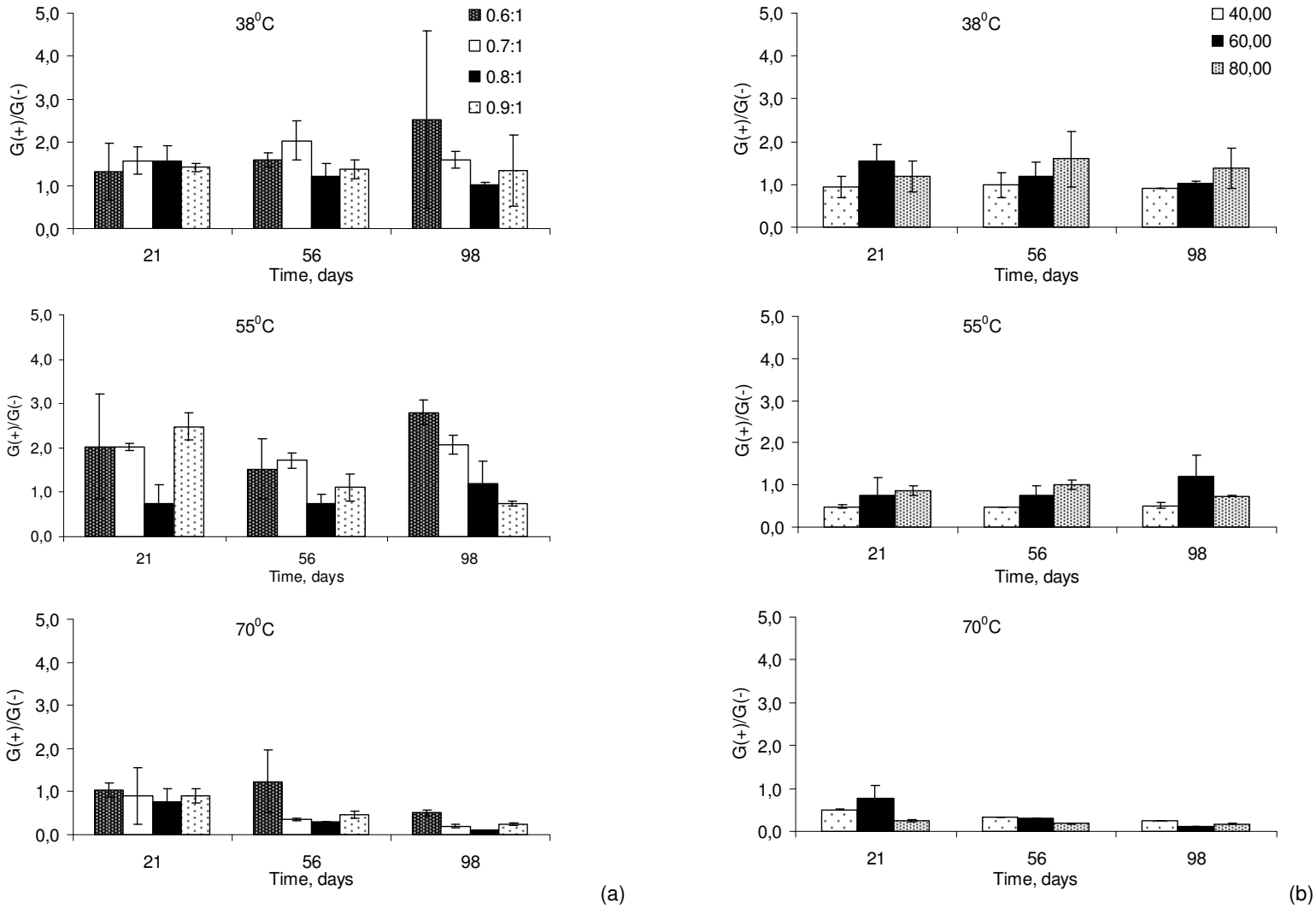


Fig. 3 Temporal profile of Gram-positive and Gram-negative bacterial biomass ratio. (a) Moisture content, MC = 60%. (b) Soil to green waste ratio, S:GW = 0.8:1.