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PLFAs of the microbial communities in composting mixtures of agro-industry sludge with different proportions of household waste

Soumia Amir [a], Rajae Abouelwafa [b], Abdelilah Meddich [b], Salah Souabi [c], Peter Winterton [d], Géorge Merlina [e], Jean-Claude Revel [e], Eric Pinelli [e], Mohamed Hafidi [b,*]

[a] Equipe Chimie Appliquée et Sciences de l'Environnement, Université Sultan Moulay Slimane, Beni Mellal, Morocco
[b] Laboratoire d'Ecologie et Environnement (Unité associée au CNRST), Département de Biologie, Faculté des Sciences Semlalia, Université Cadi Ayyad, BP 2390, Marrakech, Morocco
[c] Laboratoire Génie de l'Environnement, Faculté des Sciences et Techniques, Mohammedia, Morocco
[d] Université Paul Sabatier, 118 Route de Narbonne, 31062 Toulouse Cedex 9, France
[e] ECOLAB, Ecole Nationale Supérieure Agronomique, Avenue de l'Agrobiopole, BP 32607, Auzeville-Tolosane, F 31326 Castanet-Tolosan Cedex, France

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A B S T R A C T

Phospholipid fatty acids (PLFAs) were analysed at different time periods during composting of two waste mixtures rich in fats, M1 (22%) and M2 (39%), with the aim of monitoring changes in microbial community structure. The two mixtures consisted of a sludge sample collected from a vegetable oil refinery effluent treatment plant combined with household wastes. The PLFA profiles of both mixtures revealed that, at the start of the process, fungi and Gram-negative bacteria (G−) were more abundant in M2 than in M1. During the thermophilic phase, branched PLFA (i15:0, a15:0, i16:0, and i17:0) markers of Gram-positive bacteria (G+), became more abundant in M1, while G- bacteria were predominant in M2. The PLFA profiles in M1 representing non-specific, G+ and G− bacteria as well as fungi decreased during the cooling phase (maturation) while an increase was recorded in M2, which was richer in fats. The Shannon−Weaver diversity index (Ish) showed a greater increase during M1 composting (from 0.69 to 1.05), mainly for G+ bacteria and G− bacteria, than in M2 composting (from 0.79 to 0.84). Principal components and cluster analyses revealed a succession of different communities during composting, which varied from fungi and G− bacteria to G+ and thermophilic and thermotolerant G− bacteria. The end of composting was characterized by a reduction of all these microbial entities, especially for M1, except actinomycetes, which are associated with compost stability.

1. Introduction

Composting is a process involving the decomposition and transformation of biodegradable organic waste under the action of various microbial populations occurring in damp aerobic environments. The initial phase is characterised by the activity and growth of mesophilic microorganisms leading to a rapid increase in temperature (Finstein and Morris 1975; De Bertoldi 1981; Miller 1993). Then, thermophilic microorganisms ensure the degradation process, the activity of non-heat-tolerant species being halted. The final phase, which includes a period of cooling and maturation, is characterised by the development of a new community of mesophiles. Maturity and stability are words expressing very different concepts: The maturity of compost refers to its degree of humification, and stability concerns the level of activity of the microbial biomass (Iannotti et al. 1993). Analysis of phospholipid fatty acids (PLFAs) is an accurate method of examining the changes occurring in the microbial community during composting.

Analysis of PLFAs is a method of choice for many reasons. One is that most environmental microorganisms, although viable, cannot be cultured (Xu et al. 1982; McCarthy and Murray 1996; White et al. 1997). In addition, microorganisms contain phospholipids in their membranes that are not stored but have a relatively high turnover during metabolism (White et al. 1997). Consequently, the PLFAs present in a sample are representative of the viable microbial biomass contained in that sample (Vestal and White 1989). Moreover, some PLFAs can be considered to be taxonomic or physiological biomarkers for certain genera of microbes (White 1995; Zelles et al. 1995). Several studies, such as that of Herrmann and Shann (1997), have used PLFA profile variations to monitor changes occurring in the microbial community as composting progresses. They found that during the mesophilic phase, fatty acids (FAs) from eukaryotes and from mycetes were the most abundant. In the thermophilic phase, FAs from actinomycetes and

* Corresponding author. Tel.: +212 5 24 43 76 65.
E-mail address: hafidi@ucam.ac.ma (M. Hafidi).

Gram-negative (G−) bacteria were predominant. In the samples collected at the end of the process, they found biomarkers from mycetes and actinomycetes. Furthermore, when using very old samples for composting they found that the changes occurring in FA profiles during the process were similar. This suggests that PLFA analysis provides a reliable and powerful tool for evaluating the maturity of compost. However, it is not possible from current literature to get a clear picture of the microbial communities' variations during composting. These communities vary according to the composition of the initial mixture that is intended for composting and the environmental conditions during composting.

The major goal of the present study was to monitor the variations in microbial communities during composting of sludge samples collected at the outlet of a vegetable oil refinery wastewater treatment plant located at Ain Harouda in Morocco. This sludge tends to vary in quality and in quantity from day to day. The type of effluent depends on which oils are being processed (soybean, palm, sunflower, rapeseed) and the processes they undergo (soap making, production of distilled fatty acids, production of cake) (Bhaih and Sidibe 2002; Soudi and Maftouh 2003; Battachi 2006; Lachhab and Herras 2006). There can also be lipid spills during the different stages of refining, which increase the level of fats in the sludge. On occasions, owing to the increase in production from the refinery – when a third production line is put into operation – the input of wastewater exceeds the treatment plant's capacity (1450 m^3/day). In a study by Lachhab and Herras (2006), it was reported that the input to the treatment plant in May 2006 ranged from 350 $m^3\ day^{-1}$ to 1680 $m^3\ day^{-1}$, and the level of fats in the effluent varied from 3054 to 6634 $mg\ l^{-1}$.

In this work we studied the change in fats in the course of composting of two samples of the Ain Harouda sludge, initially containing different levels of lipids. In order to reduce the amount of lipids, both sludge samples were mixed with household wastes. The changes occurring in the PLFA profiles were studied to determine how the microbial community varied during the composting of both mixtures.

2. Materials and methods

2.1. Compost substrate

The substrate consisted of a mixture of sludge and household waste

- The sludge samples were taken from the vegetable oil refinery effluent treatment plant of Lesieur-Cristal Co. Ltd. (Ain Haroudda, Morocco). They were produced after the liquid effluent had undergone three types of treatment: physical, chemical, and biological.
- The household waste was taken from the municipal dump of the town of Mohammedia (Morocco). It was sorted to remove non-biodegradable material.

The two sludge samples were collected at two different times during the effluent treatment. The proportion of organic carbon (43%) of the sample collected at the later step of the treatment, S2, was higher, and the nitrogen content (0.72%) and available phosphorus (885 ppm) were lower than the sludge sample S1, which was collected at an earlier treatment step (Table 1). Owing to these differences, two mixtures were prepared with different proportions of sludge and household waste:

- Mixture M1: 25 kg (dry weight) Lesieur sludge S1 (collected during an early step of the treatment) were mixed with 40 kg (dry weight) sorted household rubbish.
- Mixture M2: 77 kg (dry weight) Lesieur sludge S2 (collected 15 days after S1 sampling) were mixed with 28 kg (dry weight) household rubbish sorted to remove non-biodegradable items.

Table 1
Physical–chemical characteristics of the material to be composted (sludge samples S1 and S2, household waste (HW), and their mixtures M1 and M2).

	Mixture M1			Mixture M2		
	Sludge S1	HW	Mixture	Sludge S2	HW	Mixture
Moisture (%)	75.86	80.45	77.64	45.97	72.11	63.01
PH	5.16	4.82	4.96	5.24	5.85	5.70
Electrical conductivity ($ms\ cm^{-1}$)	4.1	5.95	5.42	1.5	5.2	1.86
Total Kjeldhal nitrogen (% dry wt.)	1.15	1.54	1.5	0.72	3.46	1.3
Total organic carbon (%)	35.91	53.22	45.18	43.33	45.18	44.27
C/N	31.22	34.55	30.12	60.18	13.05	34.05
Ash (%)	39.74	10.18	21.39	25.58	16.66	24.70
Assimilable phosphorus (ppm)	6254	1858	4688	885	986	911
Total polyphenol ($mg\ g^{-1}$ W Wt.)	0.108	0.483	0.247	nd	nd	0.254

nd: not detected.

2.2. Compost experiment

The heaps were composted outside on a purpose-built composting slab for a period of five months. They were forked over every week to maintain aeration, and moistened regularly. Samples were taken after different times of composting (T0M1 and T0M2: initial mixtures M1 and M2; T2M1 and T2M2: after 2 months; and T5M1 and T5M2: after 5 months of composting of M1 and M2, respectively). Preliminary analyses of these samples have been reported previously (Abouelwafa et al. 2008a), and the rate of decomposition was determined from the following equation:

$$\text{Decomposition } (\%) = 100\Big(\big(\text{Ash}_f - \text{Ash}_i\big)/\text{Ash}_f(100 - \text{Ash}_i)\Big)100$$

where Ash_i is the initial level of ash and Ash_f the final level.

The humic substances were extracted using 0.1 M NaOH, humic acid was isolated by 3 M HCl and recovered in 0.1 M NaOH, and its level was determined by weighing a known volume after freeze-drying.

For the lipid and fatty acid analyses, the compost was sampled at different phases during composting and the samples were transferred to a plastic bag and stored at −20 °C until analysis. Immediately before sampling, the heaps were turned to mix the contents.

2.3. Lipid extraction and separation

The lipid extraction was carried out from 15 g of accurately weighed compost samples (three repetitions). The lipids were extracted with 360 ml of a chloroform/methanol mixture (2/1, v/v) (3 × 120 ml). After 24 h of maceration at 4 °C, the mixture was filtered through glass wool. The chloroform fraction containing the lipids was recovered following the addition of 60 ml of 0.73% NaCl, according to the method of Folch et al. (1956), and it was dehydrated with anhydrous sodium sulphate. The lipids were separated on silica columns. The neutral lipids, glycolipids, and phospholipids

were eluted by chloroform, acetone, and methanol, respectively (Frostegård and Bååth 1996). The phospholipids and neutral lipids content were estimated by weighing the two extracts after evaporation and drying. Both fractions were recovered in 5 ml of $CHCl_3$.

The phospholipid fraction, further dried under a stream of nitrogen gas, was taken up in 1 ml of heptane with C10:0 as the internal standard. The fatty acids were subjected to trans-esterification after alkaline methanolysis. The fatty acid methyl esters were analysed by gas chromatography (HP 5890 series II) coupled with mass spectrometry (HP 5971): column length 30 m, internal diameter 0.530 mm. Injection used the split mode, 1 μl sample with 1 μl hexane, carrier gas helium under 21 kPa pressure; the injector temperature was 260 °C, and that of the oven: from 140 °C (4 min) to 250 °C with 4 °C min^{-1}; the detector temperature was 280 °C, and the ionisation energy was 70 eV. The methyl ester peaks obtained were compared with those of external standards corresponding to bacterial fatty acid methyl esters FAMEs (26 known bacterial FAMEs, supplied by Supelco, Bellefonte, PA).

2.4. Fatty acid nomenclature

The fatty acid nomenclature used is as follows: total number of carbon atoms::number of double bonds, followed by the position w of the double bond from the methyl end of the molecule. The *cis* and *trans* configurations are indicated by c and t, respectively. The *anteiso* and *iso* branching are designated by the prefix a or i.

The PLFA profiles recorded in this investigation were classified into specific groups of organisms according to the literature (Hellman et al. 1997; Amir et al. 2008):

NM: Not only of microbial origin, they could be also in the vegetal waste (12: 0; 14: 0; 16: 0; 18: 0; 20: 0)
NSB: Non-specific bacteria exhibit the fatty acids patterns occurring in a large number of bacteria (13: 0; 15: 0; 17: 0; 19: 0).

Fatty acids patterns specific to microbial groups:

G+: Gram-positive bacteria (i-15: 0; a-15: 0; i-16: 0; i-17: 0);
G−: Gram-negative bacteria (2-OH12: 0; 16:1ω9c; cy17: 0; 18:1ω9t; cy19: 0);
F: Fungi (18:1ω9c; 18: 2ω6,9).

2.5. Statistical treatment

Comparison of the averages was carried out by ANOVA test post-hoc Tukey. Principal components analyses (PCA) and cluster analyses were carried out between different PLFA profiles during composting.

3. Results and discussion

3.1. The properties of composts

The microbiological activity during composting of mixtures M1 and M2 led to temperatures reaching, respectively, 62.6 and 65 °C (Fig. 1); each compost achieved pathogen kill temperatures of >55 °C and remained at these temperatures for several days (Abouelwafa et al. 2008a). The physicochemical characteristics of the final composts, after 5 months of composting, were, for M1 and M2, respectively, pH: 8.5 and 7.1; C/N: 10 and 16; proportion of decomposition: 78 and 55%; NH_4^+/NO_3^-: 0.78 and 1.02. Total polyphenol concentrations decreased during composting (Abouelwafa et al. 2008a), falling by 75% and 76% for M1 and M2, respectively. The levels of cellulose, hemicellulose, and lignin fell in M1 by 62, 63, and 67% and in M2 by 39, 49, and 39% respectively. This was paralleled by a rise in the humic acid content, which reached 22 and 36 mg g^{-1} in M1 and M2, respectively.

3.2. Changes in total fats, phospholipids, and neutral lipids during composting

Lipid analysis indicates that sludge sample S2 contained a higher amount of fats (49%) than did S1 (31%) (Table 2); their mixtures with household waste paralleled these values. The lipid composition of the two mixtures in the initial sludge samples and at different phases during composting differed significantly. The respective percentages of neutral lipid comprised in total lipids (TL) varied from 27 and 29% before composting to 79 and 53% after composting for M1 and M2. In the case of phospholipids, maximal values did not exceed 10% of the total lipids except in the case of T5M1 (Table 2). As reported by Malosso et al. (2004), neutral lipids serve as energy storage in microbial organisms. However, the possibility that they originated from the refinery wastewater cannot be excluded.

During composting, TL degradation was extensive in both mixtures, reaching 81% and 72% for M1 and M2 respectively, although a non-negligible quantity of lipids (11% dw) remained in mixture M2. The degradation of neutral lipids reached 92% for M1 and 75% for M2 after 150 days of composting. By contrast, phospholipids fell slightly in compost M2 and increased in M1. The change in phospholipid levels can be well explained by the fact that the phospholipids originate mainly from the membranes of microorganisms which were abundant during the high-temperature phase of composting, as reported by Bai et al. (2000), Boulter et al. (2002), Barje et al. (2008), and Amir et al. (2008). Similar results were reported by El Hajjouji et al. (2008) during the aerobic treatment of olive mill wastewater (OMW), when PLFAs increased owing to the growth of a new population of microorganisms.

3.3. Phospholipid fatty acid analyses

Table 3 shows the concentrations of the phospholipids and fatty acids detected in sludge S1 and S2 and in mixtures M1 and M2 before and after 2 months and 5 months of composting. In Table 3, the fatty acids are organized according to the groups of organisms in which they occurred (White 1995; Zelles et al. 1995). On the basis of PLFA profiles of the sludge samples, the microbial biomass of sludge S1 is richer than that of S2. The higher concentration of fats in the latter is probably hindering its development (Table 3). In fact, the concentrations of bacterial biomass (fungal and G− bacteria PLFAs) were much higher at the time of mixing S2 with household waste: T0M2 compared to T0M1 (Table 3). This is corroborated by the microbiological analysis reported in a previous study, which showed that T0M2 exhibited a higher microbial diversity with respect to total mesophilic flora, fungi, spore-forming bacilli, and actinomycetes compared to T0M1 (Abouelwafa et al. 2008b). In addition, PLFA analysis showed a higher level of PLFAs in T0M2 than in T0M1 (Table 3). This could be explained by the better conditions for the development of these microorganisms in M2 compared to M1 obtained by reducing the concentrations of fats through addition of more household waste, the presence of more readily available compounds for decomposition, and mesophilic temperature of M2 compost at the initial time (Fig. 1). As a consequence, the high microbial activity in M2 led to the emergence of an anaerobic state, as demonstrated by the presence of hydroxy fatty acids 2 OH-12:0 in T0M2 (Myers et al. 2001) (Table 3). In fact, the higher NH_4^+/NO_3^- ratio in M2 compared to M1 is further evidence that composting of M2 reaches an anaerobic state (Wang et al. 2008).

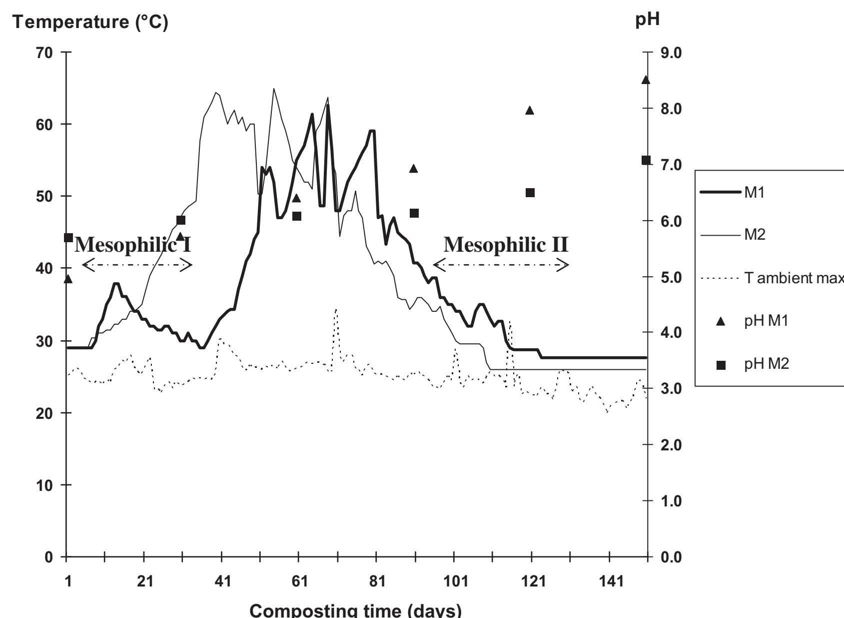

Fig. 1. Temperature and pH changes during composting of mixtures M1 and M2 consisting of mixtures of agro-industrial sludge samples and household wastes.

However, at the beginning of the thermophilic phase of composting, the amount of lipids associated with G+ and G− bacteria increased significantly ($P < 0.01$) in M1 (T2M1) (Fig. 2), and those associated with fungi and NSB were reduced ($P < 0.01$). A similar pattern occurred in M2 except that lipids associated with G− bacteria decreased (T2M2) (Fig. 2). Many authors have reported the use of increased *iso* and *anteiso* branched PLFAs (e.g., i-15:0; a-15:0; i-16; i-17) as indicators of the growth of G+ bacteria during the stabilisation phase of composting (Klamer and Bååth 1998; Carpenter-Boggs et al. 1998; Cahyani et al. 2002). The strong increase in 15- and 17-carbon chains is considered to be a good indication of the presence of thermophilic *Bacillus* sp [12]. According to these authors, a high proportion of branched *iso*-FAMEs indicates the continuous and extensive survival of G+ bacteria in a strictly thermogenic phase of composting. In a recent study, Amir et al. (2008) examined the lipid variations during composting of sewage sludge and straw. They found that the branched fatty acids with *iso*- and *anteiso*-forms (i-15:0; a-15:0; i-16; i-17) increased mainly in the thermophilic phase, but decreased immediately afterwards. However, the decrease in M2 (T2M2) of G− bacteria PLFAs (mainly 16:1 and 18:1) was probably caused by a decrease in available carbon, which is evidenced by the observation that the C/N ratio and the carbon compounds (polyphenols, cellulose, hemicellulose, and lignin) decreased significantly during the thermophilic phase (Abouelwafa et al. 2008a). Zelles et al. (1992) reported that monounsaturated fatty acids 16:1 and 18:1 from G− bacteria are strongly related to high substrate availability.

In the maturation phase, in M1, all microbial entities: G+ and G− bacteria and fungi decreased (T5M1), in contrast to M2 (T5M2), where all entities increased ($P < 0.01$) (Fig. 2). This is an indication that at the end of the thermophilic phase, compost M1 was

Table 2
Proportion of total lipids, phospholipids, and neutral lipids in sludge S1 and S2 and in mixtures M1 and M2 after different times of composting.

Lipids (% dry wt)	Mixture or sludge	Sludge	Mixture 1 day	Mixture 2 months	Mixture 5 months
Total lipids	(1)	30.6	21.7	7.7	4.1
	(2)	48.8	38.7	14.0	10.7
Phospholipids	(1)	1.9	0.1	0.7	1.2
	(2)	1.9	1.9	1.5	0.7
Neutral lipids	(1)	16.6	17.1	2.1	1.3
	(2)	14.1	20.4	5.1	5.3

All values are the means of three repetitions.

Table 3
PLFA concentrations in sludge S1 and S2 and in mixtures M1 and M2 at time 0 (T0), during the hottest phase (T2: 2 months), and at the end (T5: 5 months) of composting.

PLFA (μmol g^{-1} d.w)	M1				M2			
	S1	T0M1	T2M1	T5M1	S2	T0M2	T2M2	T5M2
NM								
12: 0	0.0	0.0	0.0	1.6	0.0	0.0	0.0	0.0
14: 0	34.2	4.7	7.6	3.3	3.8	4.9	2.5	5.8
16: 0	96.1	12.1	26.8	13.8	39.1	20.7	19.0	34.7
18: 0	9.9	4.2	4.4	3.7	13.7	5.3	4.8	9.0
20: 0	4.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0
NSB								
13: 0	0.0	2.6	0.0	0.0	0.0	0.0	0.0	0.0
15: 0	8.7	0.0	0.7	0.9	0.0	0.0	0.0	0.0
17: 0	7.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
19: 0	34.8	69.5	0.0	0.0	0.0	0.0	0.0	0.0
BG+								
i-15: 0	20.0	0.0	12.7	5.1	0.0	0.0	1.9	5.9
a-15: 0	37.3	1.4	13.1	4.2	0.0	0.0	1.3	5.4
i-16: 0	0.0	0.0	5.2	2.3	0.0	0.0	0.0	2.8
i-17: 0	0.0	0.0	1.4	1.2	1.8	0.0	0.0	0.0
Fungi								
18:1ω9c	60.9	20.1	17.6	10.2	48.8	46.4	23.5	38.8
18: 2ω6,9	74.6	12.1	7.4	3.7	0.0	29.8	3.7	5.3
BG$^-$								
2-OH12: 0	0.0	0.0	0.0	0.0	0.0	27.0	0.0	0.0
16:1ω9c	20.5	0.0	13.2	5.3	0.0	4.1	3.2	2.0
cy17: 0	0.0	0.0	0.9	1.5	0.0	0.0	0.0	0.0
18:1ω9t	127.8	10.3	30.8	13.5	9.9	22.4	16.0	35.2
cy19: 0	0.0	0.0	1.4	3.8	0.0	0.0	1.5	5.4

NM: non-microbial lipids; NSB: non-specific bacteria; BG+: Gram+ Bacteria; BG−: Gram− Bacteria.

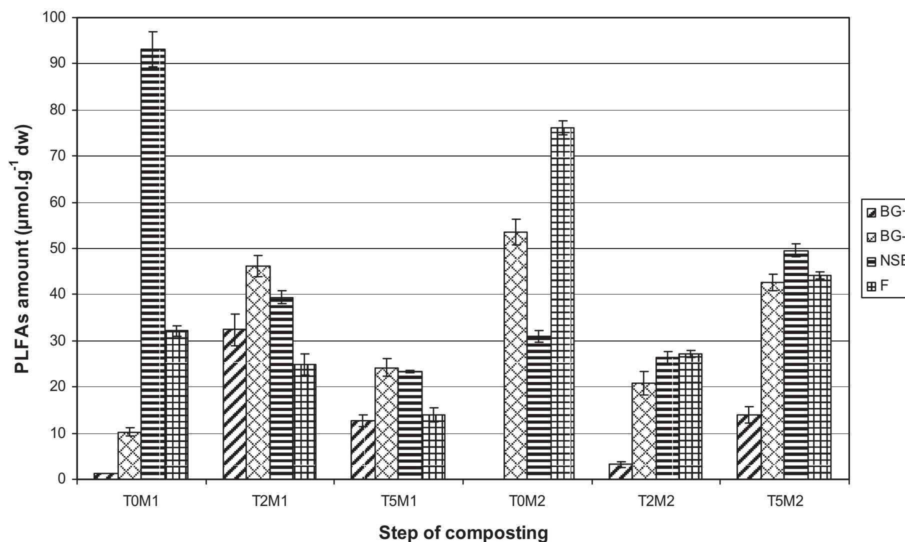

Fig. 2. Changes in bacterial population based on PLFA profiles during composting of mixtures M1 and M2. T0, T2, and T5 refer to time 0, 2 months, and 5 months of composting. BG−, G negative bacteria; BG+, G positive bacteria; NSB, non-specific bacteria; F, fungi.

stabilized, unlike M2, where the thermophilic microorganisms were replaced by an active mesophilic microbial population (Herrmann and Shann 1997).

Major conclusions can be drawn on the basis of the relative abundance of each group of microorganisms. The first is that bacteria were much more abundant than fungi in M1 than in M2; the bacteria/fungi ratio was of the order of 3.2–4.7 in M1 and about 1.0–2.4 in M2. This difference could, in all likelihood, be attributed to the high amounts of fatty acids in sludge S2, and therefore compost M2, that favour the presence of fungi. A second conclusion is that all bacteria (G+ plus G−)/fungi or G+/G− ratios increased and then decreased at the end of M1 composting, but in M2 these ratios continued to increase, which confirms that in M2, composting had not yet ended.

The Shannon-Weaver index (Ish) was used to calculate the degree of microbial diversity during composting. Low values of Ish correspond to low diversity, while a high index corresponds to a complex system. This ecological index was used by Alcaniz et al. (1983) and Ayuso et al. (1996) to examine the organisation of organic compounds in soil and humic acids.

The relative abundance, Pi, of each fatty acid is calculated from the ratio between the area of its corresponding peak to the sum of the areas of all the peaks considered in the chromatograms (Ayuso et al. 1996).

$$\mathrm{Pi} = \frac{a_i}{\sum_1^n a_i}$$

The Ish index was calculated as follows: $\mathrm{Ish} = -\sum_1^n \mathrm{Pi}\log_2 \mathrm{Pi}; \quad 0.5 < \mathrm{Ish} < 4.5$.

As seen in Table 4, the diversity index differed between sludge S1 and S2. The total microbial diversity $\mathrm{Ish_{tot}}$ increased more in compost M1 (from 0.69 to 1.05) than in compost M2 (from 0.79 to 0.84). The diversity index increased principally for the G+ and G− bacteria, whereas the diversity index for fungi did not increase.

The fatty acid profile variations during composting of mixture M1 were analyzed using the principal component analysis (Fig. 3a). The plots of the principal component scores are divided into three domains (I, II, and III). The first axis, PC1, explains 73.6 % of the variability between domain I of PLFA profiles (18:1ω9c; 18:2 ω 6,9; 19:0; 13:0) and domain II (16:0, 18:1ω9t; a15:0; i 15:0; 16:1ω9c; i 16:0; i 17:0). NSB and fungal PLFAs are clustered into domain I, whereas PLFAs of G+ and G− bacteria, except for the G− molecules cy17:0 and cy19:0, are clustered into domain II. Thus, the high temperature contributes to the first principal components, separating thermo-sensitive species (PLFA profiles domain I) and thermophilic or thermotolerant species (PLFA profiles domain II). The PLFA profiles of domain II are presumed to express the index of stability of the material in the thermophilic phase of composting. The G− PLFAs (cy17:0; cy19:0) present with 15:0 in domain III can be distinguished from domain II by taking PC2 (26.4 %) into consideration. The molecules cy17:0, cy19:0, and 15:0 increased during composting from 0 to 1.5, 3.8, and 0.9 μmol g^{-1} dry wt in T5M1, respectively. However, only cy19:0 increased from 0 to 5.4 in T5M2. Herrmann and Shann (1997) reported that cy17:0 and cy19:0 underwent a consistent increase as the compost aged. These authors suggested that high levels of these PLFAs would be associated with a decrease in metabolic activities as the readily degradable fraction of the compost is depleted. Therefore, the presence and level of phospholipids cy17:0 and cy19:0 have been suggested to provide a means of determining the age and maturity of a composted material. The PLFA 15:0 is generally associated with bacteria and can be found in small amounts in completely different bacterial groups (Bååth 2003).

Similar observations were made from the PCA analysis for the PLFA profiles of compost M2, where the plot of the principal component scores was divided into two domains (I and II) (Fig. 3b). The first axis PC1 explains 73.6 % of the variability between PLFA profiles of domain I (fungal PLFAs: 18:1ω9c; 18:2ω6,9 and G− bacteria PLFAs: 2 OH-12:0; 16:1ω9c) and those of domain II (Gram+ bacteria: a15:0; i15:0; i16:0; Gram−: 18:1ω9t; cy19:0;

Table 4
The Shannon-Weaver index (Ish) calculated between all identified microorganisms (Ish tot) and within each microbial group during composting of mixtures M1 and M2 at time 0 (T0), 2 months (T2), and 5 months (T5).

	S1	T0M1	T2M1	T5M1	S2	T0M2	T2M2	T5M2
Ish tot	0.95	0.69	0.97	1.05	0.59	0.79	0.80	0.84
Ish NSB	0.63	0.38	0.39	0.52	0.34	0.38	0.33	0.35
Ish BG+	0.28	0.00	0.51	0.55	0.00	0.00	0.29	0.46
Ish Fungi	0.30	0.29	0.26	0.25	0.00	0.29	0.17	0.16
Ish BG−	0.17	0.00	0.35	0.49	0.00	0.39	0.30	0.25

NSB: non-specific bacteria; BG+: Gram+ Bacteria; BG−: Gram− bacteria.

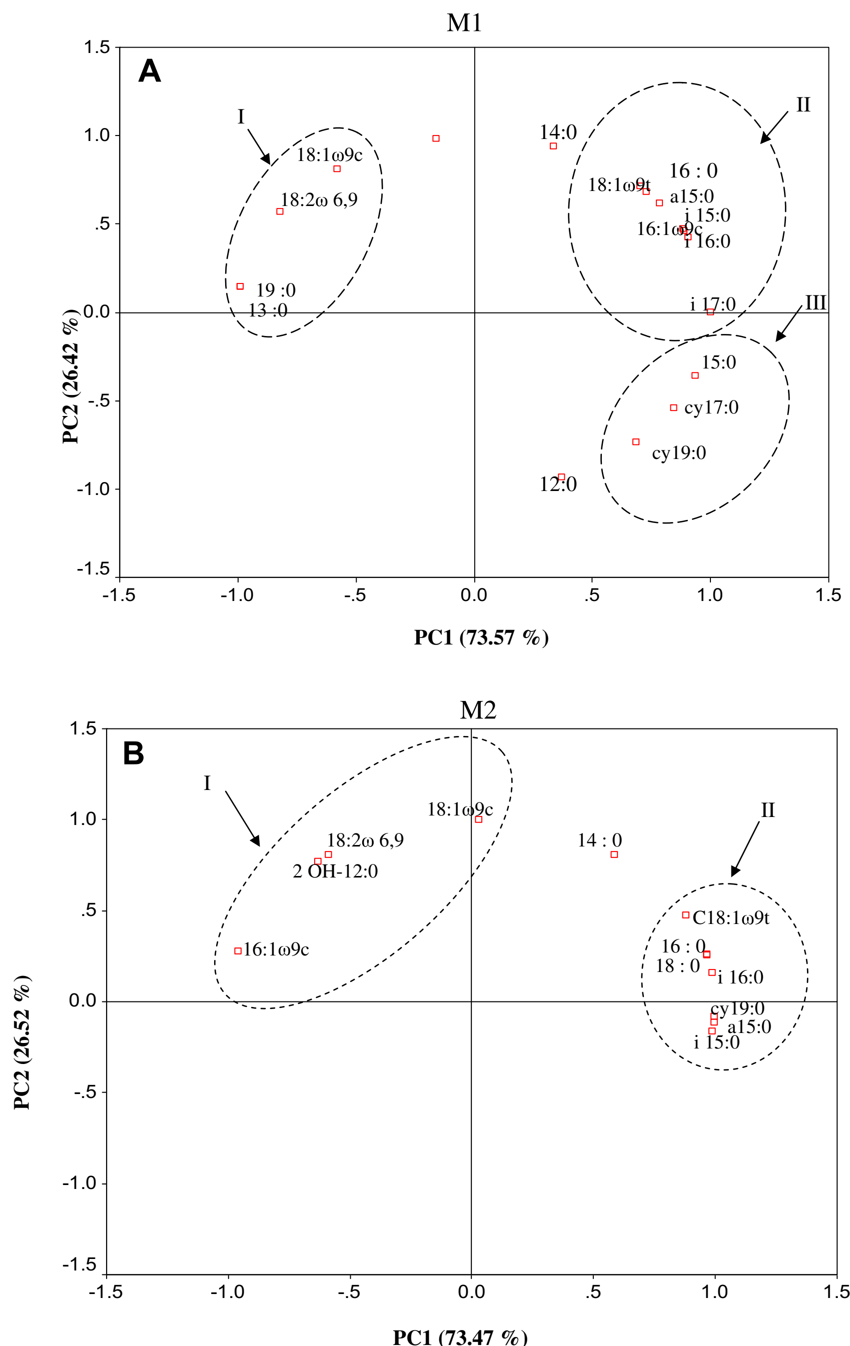

Fig. 3. Principal components analysis of PLFA profile variations during composting of (a) mixture M1, and (b) mixture M2.

NSB: 16:0, 18:0). Domain I appears to include the PLFAs of thermosensitive microorganisms in which the temperature is a selective factor for their development. In contrast, domain II gathers PLFA profiles of bacterial entities whose abundance increases in the first phase of composting, i.e., those specific to thermotolerant species or microorganisms contributing to the stabilisation of the material to be composted. PC2 separates the G− bacteria PLFA C18:1ω9t from G+ PLFAs in domain II, as well as G− PLFA 16:1ω9c from fungal PLFAs in domain I.

The cluster analysis based on the PLFA profile variations during composting of mixture M1 shows two major clusters (Fig. 4a). Each one was divided into two sub-clusters, each corresponding to specific microbial groups: cluster I comprised NSB PLFAs (sub-cluster 1) and fungal PLFAs (sub-cluster 2); cluster II comprised PLFAs profiles of G− bacteria (sub-cluster 3) and of G+ bacteria (sub-cluster 4). Similar results were obtained for the analysis of mixture M2 composting (Fig. 4b); the PLFA profiles were divided into two large clusters, the first comprising the PLFA profiles of G+ and G− bacteria, and the second, the fungal PLFAs. One exception is the presence of 2 OH-12:0 in the cluster assembling the fungal PLFAs. The cluster division clearly demonstrates that different groups of microorganisms proliferate in the compost and that the PLFA profiles detected during composting depend upon the conditions prevailing at each phase of treatment. In fact, it has been reported that, at the start of composting, eukaryotes, fungi, and some G− bacteria are less abundant in the compost biomass (Steger

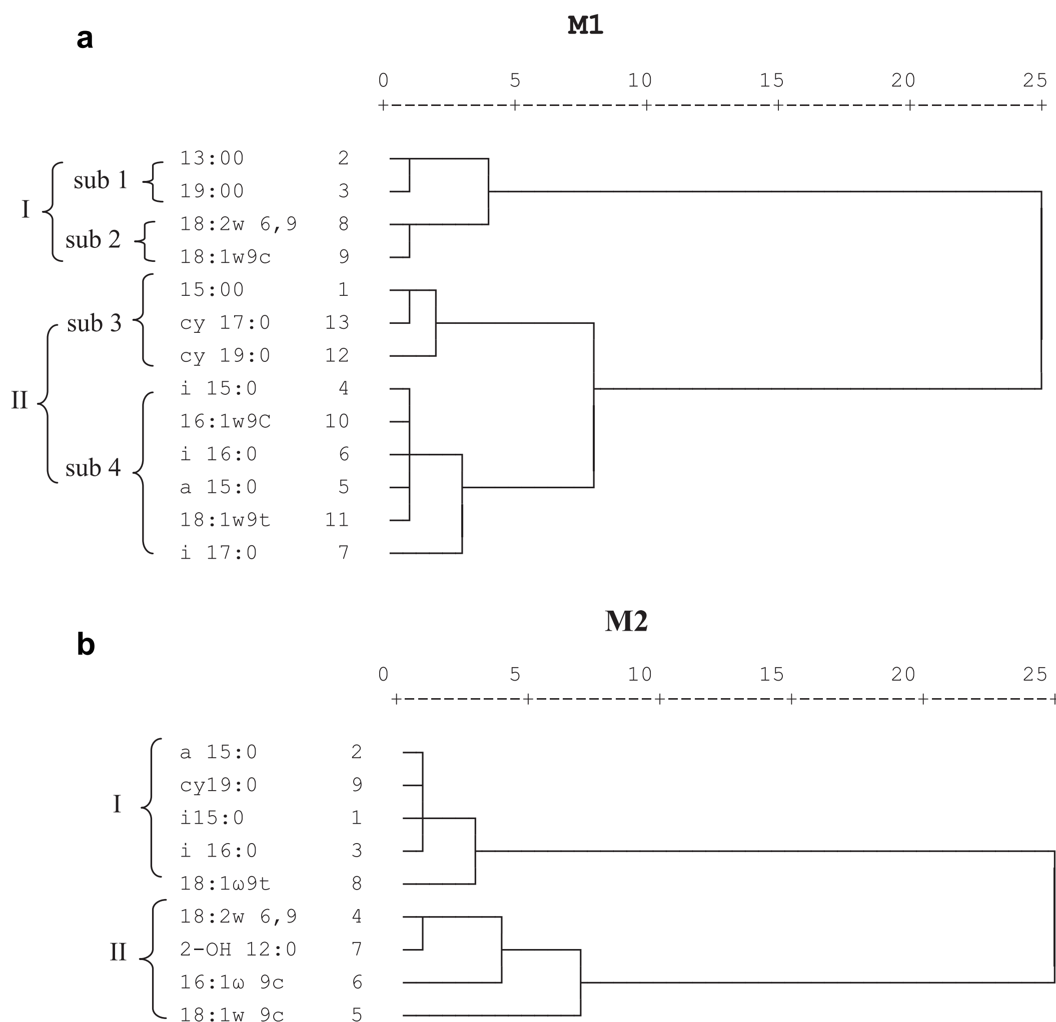

Fig. 4. Cluster analysis of PLFA profiles during the composting of (a) mixture M1, and (b) mixture M2.

et al. 2003). The other G− bacteria are thermotolerant (Rhee et al. 2000), but G+ bacteria represent the strict thermophilic bacteria that proliferate especially during the high-temperature phase.

Altogether, PCA and cluster analyses demonstrate the changes occurring in each group of microorganisms during the course of composting. The latter process is controlled by a succession of communities varying from fungi and G− to G+ bacteria and other thermophilic and thermotolerant G− bacteria (Herrmann and Shann 1997; Klamer and Bååth 2004). The actinomycetes, considered by many authors to be an index of compost maturity, showed a continuous increase in the late steps of composting in M2, while in M1 they became stabilized from day 60 (Steger et al. 2007; Abouelwafa et al. 2008b).

From the PLFA changes that occurred in the course of composting, it appears that the succession of microbial communities was fairly similar in both composts, but differences in microbial composition were recorded depending on the initial chemical composition of the mixtures to be treated as well as the selective conditions and the degree of maturity achieved in each compost. PLFA analyses showing that mixture M1 reached a state of maturity during composting was corroborated by other analytical data based on physicochemical properties such as the spectroscopic analysis of humic substances (not shown). In contrast, in M2 the high fatty acid content hindered rapid stabilisation and maturation (Abouelwafa et al. 2008a).

This difference between the two mixtures was especially due to the composition of the initial mixture and the resulting temperature changes. Temperature showed a rapid increase in M2 from the start of composting, while in M1 a latency step and mesophilic phase was recorded before the thermophilic phase.

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