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Molecular Characterization of Compost at Increasing Stages of Maturity. 1. Chemical Fractionation and Infrared Spectroscopy

RICCARDO SPACCINI* AND ALESSANDRO PICCOLO

Dipartimento Scienze del Suolo, della Pianta, e dell'Ambiente, Universitá di Napoli Federico II, Via Universitá 100, 80055 Portici, Italy

Composted organic biomasses at 60, 90, and 150 days of maturity were studied for changes in molecular composition. Compost samples were subjected to a mild sequential fractionation based on (1) organic solvent extraction, (2) transesterification with boron trifluoride in methanol (BF_3-CH_3OH), and (3) methanolic alkaline hydrolysis ($KOH-CH_3OH$). The general chemical variations in compost residues following fractionation were monitored by DRIFT spectroscopy, whereas the molecular components separated along the fractionation steps were identified by GC-MS. DRIFT spectra suggested a progressive decrease of biolabile compounds such as alkyls, carbohydrates, and proteinaceous materials with compost maturity. Extraction of unbound components in an organic solvent indicated a considerable reduction of linear and branched alkanoic acids, both saturated and unsaturated, *n*-alkanes, and *n*-alkanols with enhancing compost maturity. Extracts of weakly bound molecules by transesterification revealed a decrease, with compost maturity, of components from more recalcitrant plant polyesters, such as ω-, di-, and trihydroxy acids, dioic acids, and *n*-alkanols. Extracts of strongly bound molecules by alkaline hydrolysis indicated a lower decrease of the same components, suggesting their reduced availability when in stable hydrophobic domains of progressively mature compost. The largest decrease in molecular components occurred when compost was stabilized from 60 to 90 days, whereas its composition did not significantly vary after stabilization at 150 days. The molecular structures of a number of steroids and terpenes appeared to be less susceptible to transformation with composting maturity, thereby resulting as useful biomarkers to trace the fate of composted organic matter in the environment. This work showed that a detailed molecular characterization of compost by a stepwise chemical fractionation enables the evaluation of compost maturity and origin of composted biomasses, as well as the identification of environmental tracers.

KEYWORDS: Compost maturity; biomass recycling; molecular characterization; sequential molecular fractionation; hydrophobic components

INTRODUCTION

Composting is a widespread practice for recycling organic wastes in the soil environment. The main advantage of compost amendment resides in the long-term improvement of soil physical stabilization through an increased accumulation of organic matter. Hence, the more biologically stable the compost, the greater is its effect on soil quality.

Research has generally focused on the changes, with composting time, of total organic carbon and nitrogen (*1*) and molecular characteristics of the dissolved organic matter (DOM) fraction (*2*), as well as the colloidal humic-like components (*3*, *4*). However, it is doubtful that an evaluation of the very general chemical properties of DOM and humic extracts may be sufficient to evaluate the development of compost maturity and its transformation in soil. Both of these processes may be better understood when a molecular comprehension of compost composition can be reached. Nevertheless, little attention has been so far given to the transformation and composition of compost at the molecular level (*5*).

A possible approach may be to follow the progressive change of alkyl components with different maturity stages of an aerobically digested compost. The identification of hydrophobic compounds is used to evaluate the diagenetic processes in sediments of terrestrial and marine environments (*6*) and modifications of plant tissues, litter (*7*), and persistent organic matter in soil (*8*−*11*). Even though alkyl molecules are only a minor fraction of the fresh organic matter reaching soil on seasonal bases, it has been shown that they play an essential role in the accumulation and stabilization of organic matter in the soil environment (*12*−*15*).

The heterogeneity of hydrophobic components in compost requires a sequence of chemical treatments that may progres-

* Corresponding author (e-mail riccardo.spaccini@unina.it).

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sively reduce the complexity of extracts and facilitate the identification of their molecular structure. A stepwise fractionation may imply a preliminary extraction in an organic solvent of unbound or free components. Then, the unsoluble biopolyesters deriving from vegetal tissues may be depolymerized by a mild acidic methyl transesterification (*16*−*18*), thereby solubilizing weakly bound molecules. Finally, an alkaline hydrolysis may dissolve the residual components strongly bound in a matrix of more complex polyesters (*19*−*21*).

The objective of this work was, thus, to obtain information on the molecular changes occurring in compost subjected to different periods of aerobic stabilization by applying a sequential chemical fractionation of the hydrophobic components of compost. An advanced understanding of the molecular changes of compost may also lead to the identification of natural biomarkers, which may become useful to define an index of compost maturity and trace the environmental fate of compost constituents after application to soil.

MATERIALS AND METHODS

Compost Samples. The organic biomasses used in compost production (GeSeNu SrL, Perugia, Italy) had the following composition: 50% domestic organic wastes, 40% refuse from plant trimming, and 10% vegetal residues from tobacco and aromatic plants. These organic materials were mixed, ground, and sieved at 12 mm. The final products were obtained through an aerobic composting process consisting of 30 days of a common active phase, followed by three different curing phases of 30, 60, or 120 days of maturity, which yielded, respectively, compost samples 60, 90, and 150. Compost was oven-dried at 40 °C until constant weight and sieved at 500 μm. Each compost sample was subjected to the fractionation sequence in five replicates.

Organic Solvent Extraction. The free or unbound components were extracted by shaking compost (1 g) for 2 h at room temperature with a 15 mL solution of dichloromethane and methanol (DCM/CH_3OH, 2:1, v/v). The extract was separated from residue through centrifugation (15 min, 5000 rpm) in Teflon tubes and the supernatant removed. The residue was further extracted with the same extracting solution (DCM/CH_3OH) overnight at room temperature, and the supernatant was separated again by centrifugation. The extracts were combined, dried by rotoevaporation, and added with a minimum amount of a DCM/isopropanol (2:1 v/v) solution. This extract was separated in two fractions, a neutral and an acid fraction, by using a solid-phase extraction (SPE) cartridge filled with a bonded aminopropyl solid phase (Strata NH_2 500 mg of sorbent, 3 mL elution capacity; Phenomenex) and previously conditioned with *n*-hexane The DCM/isopropanol solution was slowly loaded onto the SPE cartridge, and the neutral fraction was eluted with an additional 8 mL of the DCM/isopropanol solution. The cartridge was then flushed with 2% v/v of acetic acid in diethyl ether (8 mL) to elute the acidic fraction. The recovery capacity from the SPE cartridge, controlled with sitosterol and docosandioc acid as standards, was >95%. Both eluted fractions were dried under a gentle stream of nitrogen and subjected to GC-MS and GC-FID analyses.

Transesterification. The solid residue remaining from the organic solvent extraction was air-dried and placed in a screw-stoppered Teflon tube. Fifteen milliliters of a 12% solution of boron trifluoride in methanol (BF_3−CH_3OH) was added to the tube, which was then flushed with N_2 and, after sealing, heated at 90 °C overnight. This treatment was repeated twice. The supernatants were first separated by centrifugation (15 min, 5000 rpm) and filtered over a glass filter, and the residue was deposited on the filter rinsed with an additional 15 mL of CH_3OH to completely remove the released compounds. The liquid extracts were combined, treated with an excess of deionized water to destroy the residual BF_3−CH_3OH complex, and then liquid−liquid extracted twice with 10 mL of chloroform in a separation funnel. The organic phase was concentrated, dehydrated with anhydrous Na_2SO_4, and evaporated at reduced pressure for the GC-MS and GC-FID analyses.

Methanolic Alkaline Hydrolysis. The compost residue from the two previous steps was hydrolyzed for 1 h under reflux at 75 °C with 20 mL of a 1 M KOH−CH_3OH solution in a three-neck round flask under inert N_2 atmosphere. After recovery of the supernatant by centrifugation (15 min, 5000 rpm) and filtration, the residue was again refluxed twice with 20 mL of CH_3OH for 30 min. The combined methanolic extracts were concentrated by rotoevaporation, acidified to pH 5 with a 3 M HCl solution, and transferred in a separation funnel with 30 mL of deionized water. The released organic compounds were finally extracted three times with 30 mL of chloroform. The organic phase was concentrated, dehydrated with anhydrous Na_2SO_4, and evaporated at reduced pressure for the GC-MS and GC-FID analyses.

Derivatization. Before GC analyses, aliquots of the extracts obtained through fractionation steps were derivatized by methylation (except for the extract from BF_3−CH_3OH transesterification) and silylation procedures, following addition of known amounts of tridecanoic acid as internal standard. Methylation of acidic functional groups was carried out by refluxing the dried extracts with acetyl chloride in an excess of methanol for 30 min at 60 °C. The solution was concentrated by rotoevaporation, transferred in a 2 mL glass vial, and dried under N_2 to be ready for the subsequent silylation. The other polar functional groups (mainly OH of straight-chain alcohols, sterols, hydroxy fatty acids, and phenols) were silylated by adding of 200 μL of *N*,*O*-bis-(trimethylsilyl trifluoroacetamide) (BSTFA) reagent with 1% of trimethylchlorosilane (TMS) and heating for 1 h at 70 °C. The excess of the sylilating agent was removed by streaming N_2, and 100 μL of *n*-hexane was added to the products before GC-FID and GC-MS analyses.

GC-MS and GC-FID. Alkyl compounds were analyzed by GC-FID on a Perkin-Elmer (PE) Autosystem XL equipped with a ZB-5 WCOT capillary column (Phenomenex, 30 m × 0.25 mm; film thickness = 0.25 μm). The temperature program was from 100 to 320 °C (10 min isotherm) at a 4 °C min^{-1} rate. He was the carrier gas at 1.60 mL/min, the injector temperature was at 250 °C, the split injection mode had a 30 mL/min of split flow, and the FID temperature was set at 300 °C. The GC-MS analyses were carried out with the PE Autosystem XL equipped with an RTX-5MS WCOT capillary column (Restek 30 m × 0.25 mm; film thickness = 0.25 μm) and coupled, through a heated transfer line (250 °C), with a PE Turbomass-Gold mass spectrometer. The chromatographic conditions were the same as for GC-FID analysis. Mass spectra were obtained in EI mode (70 eV), scanning in the range *m*/*z* 45−650, with a cycle time of 1 s. Compound identification was based on comparison of mass spectra with the NIST library database, published spectra, and real standards.

For quantitative analysis, due to the large variety of detected compounds with different chromatographic responses, external calibration curves were built using mixtures of the following standards: tridecanoic acid, octadecanol, 16-hydroxyhexadecanoic acid, docosandioic acid, β-sitosterol, and cinnamic acid. Tridecanoic acid was also used as internal standard to evaluate derivatization yields and steadiness of chromatographic response.

Infrared Spectroscopy. Diffuse reflectance infrared Fourier transform spectroscopy (DRIFT) spectra of bulk compost samples and solid residues resulting from the three fractionation steps were recorded with a PE Spectrum-One spectrometer, equipped with a diffuse reflectance accessory, accumulating up to 100 scan with a resolution of 4 cm^{-1}. Before DRIFT analysis, dry samples were finely ground and diluted with a KBr powder (5/100, w/w), an agate mortar.

RESULTS AND DISCUSSION

Spectroscopic Characterization. The DRIFT spectra of bulk compost samples 60, 90, and 150 are shown in **Figure 1**, whereas those of solid residues remaining from fractionation of compost 60 are reported in **Figure 2**. The band assignment in DRIFT spectra are based on the literature (*22*).

A large content of alkyl compounds was revealed in DRIFT spectra of less mature compost 60 (**Figure 1**) by the strong absorptions in the 2926−2853 cm^{-1} interval and the intense peaks in the 1452−1368 cm^{-1} region, which represented, respectively, the symmetrical and asymmetrical stretching and bending vibrations of CH_2 groups in long-chain aliphatic

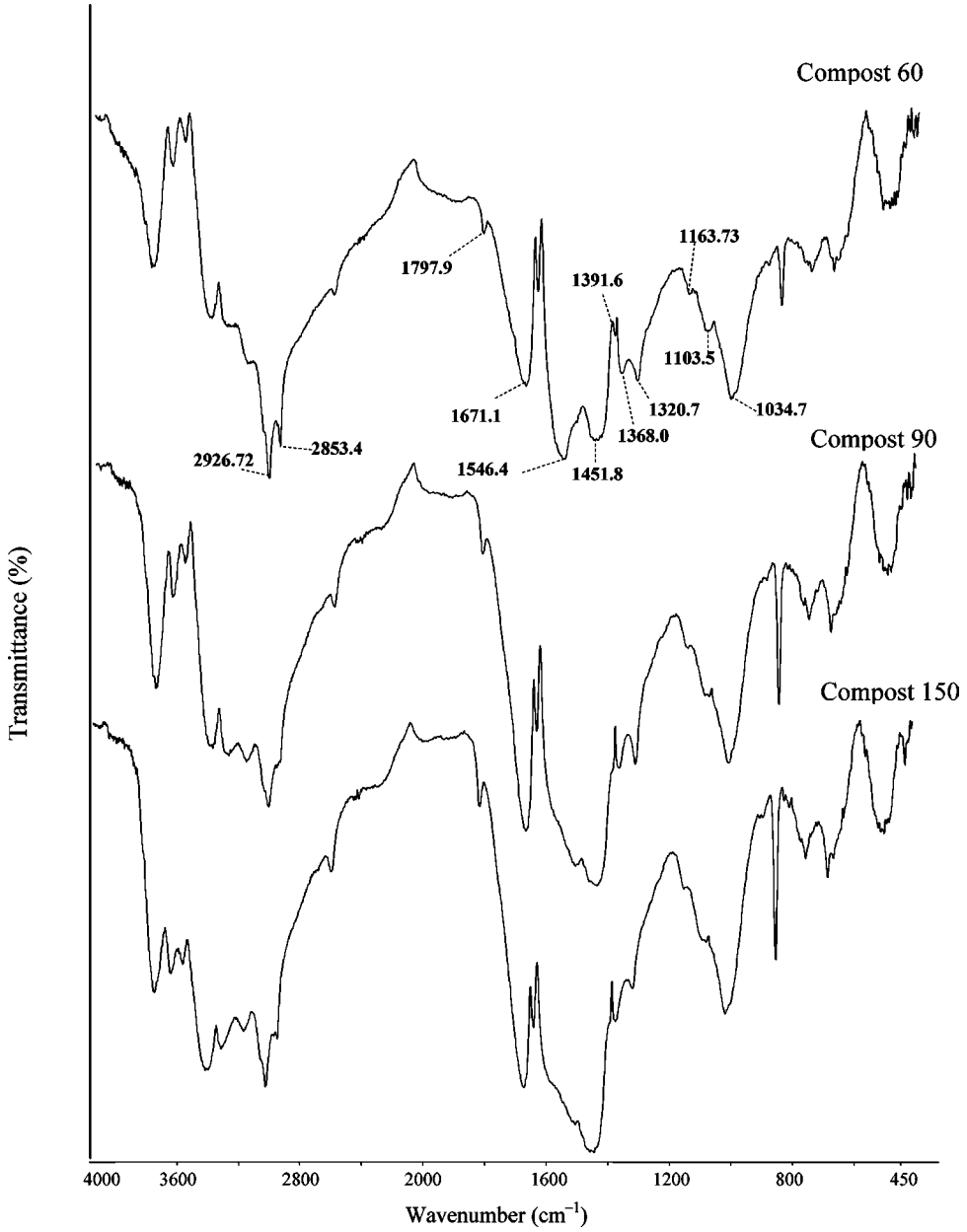

Figure 1. DRIFT spectra of bulk compost samples at different periods of curing phase: compost 60 = 60 days; compost 90 = 90 days; compost 150 = 150 days.

molecules. Moreover, the signals at 1671 and 1321 cm^{-1}, assigned to C═O and C—O groups in aliphatic carboxylic acids, may be related to alkyl acids present as carboxylate ions and/or involved in intermolecular hydrogen bonding. Both forms lower the stretching frequency usually found for carbonyl vibration in protonated acids. The shoulder at 1798 cm^{-1} was tentatively assigned to stretching vibrations of carbonyl groups in alkyl and alkyl−aryl polyester bonds of cutin and suberin plant biolpolymers. Other evident signals were the intense band at 1546 cm^{-1} (amide II band) related to peptidic material and peaks at 1034, 1103, and 1164 cm^{-1}, usually assigned to C−O bonds in both polyalcoholic and ether functional groups, such as those of polysaccharides and simple carbohydrates.

A large decrease of alkyl signals in the 2926−2853 cm^{-1} region was shown by the DRIFT spectra of bulk compost samples at 90 and 150 days of maturity (**Figure 1**). Other variations were the progressive disappearance of the peak at 1546 cm^{-1} and a slight reduction of band intensity for alcoholic functional groups (1030−1160 cm^{-1}). These alterations suggest a progressive degradation, with composting process, of the most bioavailable compounds such as free lipids and proteinaceous and carbohydratic substances. On the other hand, the persistence in the mature compost (**Figure 1**, compost 150) of the band related to methylenic groups in alkyl chains and the almost unvaried bands around the 1360−1452 cm^{-1} interval and at 1671 and 1795 cm^{-1} indicate the presence of significant amounts of stable alkyl compounds in the final composting product.

The progressive removal of alkyl components by chemical fractionation was revealed by the spectra of solid residues remaining after each step. **Figure 2** shows the structural changes for only compost 60, those of samples 90 and 150 being very similar. The DRIFT spectrum of bulk sample (**A**) as compared to that obtained after organic solvent extraction (**B**), BF_3−CH_3-OH transesterification (**C**), and final KOH−CH_3OH hydrolysis (**D**) indicates a progressive decrease of bands associated with alkyl substances. The organic solvent extraction affected mainly the 2926−2853 cm^{-1} region and the peak centered at 1660 cm^{-1}. These bands showed a continuous decrease with the

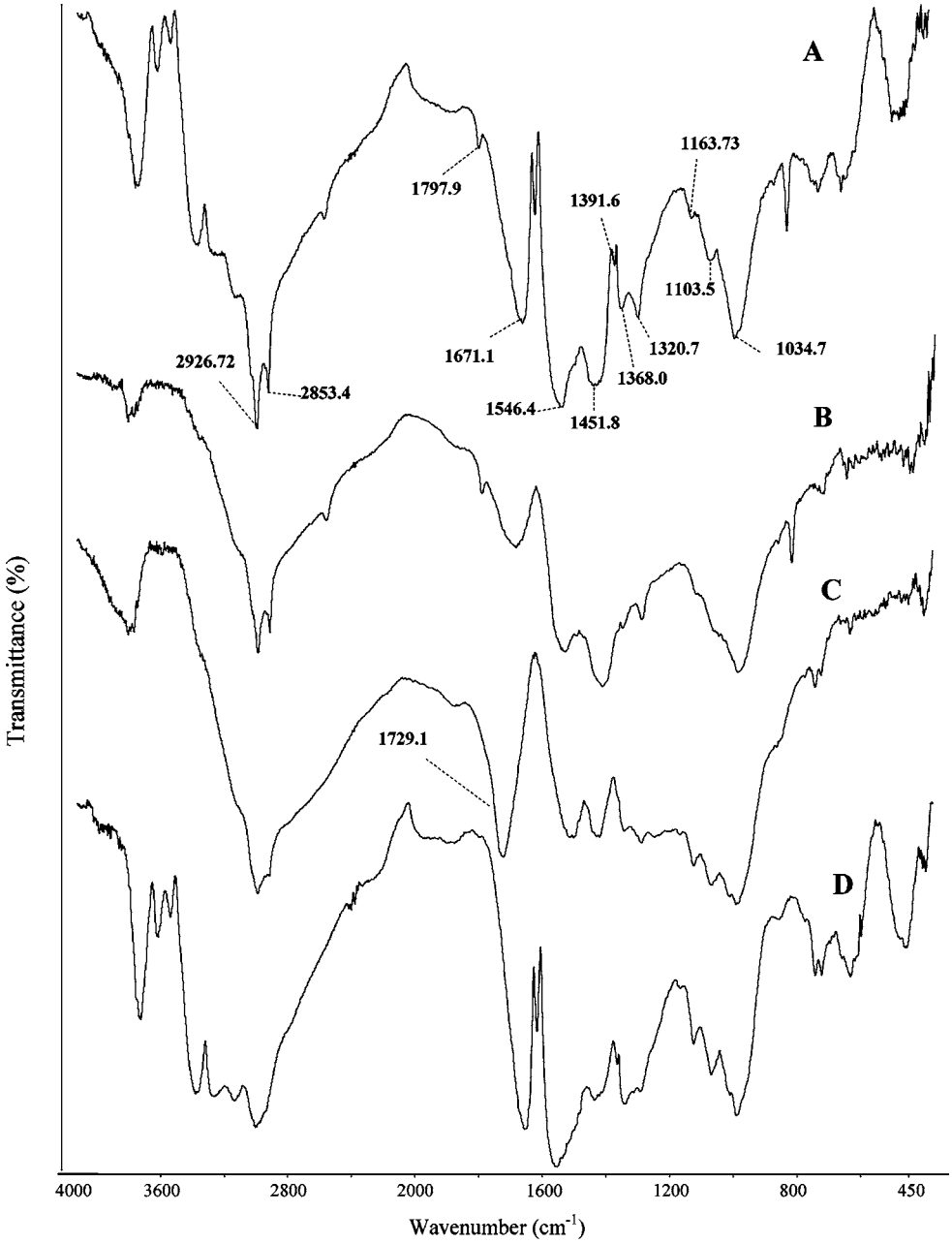

Figure 2. DRIFT spectra of compost 60 at different steps of chemical fractionation: (**A**) initial bulk sample; (**B**) after organic solvent extraction; (**C**) after BF_3–MEOH transesterification; (**D**) after KOH–MeOH base hydrolysis.

additional fractionation steps (**Figure 2B−D**). Methanolic transesterification and alkaline hydrolysis completely removed the band assigned to carbonyl group in esters (1798 cm^{-1}) and significantly reduced methylenic bending vibration around the 1448−1369 cm^{-1} region. These structural changes suggested the loss of long-chain carboxylic acids associated in compost with complex polyesters. The absorption shift to higher frequencies found for protonated carboxylic groups in solid residue after BF_3-CH_3OH treatment (the 1729 cm^{-1} band in **Figure 2C**) may be explained by the pH decrease resulting from the acidic transesterification reaction.

Unbound Components. The alkyl compounds released from the compost matrix at increasing maturity, their extraction yield, dimensional range, and dominant homologues are shown in **Table 1**. The most abundant components in the less mature compost 60 were in the order fatty acids, alkanes, alcohols, sterols, and diterpenoids.

The fatty acid methyl esters (FAME) obtained from sample 60 (**Figure 3**) had chain length varying from C_{12} to C_{28}, with a marked predominance of even over odd carbon numbers. Whereas this characteristic is typical of higher plant lipids (*23*), the large yield of FAME may be an indication of their multiple origin. In fact, about 90% of total FAME was represented by hexadecanoic (C16) and octadecanoic (C18) unsaturated and saturated homologues, which are usually found in different substrates (*24*). The short-chain fatty acids are among the main products of microbial activity, whereas straight midchain compounds have multiple origins, with unsaturated homologues also deriving from bacterial desaturation (*25*). Therefore, alkyl metabolites of microflora and fungi may also contribute to compost unbound components.

Different origins have also been suggested for long-chain (>C20) alkylcarboxylic acids in degraded organic matter. The longer chain FAME may derive from the breakdown of aliphatic

Table 1. Yields (Micrograms per Gram of Dry Weight)[a] and Composition[b] of the Organic Solvent Extract from Compost at Increasing Maturity

	compost 60	compost 90	compost 150
n-alkanoic acids	24225; $C_{12} \div C_{28}$ ($C_{18:1}$)	6288; $C_{12} \div C_{28}$ (C_{16}, $C_{18:1}$)	2770; $C_{12} \div C_{28}$ (C_{16}, $C_{18:1}$)
unsaturated (%)	51.9	36.5	32.2
long chain (%)	5.2	15.4	25.8
branched alkanoic acids	472; $C_{15} \div C_{19}$ (C_{17})	110; $C_{15} \div C_{19}$ (C_{17})	90; $C_{15} \div C_{19}$ (C_{16})
n-alkanes[c]	7540; $C_{25} \div C_{33}$ (C_{29}, C_{31}) CPI = 3.8; ACL =29.8	3527; $C_{25} \div C_{33}$ (C_{31}, C_{33}) CPI = 4.2; ACL =30.3	2944; $C_{25} \div C_{33}$ (C_{31}, C_{33}) CPI =5.0; ACL =30.5
n-alkanols	2370; $C_{22} \div C_{28}$ ($C_{24, 26}$)	850; (C_{26})	700; (C_{26})
steroids[d]	810	684	670
diterpenes[d]	530	505	485

[a] Overall coefficient of variations lower than 15%; $n = 5$. [b] Total range varying from C_i to C_j carbon numbers; compounds in parentheses are the most dominant homologues in the class; numbers after colon refer to double bond. [c] CPI = carbon preference index = $(\Sigma_{odd}\, C_{25} \div C_{31} + \Sigma_{odd}\, C_{27} \div C_{33})/2\Sigma_{even}\, C_{26} \div C_{32}$. ACL = average chain length = $\Sigma([C_i] \times i)/\Sigma[C_i]$; $[C_i]$ = concentration of *n*-alkane containing *i* carbon atoms. [d] Representative compounds listed in Table S1 in the Supporting Information.

esters that, together with *n*-alkanes and steroids, form the external protective wax layer in aerobial plant tissues (*11*). Alternatively, they can originate from the microbial oxidation of other alkyl molecules such as long-chain *n*-alkanes and alcohols (*23*, *26*). Conversely, a direct input from microbial activity was revealed by the detection, among the lightest components, of branched-chain FAME (**Table 1**). The most important compounds were the 12- and 13-methyltetradecanoic and hexadecanoic acids (iso and anteiso C_{15} and C_{17} FAME in **Figure 3**), which are common microbial constituents of natural organic matter in terrestrial and marine environments.

The distribution of *n*-alkanes in compost 60 (**Figure 4**) revealed a prevalence of longer chain components (from C_{25} to C_{33}) and a distinct predominance of odd versus even carbon numbers. The origin of linear hydrocarbons may be estimated through the evaluation of two dimensionless indices: the carbon preference index (CPI) and the average chain length (ACL). They represent, respectively, the ratio between the concentration of odd versus even members and the weight-average value of a compound with a specific chain length. The high values found for these parameters (**Table 1**) are due to the large concentrations of nonacosane, hentriacontane, and tritriacontane, which are typical components of wax layers of higher plants (*27*, *28*).

The short range of aliphatic alcohols, varying from C_{22} to C_{28} and centered around tetracosanol and hexacosanol dominant homologues, also suggests a plant contribution (*29*). This origin was supported also by the presence of tetra- and pentacyclic triterpenes and tricyclic diterpenes in the extracts from compost 60 (**Table 1**). Two compounds (stigmasta-3,5-dien-7-one and urs-12-en-28-oic acid, 3-methoxymethyl ester) were tentatively identified by their chromatographic retention times, and mass spectra are shown in Figure S1 in the Supporting Information. Both tetracyclic and pentacyclic triterpenes are among the main lipid components of aerobial and root plant tissues (*10*, *29*, *30*). The relatively low amount found in compost 60 could be related to the biotic and abiotic degradation during composting. It was already pointed out that sterol and triterpenol undergo a rapid decline once exposed to microbial activity in soil (*11*, *29*). Other less abundant molecules found in the organic solvent extract from compost 60 were assigned to diterpenoid compounds (**Table 2**), such as abietane, pimarane, isopimaranes, and labdane structures, the main mass fragments and molecular structures of which are shown in Table S1 and Figure S2 of the Supporting Information.

Both diterpenoid and triterpenoid compounds are useful indicators of coniferous and angiosperm plant input in terrestrial and marine environments (*31*). Despite the various alteration processes, these molecules usually preserve the structural characteristics of their original precursors. Thus, *bio-terpenoids* may be converted into *geo-terpenoids* and used as valuable biomarkers for tracing biological sources in natural organic matter (*30*, *32*).

The more mature compost samples showed a large decrease of unbound components, the total yield of which passed from 35960 μg g^{-1} in the starting sample to 11960 and 7660 μg g^{-1} in composts 90 and 150, respectively (**Table 1**). Aliphatic alcohols were reduced significantly already after 90 days, whereas the largest absolute decrease was shown by *n*-fatty acids, which showed losses of 73 and 56% after 90 and 150 days, respectively. Both hexadecanoic and octadecanoic acids showed substantial losses, being reduced from 89 to 96% and from 86 to 90.5% for unsaturated and saturated homologues, respectively. Long-chain alkylcarboxylic acids ($>C_{20}$) were lost relatively less, thereby passing from 5 to 26% of the total amount of FAME (**Figure 3**). The yield of methyl-branched fatty acids largely decreased by passing from compost 60 to compost 90 and remained stable in the most mature, compost 150 (**Table 1** and **Figure 3**). This suggests the attainment of a steady microbial activity in the interval between the active phase and the start of the curing phase.

Linear alkanes were lost mainly after 90 days of composting (sample 90 in **Table 1** and **Figure 4**). However, they still accounted in compost 150 for 40% of their initial content and for almost 40% of total unbound components. The distribution of hydrocarbons revealed a persistence, during the stabilization process, of the heaviest compounds with odd carbon numbers, as shown by the steady increase of both ACL and CPI indices (**Table 1**). The content and composition of sterols and triterpenols did not vary substantially during composting, although a slight loss was noted by passing from compost 60 to compost 150 (**Table 1**).

Weakly and Strongly Bound Components. The extraction yield and composition of compost extracts after both boron trifluoride−methanol (BF_3-CH_3OH) transesterification and alkaline methanolysis ($KOH-CH_3OH$) are reported in **Table 2**. Although similar compounds were released by both treatments, the quantitative distributions were different. The BF_3-CH_3OH treatment produced mainly linear and hydroxy-substituted aliphatic and aromatic acids, whereas larger amounts of alkanedioic acids, linear hydrocarbons, and steroid compounds were extracted by alkaline methanolysis. Similar amounts of aliphatic alcohols were produced by both treatments (**Table 2**).

The two treatments most abundantly extracted ω-hydroxy- and di/trihydroxy-substituted aliphatic acids, which derive from complex bio-polyesters of higher plants, such as cutin and

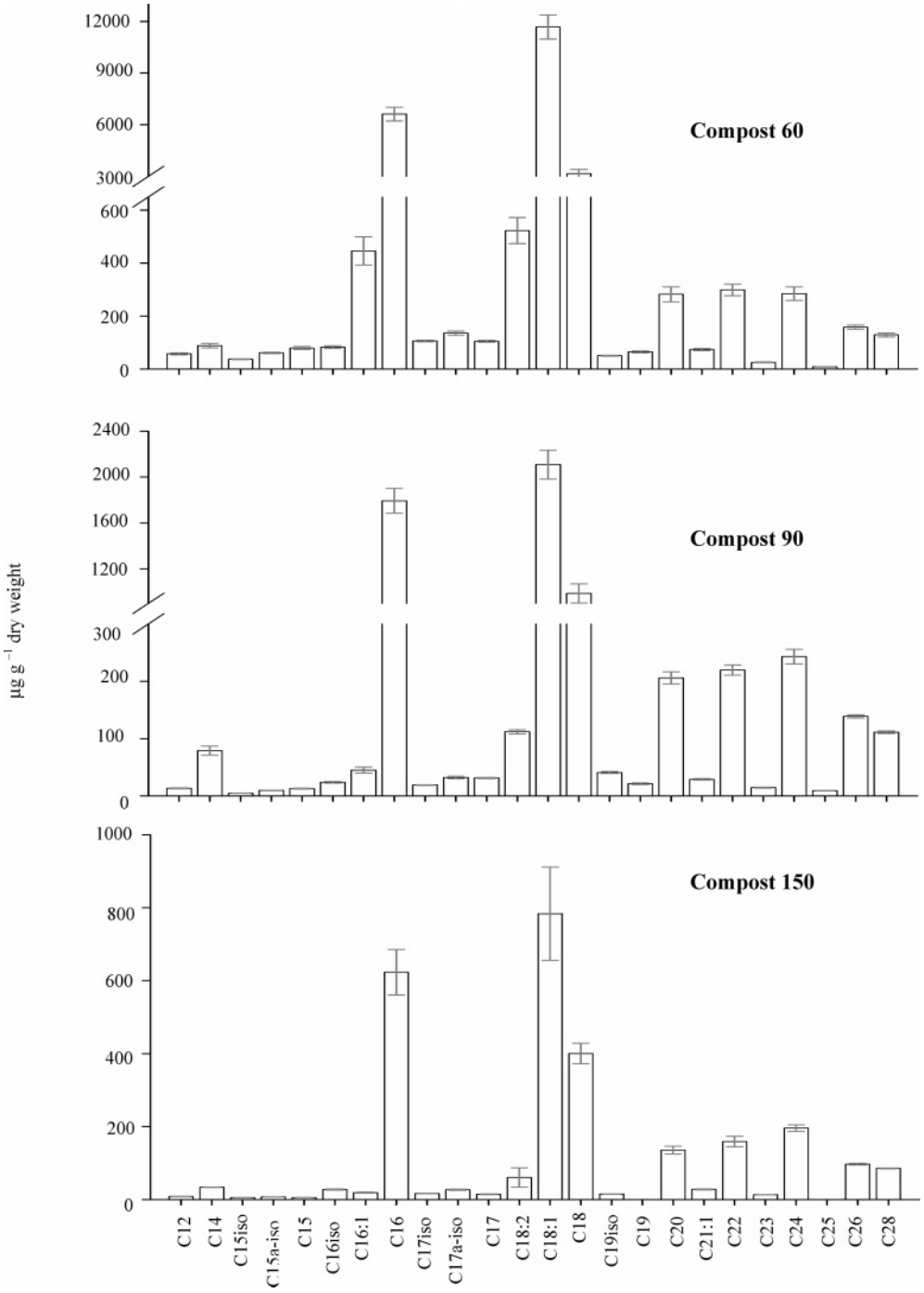

Figure 3. Absolute concentration (micrograms per gram of dry weight) and distribution of fatty acids detected in organic solvent extracts of compost samples.

suberin (*33*, *34*). The major products were the even-carbon-numbered (from C_{12} to C_{26}) ω-hydroxy fatty acids, with a prevalence of C_{16} and $C_{18:1}$ species (**Figure 5**). The short-chain molecules ($C_{12}-C_{16}$) are usually related to cutin, whereas the long-chain compounds are commonly associated with suberin, of which they may represent up to 45% (*9*, *34*). About 33% of the ω-hydroxy acids consisted of the two 18-hydroxyoctadec-9-enoic and 22-hydroxydocosanoic acids, which had been found in suberin depolymerisates of both herbaceous and perennial plants (*19*, *24*).

Di- and trihydroxy acids were represented by C_{16} and C_{18} homologues, which were also reported in depolymerized digests from shoot and root tissues of various plant species (*10*, *35*, *36*). Among the hydroxy acids, the 9,16- and 10,16-dihydroxy-hexadecanoic isomers were the most abundant, followed by the 9,10-epoxy-18-hydroxy- and 9,10,18-trihydroxyoctadecanoic acids (**Figure 6**). The latter compound may also result from the hydrolysis of the corresponding C_{18} epoxide, which remained unaltered after transesterification. In fact, a possible modification of the epoxide by transesterification may yield mainly two isomers: the 9,18-dihydroxy-10-methoxyoctadecanoic acid methyl ester and the 10,18-dihydroxy-9-methoxyoctadecanoic acid methyl ester (*16*). Conversely, the opening of the epoxidee ring due to alkaline hydrolysis leads to formation of the trihydroxy isomer, which was identified here as the trimethylsilyl ether derivative.

Also, α- and β-hydroxy fatty acids ($C_{21}-C_{26}$) were detected in both BF_3-CH_3OH and $KOH-CH_3OH$ extracts of compost

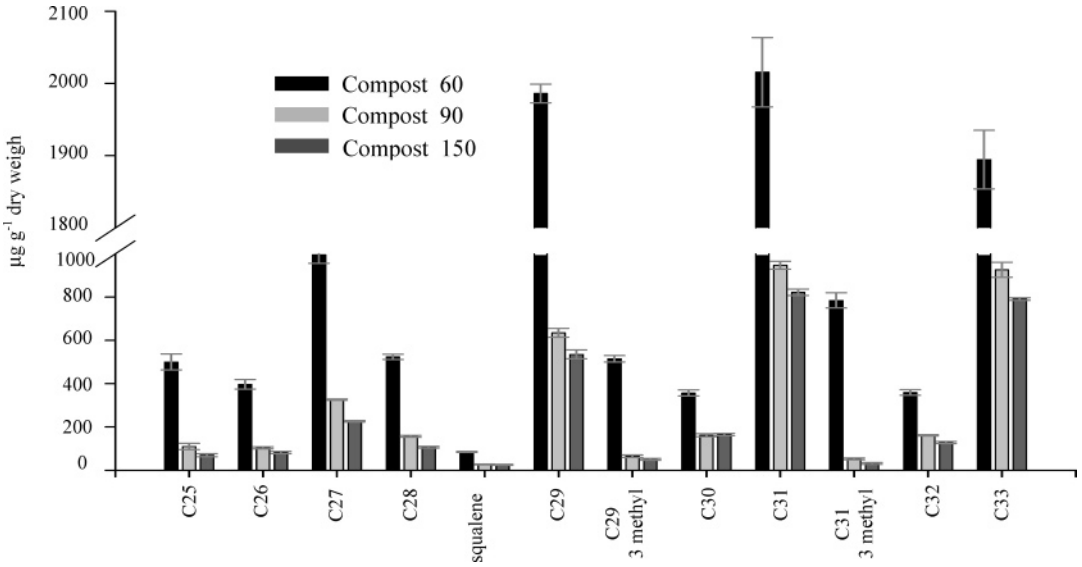

Figure 4. Absolute concentration (micrograms per gram of dry weight) and distribution of alkanes detected in organic solvent extracts of compost samples.

Table 2. Yields (Micrograms per Gram of Dry Weight)[a] and Composition[b] of Main Molecules Released after Transesterification and Basic Methanolic Hydrolysis of Compost at Different Maturities

	compost 60		compost 90		compost 150	
compound	BF_3–MeOH	KOH–MeOH	BF_3–MeOH	KOH–MeOH	BF_3–MeOH	KOH–MeOH
ω-hydroxy acids	6103 (C_{16}, $C_{18:1}$) C_{12}–C_{26}	4068 ($C_{18:1}$) C_{14}–C_{26}	4597 (C_{16}, $C_{18:1}$) C_{12}–C_{26}	2163 ($C_{18:1}$) C_{14}–C_{26}	4216 (C_{16}, $C_{18:1}$) C_{12}–C_{26}	2270 ($C_{18:1}$) C_{14}–C_{26}
di/trihydroxy acids	7296 (C_{16}, C_{18})	2432 C_{16}, C_{18})	4459 (C_{16}, C_{18})	2196 (C_{16}, C_{18})	4296 (C_{16}, C_{18})	1670 (C_{16}, C_{18})
alkanedioic acids	3068 (C_{16}) C_8–C_{24}	5645 ($C_{18:1}$) C_{14}–C_{24}	2037 (C_{16}) C_8–C_{24}	4398($C_{18:1}$) C_{14}–C_{24}	1634 (C_{16}) C_8–C_{24}	3751 (C_{22}) C_{14}–C_{24}
α/β-hydroxy acids	858 C_{10}–C_{26}	482 C_{14}–C_{26}	630 C_{12}–C_{26}		510 C_{12}–C_{26}	
n-alkanoic acids	3770 (C_{18}) C_{12}–C_{28}	1616 (C_{24}) C_{14}–C_{34}	2978 (C_{18}) C_{12}–C_{28}	1675 (C_{24}) C_{14}–C_{34}	1920 (C_{20}) C_{12}–C_{28}	1227 (C_{24}) C_{14}–C_{34}
n-alkanols	610 (C_{16}) C_{12}–C_{24}	509 (C_{22}) C_{12}–C_{24}	578 (C_{16}) C_{12}–C_{24}	465 (C_{22}) C_{12}–C_{24}	520 (C_{16}) C_{12}–C_{24}	430 (C_{22}) C_{12}–C_{24}
aromatic acids	620		510		520	
n-alkanes[c]		511; C_{25}–C_{31} CPI = 3.9 ACL = 29.2		490; C_{25}–C_{31} CPI = 4.0 ACL = 29.7		476; C_{25}–C_{31} CPI = 3.8 ACL = 29.6
sterols and triterpenols[d]		284		276		280

[a] Overall coefficient of variations lower than 15%; $n = 5$. [b] C_i–C_j indicates the range of variation in carbon atoms in the chain. Compounds in parentheses are the most dominant homologues. Number after colon refers to double bond. [c] CPI = carbon preference index = $(\Sigma_{odd}\ C_{25} \div C_{31} + \Sigma_{odd}\ C_{27} \div C_{33})/2\Sigma_{even}\ C_{26} \div C_{32}$. ACL = average chain length = $\Sigma([C_i] \times i)/\Sigma[C_i]$; $[C_i]$ = concentration of *n*-alkane containing *i* carbon atoms. [d] Representative compounds listed in Table S1 in the Supporting Information.

60 (**Table 2**). These molecules are typical microbial biomarkers in natural organic matter that has undergone biological transformation (*15*, *37*, *38*). They steadily decreased in transesterified extracts with compost maturity, whereas they were found in hydrolysis extracts only for compost 60. This suggests that microbial metabolites may be actively incorporated in compost hydrophobic domains that are only weakly bound to the compost matrix, whereas their repartition in the inner domains of strongly associated bio-polyesters is reduced when microbial activity subsides as in composts 90 and 150.

Alkane dioic acids were found in extracts of both transesterification and hydrolysis for all compost samples (**Figure 6**). The long-chain homologues, which are constituents of suberified tissues of higher plants (*8*, *33*), also including monounsaturated C_{18} chains (*16*), were relatively more present in hydrolysis extracts, thereby suggesting their stronger interaction with complex hydrophobic matrices. Conversely, the short-chain homologues, which are not usually found in plant tissues, were detected only in BF_3-CH_3OH compost extracts and appear to be weakly bound products of biotic oxidation of the long-chain monounsaturated acidic compounds (*39*). Both homologues decreased substantially with the progressive compost maturity.

A similar behavior was shown by linear alkanoic acids, which were detected in a wide range of chain lengths ($C_{12}-C_{34}$) in both fractionation steps (**Table 2**). They were prevalently even-carbon-numbered chains with low amounts of unsaturated molecules. A larger content of long-chain ($\geq C_{20}$) *n*-alkanoic acids was found in the extract from alkaline hydrolysis, being about 58% of total yield. These compounds, together with short-range *n*-alkanols ($C_{16}-C_{24}$) and various cinnamic and ferulic

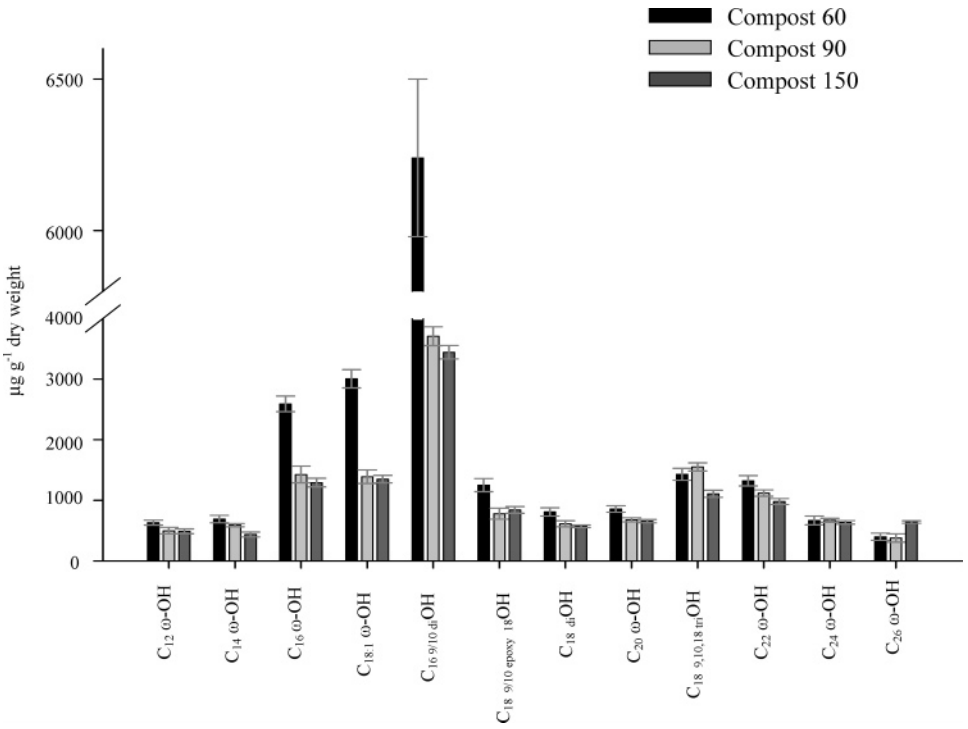

Figure 5. Absolute concentration (micrograms per gram of dry weight) and distribution of ω-hydroxy- and di/trihydroxyalkylcarboxylic acids found in BF_3–MeOH and KOH–MeOH extracts of compost samples.

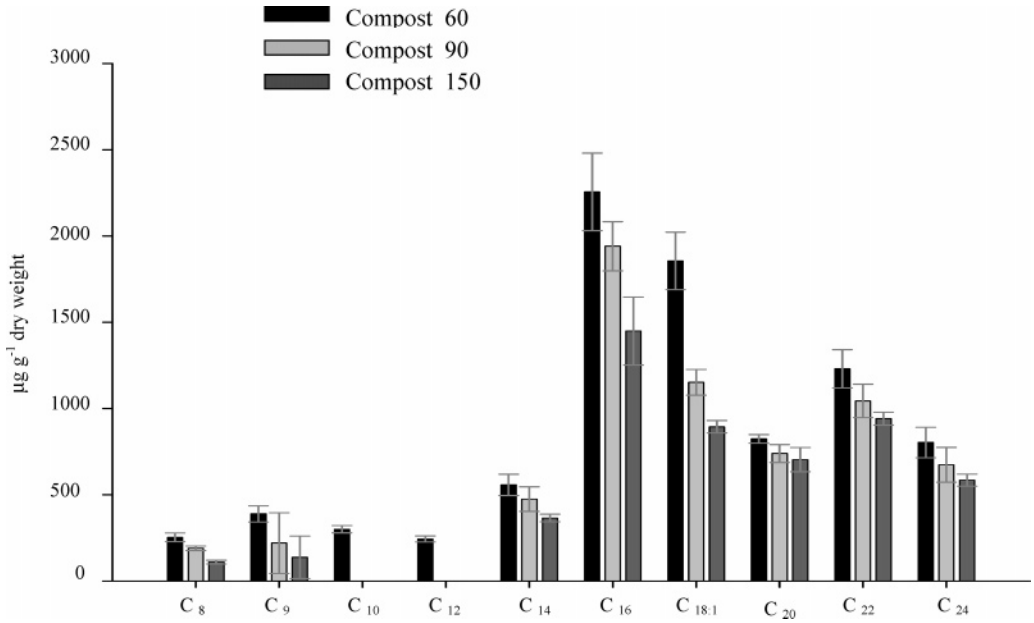

Figure 6. Absolute concentration (micrograms per gram of dry weight) and distribution of alkanedioic acids found in BF_3–MeOH and KOH–MeOH extracts of compost samples.

acid isomers as unique aromatic products, indicate plant suberins as the prevalent source of these minor compost components (*24*, *40*). However, the aromatic structures, which were released only by transesterification, appeared to be less strongly bound to the compost hydrophobic matrix and less influenced by the compost biological transformation.

Low but significant amounts of *n*-alkanes and tetra- and pentacyclic steroids were released by alkaline methanolysis (**Table 2**). The absolute similarity of steroid compounds with those obtained from organic solvent extraction (**Table 1**) and the higher values of CPI and ACL indices found for linear hydrocarbons (**Table 2**) suggested a direct inheritance of these compounds from the corresponding homologues among the unbound components. A possible explanation is the progressive repartition of these molecules into the hydrophobic complex matrices of compost. This hydrophobic protection prevents their direct availability in an organic solvent, whereas their extraction becomes possible only after the alkaline hydrolysis disrupts the complex protective domains. Similarly, linear hydrocarbons had been extracted from soil humic substances only after hydrolysis or by thermochemolysis reactions (*41*, *42*). Moreover, steroid compounds were detected only when mineral horizons in forest soil were extracted in alkaline or subacid media after base hydrolysis (*11*, *21*).

The increasing compost maturity imposed a selection between microbial bioproducts and plant-derived compounds. More than 65% of shorter chain (<C12) dioic acids already disappeared in the hydrolysates from compost 90 (**Figure 6**). Moreover, a slightly lower, but steadily significant, decrease was observed for both α- and β-hydroxy acids (**Table 2**). As noted for the unbound components, this behavior confirms the progressive decline of bio-oxidative processes after the first period of compost stabilization. Conversely, an overall biochemical stability was a property of plant-derived products, because their total yields, after the initial decrease, remained almost unvaried in the more mature composts 90 and 150.

The di- and trihydroxy fatty acids of compost 90 showed a distinctly lower yield only for the most abudant C_{16} compound, with C_{18} homologues being less affected by the biological transformation (**Table 2** and **Figure 5**). Although the presence of a midchain oxygen functionality has been related, in these compounds, to an increased reactivity in decomposition processes (*8*, *35*, *43*), their yield in the final compost 150 of this study still represented 61% of their initial amount.

The main components extracted during the last two fractionation steps also showed persistence with increasing compost maturity. Both ω-hydroxy fatty acids and alkanedioic acids remained in compost 150 at about 68% of their respective initial content (**Table 2**). For both molecular classes, the degradation affected mainly the monounsaturated C_{18} compounds, due the biolability of their internal double bond, whereas lower losses were shown by the long-chain saturated homologues (**Figures 5** and **6**).

The *n*-alkanoic acids extracted from compost 150 showed a general 42% loss with respect to compost 60 (**Table 2**), although the longest chain components relatively increased during the same composting period. Similar results have been obtained in studies of natural organic matter, where the selective preservation of long-chain linear, dioic, and hydroxyalkyl acids and hydrocarbons was recognized among the main factors leading to accumulation of recalcitrant organic matter (*15*, *17*, *42*).

Rather constant yields were found for the other minor components of total hydrolysates (**Table 2**). Alkyl alcohols and aromatic acids showed a total loss ranging from 20 to 15% of their respective initial content. Moreover, neither quantitative nor qualitative changes were found for linear hydrocarbons and steroid compounds, thereby implying incorporation of these biolabile molecules into protective hydrophobic domains formed during composting (*14*).

The chemical fractionation sequence applied here enabled the molecular changes associated with the compost stabilization to be followed. Large losses of compounds were found in the intermediate stage between 60 and 90 days of the composting period. Although the decreasing rate was compound-specific, it affected mostly the more biolabile and bioavailable fraction, such as the unbound compounds. Nevertheless, a large amount of hydrophobic components was still present in the final organic material. In fact, both DRIFT spectroscopy and molecular results from chemical fractionation revealed the selective preservation, in the mature compost sample, of recalcitrant hydrophobic molecules, such as plant waxes and plant bio-polyesters. The sequential chemical fractionation adopted here allowed a precise identification of plant biomarkers and the determination of molecular indices related to the stability of different molecules. The evaluation of these molecular parameters represents a valuable tool to trace both the origin of compost and their fate in the environment after compost application to soils.

Supporting Information Available: Mass spectra of tetracyclic ketosteroid and pentacyclic triterpenyl acid; mass fragments of main tricyclic diterpenes and tetra- and pentacyclic triterpenes released by stepwise fractionation; chemical structures of main tricyclic diterpenes, found in organic solvent extracts of compost samples. This material is available free of charge via the Internet at http://pubs.acs.org.

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