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Microbial community dynamics during composting of sewage sludge and straw studied through phospholipid and neutral lipid analysis

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ABSTRACT

The composting process involves a succession of different communities of microorganisms that decompose the initial material, transforming it into a stable final product. In this work, the levels of phospholipid fatty acid (PLFA), neutral lipid fatty acid (NLFA) and sterol were monitored in compost versus time, as indicators of the activity of various microorganisms (Gram-positive or Gram-negative bacteria, fungi, etc.). During composting, the PLFA and NLFA from Gram-negative bacteria and eukaryotes (2-OH 10; 3-OH 12; 2-OH 14; 13:0; 16:1; 18:1 *trans*) as well as some sterols of plant origin (e.g. monostearin sterols) decreased until the end of composting. In contrast, 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 right after. The PLFA 18:2 (6; 9), which is used as an index of the occurrence of some fungi, rose strongly at the beginning of composting, but fell after peak heating. In contrast, the other main sterol indicative of fungi, ergosterol, decreased at the beginning of the thermophilic phase, but increased strongly by the end of composting. Accordingly, cluster and PCA analysis separated the PLFA of Gram-negative bacteria and eukaryotic cells from those of Gram-positive bacteria and long-chain fatty acids. The fungal PLFA considered, 18:2 (9, 12), was clustered more closely to *iso*- and *anteiso*-branched PLFAs. Stigmasterol, squalene and cholesterol occurred in the lower right part of the loading plot and were clustered more closely to *iso*-, *anteiso*-branched PLFAs and 18:2w6,9 suggesting their relationship to microbial activities. We also observed the tendency of resistance of fatty acid PLFAs and NLFAs of long chain (19:0 (*cis*-9); 20:0) and some recalcitrant sterols, e.g. sitosterol, at the end of composting. The presence of high levels of the latter in the final stage indicates their contribution to the structural stability of organic matter fractions. These recalcitrant components were more clustered and occurred in the lower right part of the loading plot.

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1. Introduction

Composting is defined as the microbial degradation of heterogeneous organic material under moist, self-heating, aerobic conditions. It is basically a biooxidative process characterised by a succession of different microbial populations. A large variety of mesophilic, thermotolerant and thermophilic aerobic microorganisms (e.g. bacteria, yeasts, and fungi) are involved in the composting process [1]. The initial phase is characterised by the activity and growth of mesophilic organisms leading to a rapid increase in temperature [2,3]. In the next stage, thermophilic organisms take over the degradation process and the growth and activity of non-thermo-tolerant organisms are inhibited. The final phase, including a cooling and maturing period, is characterised by the development of a new mesophilic community. Thus, the composition of the microbial assemblage changes considerably during composting [4,5]. Numerous authors have suggested that microbial diversity is a prerequisite for a satisfactory composting process [1]. Therefore, the optimisation of compost quality is directly linked to the composition and succession of the microbial community in the composting process. Much effort has been put into understanding the changes occurring in the microbial biomass, community structure and activity during the composting process [6].

A variety of methods have been used to investigate the microorganisms participating in composting [7]. The plate-count method to count and isolate members of the microbial community is selective and not able to give a representative global picture of the microbial flora [8,9]. Biomarkers are specific chemicals produced by microorganisms, which are useful to characterise microbial biomass in various environments, such as agricultural soils and various other systems [10,11]. The most useful biomarkers are membrane lipids including fatty acids. So, certain fatty acids can be used as indica-

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Table 1
Summary of the subunits of fatty acids, their abbreviations, and their assumed indicator abilities

Abbreviation	Designation of fatty acid	Indicator for or isolated from
Straight-ch FAs	Straight-chain fatty acids	Eukaryota, widespread
Cyclopropyl FAs	Fatty acids containing a cyclopropyl ring	Gram-negatives
Branched-ch FAs	Branched-chain fatty acids in which the position of methyl branching is other than *iso-anteiso* or 10 Me	Gram-positives, actinomycetes
Iso-anteiso FAs	Position of methyl branching is *iso* or *anteiso*	Gram-positives
10 Me FAs	Methyl branching on 10th C atom	Actinomycetes
2-OH FAs	Hydroxy substitution at position 2 from carboxyl end	Pseudomonas, Gram-negative bacteria
3-OH FAs	Hydroxy substitution at position 3 from carboxyl end	Thiobacilus

tors of the microbial groups that produce them. They are essential components of all living biomass and have great structural diversity coupled with high biological specificity [12]. There are two approaches usually used to assess microbial lipids: polar phospholipid fatty acid (PLFA) analysis and total fatty acid methyl ester (total FAME) analysis [10]. Total FAME analysis, includes all saponifiable lipids present in environmental samples derived from both living and dead cells, and from all categories of life forms. Therefore, it does not discriminate between fatty acids originating from membrane lipids (PLFAs) and storage lipids (neutral lipid fatty acids: NLFA). The membrane PLFA content and composition in living bacterial cells is relatively constant over a wide range of growth conditions and PLFAs are rapidly degraded after cell death [13,14]. As microbes die, the phosphate groups in their phospholipids are mineralised. Hence, PLFA can provide an estimate of the viable microbial biomass contained in a sample. PLFA analysis is based on the fact that different subsets of microbial communities differ in their fatty acid composition though few PLFAs can be considered to be absolute signature substances for a single species or even a specific group of organisms. Accordingly, they can provide indications of overall changes in major groups, such as fungi, actinomycetes, and other Gram-positive and Gram-negative bacteria [12].

NLFA produced by the substitution of the PLFA phosphate group on the glycerol backbone for a hydroxyl, would indicate recently dead biomass, but they are also found as storage polymers in plant and animal tissue [15]. Comparing the PLFA and NLFA forms could provide information on changes in microbial structure and the turnover of microbial biomass.

In the neutral fraction, there is also a polycyclic part composed of sterols present in plants, animals and microorganisms [16]. Some sterols, like cholesterol, are responsible for structural features such as membrane fluidity and permeability and can be present in fungi. Ergosterol is widely used to estimate fungal biomass [11]. In a study by Dinel et al. [17] the main sterols extracted from duck farm compost were cholesterol and its derivatives, which constitute typical animal sterols, as well as other major sterols in plant material such as stigmasterols and β-sitosterols. These authors demonstrated the effect of neutral lipids on the aggregate stability of soil having received several applications of composted and non-composted organic waste [18]. This could influence the water retention capacity of compost-amended soils, their structural stability and the biodegradation-humification balance [19].

In the present work, all these lipid entities were followed in the course of composting. Transmethylation to recover fatty acid methyl esters was applied to study changes in fatty acids in both phospholipid (PLFA) and neutral lipid fractions (NLFA). Sterols were recovered through silylation (Table 1).

2. Materials and methods

2.1. Composting

Sewage sludge was taken from an anaerobic lagoon in an experimental wastewater treatment plant in Marrakech city, Morocco. A mixture of fresh sewage sludge (90%) and straw (10%) was composted at 54% moisture on a purpose-built platform and supervised for 6 months. To provide aerobic conditions for the composting, the mixture was turned every 2 weeks. For analysis purposes, subsamples were taken from 10 different points of the thoroughly mixed compost pile (bottom, surface, side, centre). These subsamples were pooled at each time of composting: 0 days (raw mixture); 30 days = stabilisation phase; 90; 180 days of composting = maturation phase).

2.2. Lipid extraction

Lipid extraction was carried out using the modified method of Bligh and Dyer [20]. Compost samples of 15 g dry weight were well mixed with 120 ml of chloroform–methanol mixture (2:1) by means of a Polytron homogeniser. After 12 h of contact at 4 °C, the supernatants were recovered and filtered. Two additional extractions/washings were carried out on the remaining residues (2 × 120 ml; 4 °C), the final supernatant being clear. Supernatant from three successive extractions was pooled and acidified with 20 ml of sodium chloride NaCl (0.73%) to separate the chloroform phase containing the lipids from the methanol phase containing the non-lipid compounds. The chloroform extract was dried three times by filtration through anhydrous Na_2SO_4. The total lipid content of a known volume was estimated by weighing, after evaporation and drying under N_2 vacuum.

2.3. Lipid fractionation

Phospholipids were separated from neutral lipids using silicic acid columns "Sed-pack" (WATERS; Milford, Massachussets) [21]. The columns were conditioned with 3 ml $CHCl_3$ (6 s). Lipids were then transferred to the column in 1.5 ml $CHCl_3$. Neutral lipids were eluted by 60 ml of chloroform (2 min), then 1 ml chloroform/methanol (49/1) was used to remove the monoglycerols which tended to contaminate the phospholipid fraction which was itself eluted later with 30 ml of methanol (1 min). The phospholipid and neutral lipid content was estimated by weighing the two extracts after evaporation and drying under N_2 vacuum. Both fractions were recovered in 5 ml of $CHCl_3$.

2.4. Recovery of fatty acid methyl esters of PLFA and NLFA

For transmethylation, after evaporating off the solvent, the phospholipid and neutral fractions were taken up again in 1 ml of heptane with C10:0 as internal standard and then exposed to 0.1 ml of 2 M potassium hydroxide in methanol (15 min). The upper phase containing the FAME of PLFA or NLFA was recovered for chromatographic analysis [10]. Data acquisition was carried out in the scan mode. Qualitative identification was based on external standards (26 known bacterial FAMEs, supplied by Supelco (Bellefonte, PA).

2.5. Recovery of neutral lipid through silylation

Aliquots containing about 150 μg of neutral lipid and tricaprine as internal standard were evaporated and dried under

N_2. The residue was subjected to silylation by 100 μl of Tri-Sil/BSA (Pierce Biotechnology, Rockford, Illinois) reagent (1 h). Data acquisition was carried out in the scan mode and identification was made by reference to a mixture of the external standards: monostearin, squalene, cholesterol, ergosterol, stigmasterol and lanosterol—supplied by Sigma–Aldrich (France).

2.6. GC–MS analysis

GC–MS analysis was performed with an HP-5890 series II Hewlett-Packard gas chromatograph, equipped with an MSD HP-5971 mass detector and an HP Chem data analysis station.

2.7. Statistics

The mole percent of the individual PLFAs was standardised to unit variance (scaling) before being subjected to principal component analysis (PCA) and cluster analysis to compare multivariate data obtained from PLFA profiles.

3. Results and discussion

3.1. Total lipid amounts in the course of composting

Determination of the total amounts of lipids showed a decrease from 21.4 to 8.3 mg/(g d.w.) in the course of lagooning sludge composting, which occurred mainly during the first 90 days (Fig. 1). This decrease most certainly originates from the decomposition of fat from animal and plant residues, as well as mineralisation of some dead microbial cells [22,23]. The change in the total amount of lipid cannot give any indication about changes in microbial communities during composting. Microbial community structure was assessed using membrane lipids or their fatty acids as phospholipid fatty acid and fatty acids associated with storage neutral lipid fraction (NLFA) [11,24]. The decrease of total lipid actually represents the difference between the decomposition of original fats in the waste and the variation of membrane lipids (PLFA) of microbial communities that undergo perpetual changes during composting.

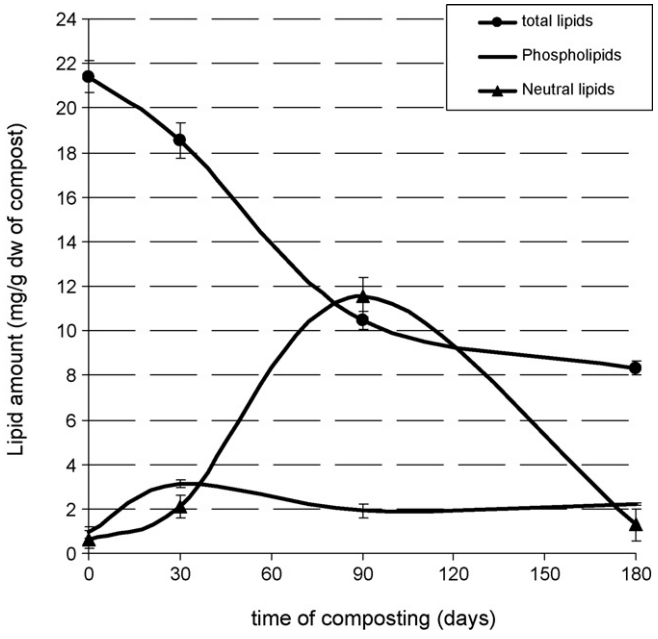

Fig. 1. Change in total lipid, phospholipid and neutral lipid levels in the course of composting of sewage sludge and straw.

3.2. PLFA and NLFA fractions

Fig. 1 illustrates the variations of PLFA and NLFA fractions in the course of composting. Both fractions showed an increase in the intermediate stages of composting and then a decrease, but the increase was of about 23.2% for PLFA and peaked at 30 days of composting, while it was greater for NLFA reaching 173% peaking at 90 days of composting. In fact, Klamer and Bååth [11] suggest that storage lipids are likely to vary much more than phospholipids, depending on the growth conditions and the C/N ratio of the substrate. For instance, Klamer and Bååth [25] reported that adding carbon-rich substrate to soil can increase fungal storage lipids 10-fold in a few days, without notably changing the phospholipid content. The pattern of both fractions agrees with phospholipid turnover occurring rapidly with respect to the storage NLFA [26,27]. Thus, the PLFA would give an estimate of the live (active) biomass in the thermophilic phase, while the storage or NLFA, would indicate the dead biomass after the active phase of composting.

3.3. Phospholipid fatty acids

PLFAs provide more information on the successional pattern of microbial groups active during the course of composting. In fact, although the sum of the quantities of PLFAs shows an increase during the stabilisation phase of composting (Fig. 2), different behaviour has been observed for specific PLFAs (Fig. 2a–f). The PLFAs can be classified into different groups presenting similar change patterns in the course of composting. The 2-OH 10; 3-OH 12; 2-OH 14; 13:0; 16:1; 18:1 *trans*, decreased and disappeared during the first 30 days of composting (Fig. 2a). The decrease of hydroxy fatty acids indicated the disappearance of Gram-negative bacteria by composting. The decrease of 16:1 and 18:1 support the suggestion of Zelles et al. [28] that monounsaturated fatty acids are strongly related to a higher substrate availability, so the decrease in their amounts implies a scarcity of bioavailable carbon during composting.

In contrast, the other PLFAs were not decreased early on in composting and even demonstrated an increase in the stabilisation step (Fig. 2b–d). The increase of *iso*-, *anteiso*-branched PLFAs (e.g. i-15:0; a-15:0; i-16; i-17) is an indicator of the growth of Gram-positive bacteria during the stabilisation phase of composting (Fig. 2b). The increase in 15- and 17-carbon chains has proved to provide a good indication of the presence of thermophilic *Bacillus* sp. [29]. Therefore, to investigate changes in the thermophilic community only PLFAs specific to the thermophilic community should be considered. Indeed, the sum of their total amounts showed an increase of about 73%, especially i-15:0, which rose by about 130%. The increase of these PLFAs in the thermophilic phase has been reported in other composting studies [10,30]. Accordingly, the results indicate intense microbial decomposition activity in the stabilisation phase [31]. Klamer and Bååth [12] suggested that the *iso*/*anteiso* ratio could be used as an index of stabilisation and maturation. In fact, the *iso*/*anteiso* ratio for the saturated molecule 15:0 increased until the temperature reached maximum levels—from 1.23 to 1.89. Then, during the last 3 months of the maturation phase, the ratio continued to increase to 1.96 ending in a clear dominance of the *iso* form.

On the other hand, the straight chain saturated fatty acids 16:0 and 18:0, which are widely distributed especially in plant matter [12,32] decreased to under half their initial levels progressively in the course of the composting process (Fig. 2c). The increase of 16:0 during the thermophilic phase could be explained by β-oxidation of other long chain fatty acids such as 18:0. Klamer and Bååth [12] and Hellmann et al. [32] noted that the large increase in 16:0 could be attributed to intense decomposition of plant matter rather than

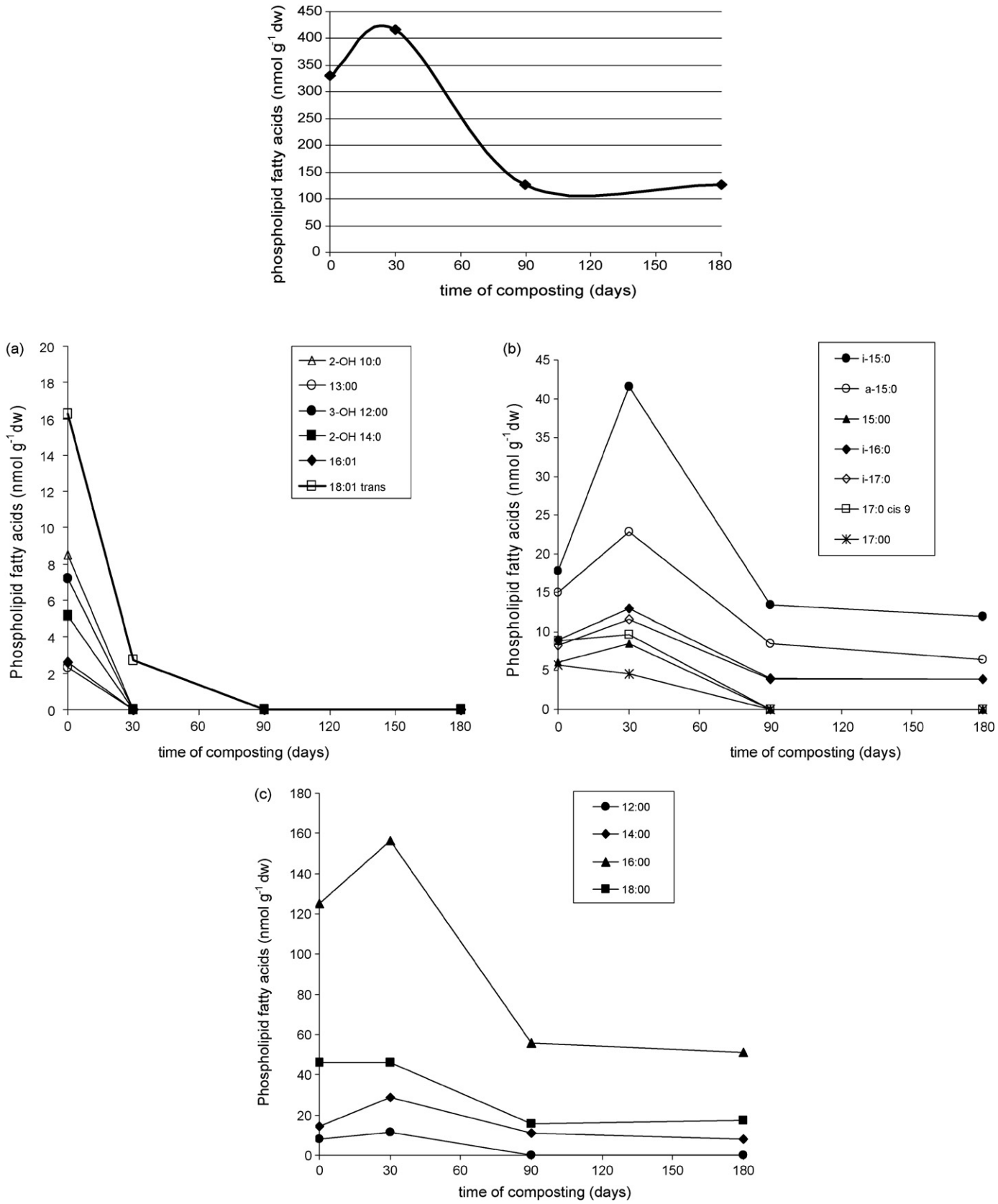

Fig. 2. Change in levels of total fatty acids of phospholipids in the course of composting of sewage sludge and straw. (a–f) Profiles of various fatty acids of phospholipids (PLFA) during composting of sewage sludge and straw.

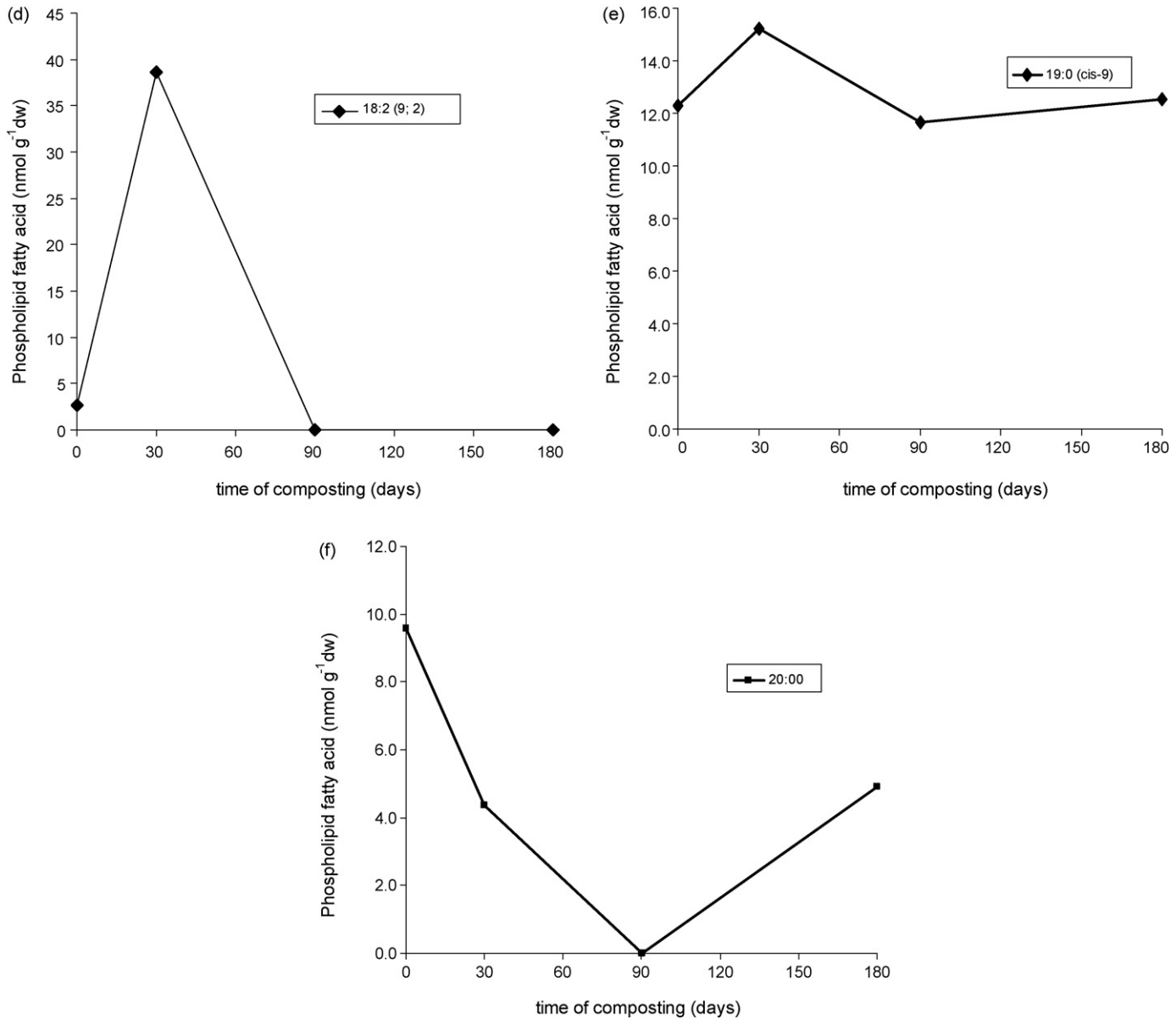

Fig. 2. (*Continued*)

to an increase in fungi. The 14:0 and 12:0 chains can also be produced by decomposition of the latter fatty acids, which decreased or disappeared at the end of composting (Fig. 2c).

The rest of the PLFAs demonstrated specific changes, 18:2 (9, 12) showed a strong increase early in composting (Fig. 2d), which could indicate a larger fungal community. Janzen and McGill [33] found that this PLFA predominated in cultures containing compost microflora. In the literature, it is shown that 18:2w6,9 increased in compost as long as temperatures remained below 50 °C; temperatures above 50 °C inhibited the growth of fungi [12]. In fact, in our study, the temperature never exceeded 52 °C.

After the thermophilic phase, by the start of the mesophilic or maturation phase, all PLFAs – except 19:0 (*cis*-9) and 20:0 – showed a decrease. In fact, in the later phases of composting, the conditions became selective and few microbes survived, so the levels of their corresponding phospholipids were reduced.

The increase in the amount of 19:0 (*cis*-9) and 20:0 at the end of composting (Fig. 2e and f) could be attributed to a gradual build-up in their levels resulting from the fact that these long chain PLAFs resist biodegradation. Dinel et al. [18] and Amir et al. [34] observed that certain lipid fractions make organic matter less biodegradable and could serve as new indicators of the degree of maturation of the organic matter of composts.

The increase of 19:0 (*cis*-9) "cy 19:0" in the stabilisation phase originates from the high temperatures reached during this phase (Fig. 2e), since these PLFAs are not characteristic of thermophilic species [32,35]. Stressful conditions, such as high temperature, decrease of pH, poor nutrient quality, insufficient aeration, etc., have been shown to stimulate the production of cyclopropyl fatty acids.

Indeed, the changes in species composition are thought to be due to adaptations to different temperature regimes [11,12,32,36–37].

3.4. Statistics

Principal component analysis with PLFA concentrations revealed a time gradient along both axes in the course of com-

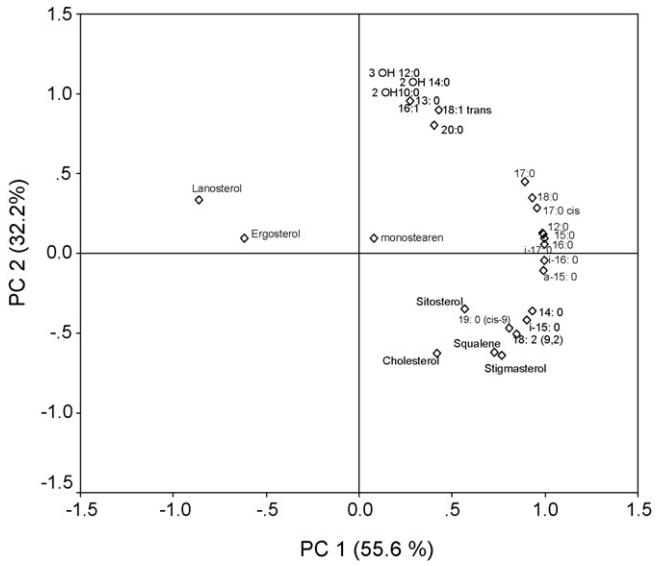

Fig. 3. Principal component analysis of PLFA profiles during composting of sewage sludge and straw.

posting (Fig. 3). In the loading plot, the fatty acids common to Gram-negative bacteria and eukaryotic cells (e.g. 2-OH 10; 3-OH 12; 2-OH 14; 13:0; 16:1; 18:1 *trans*) were present in large amounts at the start of composting and occurred in the top right part of the loading plot. The *iso*- and *anteiso*-branched, cyclopropane and 18:2 attributed to Gram-positive bacteria and fungi, are positioned in the central and lower right part of the plot.

Cluster analysis is in accordance with PCA in relationships between PLFAs according to their profiles during composting (Fig. 4). The first and second branching of the dendrogram separated the PLFA of Gram-negative bacteria and those of eukaryotic cells (e.g. 2-OH 10; 3-OH 12; 2-OH 14; 13:0; 16:1; 18:1 *trans*) from those of Gram-positive bacteria and long chain fatty acids, respectively. The fungal PLFA considered as a marker, 18:2 (9, 12), was clustered more closely to *iso*-, *anteiso*-branched PLFAs.

Accordingly, supporting the finding of other studies [38] during the stabilisation phase of composting, as temperature increased, it led to a fall in the hydroxy fatty acids and straight chain saturated and unsaturated fatty acids in favour of *iso*/*anteiso*-branched-saturated fatty acids.

3.5. Fatty acids of neutral lipids

During composting, the NLFA fraction varied in a very similar way to the PLFA fraction (Table 2), but the more significant recovery is presented in the NLFA fraction, since NLFA are used to build energy reserves. The 16:0 and 18:0 were the most prevalent NLFAs. The greatest increase was shown by NLFA 16:0 reaching 218% after 30 days of composting. The ratio NLFA/PLFA of both fatty acids 16:0 and 18:0 increased to more than six times the initial amount after 90 days of composting (Table 3). In fact, 16:0 and 18:0 are the primary storage fatty acids in triacylglycerols, and triacylglycerols are the main components of the neutral lipid fraction. Eukaryotic organisms and fungi are known to store triacylglycerols [39], whereas bacteria do not store energy as lipids but, for example, as polyhydroxybutyric acid [40].

PLFAs 18:2w6,9, which are predominant in fungi but rare in bacteria, also presented a high NLFA/PLFA ratio in the starting material (Table 3), but it decreased during the thermophilic phase. High NLFA/PLFA ratios were recorded for other fatty acids prevalent in fungi such as 16:1 and in eukaryotic organisms such as 2-OH 14:0.

Table 2
Concentration (nmol (g^{-1} d.w.)) of neutral lipid fatty acids (NLFAs) at different steps of composting of sewage sludge and straw

NLFA	0 days	30 days	90 days	180 days
12:00	42.7	0.0	0.0	32.2
14:00	77.0	197.4	258.6	62.0
i-15:0	47.8	184.3	117.5	0.0
a-15:0	48.1	163.7	148.2	66.4
15:00	39.0	0.0	137.8	29.6
2-OH 14:0	41.7	135.0	0.0	0.0
i-16:0	15.6	0.0	0.0	0.0
16:01	63.5	0.0	124.8	25.1
16:00	793.1	2564.2	2293.7	354.3
i-17:0	37.9	0.0	0.0	0.0
17:0 (*cis*-9)	102.6	156.9	171.1	60.5
17:00	175.7	249.2	89.2	217.2
18:2 (9; 2)	192.8	172.2	158.8	0.0
18:01 *trans*	87.9	0.0	0.0	0.0
18:00	289.6	863.2	572.6	118.0
19:0 (*cis*-9)	16.1	0.0	0.0	0.0
20:00	61.5	0.0	73.8	24.2

d.w.: dry weight.

Bååth [25] showed that high NLFA/PLFA ratios were found for fatty acids common in eukaryotic organisms and fungi (18:2w6,9 and 16:1) or in saturated fatty acids, common to many types of organisms.

In contrast, for fatty acids common in bacteria such as branched *iso*- and *anteiso*-fatty acids and especially cyclopropane fatty acids cy 19, low NLFA/PLFA ratios were found (Table 3), similar findings are also reported by Bååth [25].

Among fatty acids common in bacteria, the NLFA i-15:0, a-15:0, showed a strong increase in the thermophilic phase of composting reaching about 279% and 234% of the initial amounts, while NLFA i-16:0 and NLFA i-17:0 showed a decrease. NLFA i-15:0, a-15:0 presented a decrease in the later phase of composting. The ratio NLFA/PLFA of both i-15:0 and mainly a-15:0 increased at the beginning of composting, but decreased at the end. The increase is due to the slow turnover of the NLFA compared to PLFA as explained by Bååth [25].

3.6. Fate of sterols during composting

Levels of certain sterols studied during sludge composting are reported in Table 4. In the course of composting, the amount of stigmasterol, squalene and cholesterol showed an increase during the stabilisation phase (30 days of composting). In the last step of com-

Table 3
Ratio of NLFA to phospholipid concentrations at different steps of composting of sewage sludge and straw

NLFA/PLFA	0 days	30 days	90 days	180 days
12:00	5.3	0.0	0.0	-
14:00	5.4	6.9	23.1	7.8
i-15:0	2.7	4.4	8.8	0.0
a-15:0	3.2	7.1	17.5	10.4
15:00	6.4	–	–	–
2-OH 14:0	8.1	–	–	–
i-16:0	1.8	–	–	–
16:01	24.1	–	–	–
16:00	6.3	16.4	41.1	6.9
i-17:0	4.6	0.0	0.0	0.0
17:0 (*cis*-9)	11.5	16.2	–	–
17:00	30.8	54.5	–	–
18:2 (9; 2)	72.8	4.5	–	–
18:01 *trans*	5.4	0.0	0.0	0.0
18:00	6.3	18.7	36.5	6.8
19:0 (*cis*-9)	1.3	0.0	0.0	0.0
20:00	6.4	0.0	–	4.9

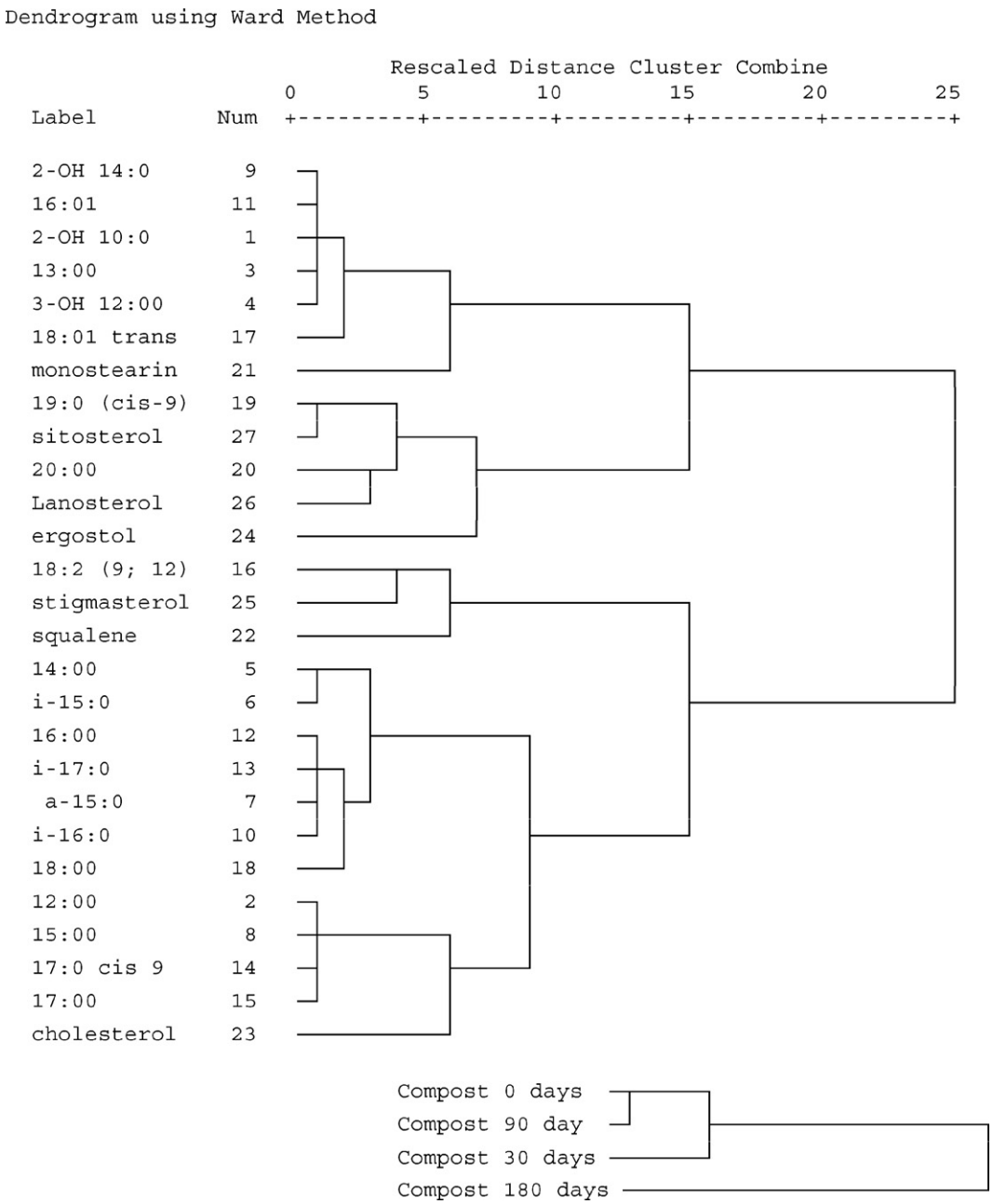

Fig. 4. Dendrogram from cluster analysis of PLFA profiles during composting of sewage sludge and straw.

posting, squalene and stigmasterol showed a decrease just after 30 days of composting. The first increase could be produced by partially decomposed and undecomposed plant and animal residues [16]. Dinel et al. [17] show that after 23 days, concentrations of fatty acids decreased by 84.6%, but those of sterols increased by 826.4%. These authors suggest that sterols were synthesised most likely by microbial activity during composting, which was confirmed by a significant increase in the number of sterols present, rising from 3 (day 0) to 12 (day 23). Indeed stigmasterol, squalene and cholesterol occurred in the lower right part of loading plot (Fig. 3) and were clustered more closely to *iso*-, *anteiso*-branched PLFAs and 18:2w6,9 (Fig. 4).

Table 4
Concentrations of some sterols at different stages of composting of sewage sludge and straw

Sterols (10^{-1} g/kg)	0 days	30 days	90 days	180 days
Monostearin	4.02	2.91	5.58	0.87
Squalene	1.28	5.97	3.02	0.26
Cholesterol	2.47	6.39	6.23	1.25
Ergosterol	12.88	9.09	9.64	29.74
Stigmasterol	0.81	7.85	2.27	0.47
Lanosterol	2.24	1.41	2.18	2.98
Sitosterol	0.46	0.52	0.43	0.49

Rather than attributing the decrease of these sterols at the end of composting to decomposition, it is more likely due to polycondensation or to their conversion to more complex and stable chemical structures [34]. Schnitzer et al. [16] suggest that during composting greater molecular cross-linking and more inter- and intra-molecular associations of the organic matter occur, forming larger and more stable molecules chemically and biologically less reactive than the starting materials. They consider that cellu-

loses, lignins and proteinaceous materials are "glued" together by the lipids and sterols. The other neutral lipid that was followed, monostearin, showed a slight decrease during the first 30 days of composting (Table 4), primarily attributed to its biodegradation during the stabilisation phase. However, the increase that occurred after the stabilisation phase could be explained by the fact that it is a storage lipid still present in recently dead biomass, and which is converted into more complex forms at the end of composting. Sitosterol, always reported as being a plant sterol [21], did not show significant variations during composting as it is more resistant to biodegradation. Fig. 4 shows that this sterol was clustered more closely to long chain fatty acids 19:0 (*cis*-9) and 20:00.

Of all the studied sterols, ergosterol was preponderant in the raw material (Table 4). In contrast to the previously mentioned sterols, this compound along with its precursor lanosterol [41] showed a decrease during the heating phase, but an increase at the end of composting. Both sterols occurred in the upper left part of loading plot (Fig. 3). The ergosterol level has been considered in numerous studies to provide an estimate of fungal biomass changes during composting, since it is generally regarded as providing a good index of fungal growth [11,24,42].

Thus, the decrease of the fungal sterols during the heating phase is attributed to the inhibition of fungal activity when the temperature reaches 50 °C [12]. After the decrease of temperature, in the maturation phase, these sterols, especially ergosterol, showed an increase at the end of composting (Table 4). This is attributed mainly to the fungal growth characterising the maturation or curing phase of composting. Taiwo and Oso [43] suggested that at the thermophilic stage the diversity of fungi is markedly reduced and that when the temperature becomes suitable once more at the maturation step, some of these organisms grow again from spores.

However, the above observations based on changes in the amount of PLFA 18:2w6 (Fig. 2d), also considered as a good index of fungal biomass, implies a contradiction in that intense growth of fungus is observed in the stabilisation phase, which disappears in the maturation phase.

In some studies, ergosterol has been shown to correlate well with the amounts of 18:2w6 [10,11,21,44]. However, in our work, no relationship was found between variations in the levels of ergosterol and 18:2w6 fatty acid. This supports the conclusions of other studies [24,45–46] that it would be erroneous to rely on only one marker such as PLFA 18:2w6 to monitor changes in the abundance of fungi. Indeed, different fungal taxa contain different amounts of 18:2w6 and under certain environmental conditions fungi may not synthesise 18:2w6 at all [11,47]. Moreover, numerous authors suggest that 18:2 cannot be regarded as signature fatty acids for fungi, because they are also widely distributed among other organisms and are found in plant matter [11,32,48]. In contrast, ergosterol is specific for higher fungi and does not occur in plants; therefore, it is frequently used as a marker for fungal content in biological samples [49,50].

It should be noted, however, that the difference in the levels of different fungal taxa in the compost could be the origin of this contradiction. Deacon [51] reported that *Rhizomucor pusillus* is a typical early coloniser of composts, in a temperature range of 20–55 °C, exploiting simple sugars, amino acids, etc. that are initially present in the plant material. It is inactivated during peak heating, and it does not recolonise afterwards. Other fungi, *H. lanuginosus* grows from 30 to 52–55 °C. It colonises composts after peak heating and persists throughout the high-temperature phase. Thus, different fungi colonise the compost during different temperature regimes [52].

Other authors have shown that the occurrence of ergosterol is generally restricted to the more advanced fungal taxa – ascomycetes and basidiomycetes – while the more primitive taxa – e.g. some zygomycetes – contain other sterols [53]. In addition, Klamer and Bååth [11] reported that the ergosterol content correlated well with the hyphal diameter of the fungi.

This shift in the composition of fatty acids and sterols during composting points to a change from Gram-negative bacteria to a community with more Gram-positive bacteria and fungi. In fact, Gram-positive bacteria are often reported as being the part of the microbial community responsible for the decomposition of organic residues in the thermophilic phase [8,38,54–55]. Some fungi continue the decomposition process instead of bacteria in the maturation mesophilic phase, because they are more efficient at breaking down more recalcitrant organic polymers such as lignins or "tough debris". Thus, at the end of composting, the decline of the bacterial population resulting from the exhaustion of readily degradable substrate, and the predominance of fungi which proliferate on the remaining less degradable organic matter indicates the good progress of decomposition and stability of the initial material to be composted, and hence the good quality of the end compost.

4. Conclusion

These findings imply that, as the different steps of the composting process take place, the successional changes of status in the microbial communities can be monitored by the composition of both fatty acids and sterols. During the thermophilic phase, the PLFA, NLFA from Gram-negative bacteria and eukaryotes as well as sterols of plant origin decreased. In contrast, branched fatty acids of *iso*- and *anteiso*-forms increased mainly in the thermophilic phase, but then decreased. The dominance of fungi in the maturation or mesophilic phase leads to the high amounts of the "ergosterol" by the end of composting. The increase in the degree of polycondensation and the cross-linking of other organic compounds present with long chain fatty acids and some recalcitrant sterols could form highly complex organic material more stable to microbial biodegradation. Overall, these variations could be indicative of compost stability and maturity, supporting the findings of previous studies.

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