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Molecular Characterization of a Compost and Its Water-Soluble Fractions

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A sequential chemical fractionation was applied to a compost, with its dissolved organic matter (DOM) extracted in water and separated in hydrophilic (HiDOM) and hydrophobic (HoDOM) components and a water extract, following oxidation of compost suspension with an oxygen flux (TEA). The components sequentially isolated by mild extractions and hydrolyses as structurally unbound (SU), weakly bound (WB), and strongly bound (SB) to the matrix of the bulk compost and its water-soluble fractions were identified in their molecular structure. The bulk compost was rich with components derived from both aromatic (phenolic compounds) and aliphatic (long-chain fatty acids, hydroxy acids, diacids, and alcohols) structures of suberins, whereas components derived from cutins were especially extracted from TEA, HoDOM, and HiDOM. The TEA sample also yielded a significant amount of oxidized products that was dominated by dehydroabietic acids. The fractionation sequence highlighted the different intermolecular interactions that bound the isolated molecular components to the compost complex matrix. While a significant part of the bulk compost was still present as a solid residue at the end of the sequential fractionation, all water-soluble fractions were almost completely hydrolyzed. These results indicate that the water-soluble components of compost may be readily separated from the compost matrix and contribute to the environmental dynamics of natural organic matter.

KEYWORDS: Compost; water-soluble components; sequential fractionation; CPMAS−^{13}C NMR spectroscopy; molecular characterization

INTRODUCTION

Compost is widely applied to arable land for improving soil quality and crop productivity. Soil amendments with compost increase the content of soil organic matter (SOM), which is the major natural source of plant nutrients (*1*) and the main factor responsible for soil physical properties, such as soil porosity, structure, and water-holding capacity (*2*–*5*). The fraction of compost that is easily soluble in water [dissolved organic matter (DOM)] is also the most biologically and chemically active and, thus, more rapidly subjected to changes than other less soluble components of compost. This implies that compost DOM is directly involved in the modification of SOM composition (*6*) and in the influence of plant growth (*7*). Although the amount of compost DOM may be small as compared to the bulk SOM, it is recognized to play a significant role in the soil microbial activity and in the transport of nutrients, metals, and hydrophobic pollutants (*8*).

Despite the growing awareness of its importance for the stabilization of organic carbon from agricultural biomasses and urban wastes, little is known on the molecular composition of compost and its DOM. Spaccini and Piccolo (*9*) have developed a chemical fractionation to selectively isolate and characterize compost molecular components according to their degree of binding to the complex matrix. The fractionation sequence implies first an extraction of unbound or free components in an organic solvent. Then, the insoluble biopolyesters of vegetal tissues are depolymerized by a mild acidic methyl transesterification (*10, 11*), thereby solubilizing weakly bound molecules. Finally, the residue is treated with an alkaline methanolic solution to further hydrolyze the components strongly bound to the compost matrix in more complex polyesters (*12, 13*). This procedure was successful in showing the molecular changes occurring during the process of compost maturity (*9*).

In view of the important role that DOM plays in both the process and maturation stages of compost, the objective of this work was to characterize, by a chemical fractionation sequence and NMR spectroscopy, the molecular composition of different water-soluble fractions of a compost. For this reason, a compost DOM was further fractionated in hydrophilic and hydrophobic

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Table 1. Relative Distribution (%) of Signal Areas over Chemical-Shift Regions (ppm) in CPMAS−^{13}C NMR spectra of Compost and Its Water-Soluble Fractions (Standard Deviation in Parenthesis)

sample	200–160	160–110	110–60	60–50	50–0	HI/HB[a]
compost	10.1 (0.69)	14.7 (0.89)	33.3 (3.40)	8.5 (0.84)	33.4 (2.54)	1.08 (0.09)
TEA	23.6 (1.61)	13.3 (0.9)	23.1 (1.61)	9.0 (0.81)	31.0 (2.72)	1.26 (0.10)
HoDOM	14.0 (0.71)	19.3 (1.18)	19.5 (0.65)	12.6 (0.16)	34.6 (1.51)	0.85 (0.07)
HiDOM	14.0 (0.7)	9.1 (0.5)	36.8 (1.8)	9.8 (0.4)	30.3 (3.1)	1.54 (0.10)

[a] Hydrophilic carbons/hydrophobic carbons = [(50–110) + (160–200)/(0–50) + (110–160)].

fractions by separation through resin adsorption, whereas another water-soluble fraction was concomitantly obtained by compost oxidization with oxygen flux.

MATERIALS AND METHODS

Compost and Its Water-Soluble Fractions. The urban waste compost used in this work was produced mechanically on an industrial scale at the Gesenu SpA composting facility in Pietramelina (Perugia, Italy). The feedstock was composed of source-separated municipal solid waste (55%, w/w), yard trimmings from pruning activities (30%), and foliage residues from the tobacco agro-industry (15%). Composting was carried out under aerobic conditions and involved a thermophilic phase of approximately 28 days, during which the feedstock was daily turned, followed by a curing phase of approximately 3 additional months in piles. A DOM fraction was extracted from the bulk compost as described earlier (*8*). Briefly, the compost was placed in contact with deionized degassed water in an extraction ratio of 1:10 (w/v) for 24 h at room temperature and subsequently centrifuged at 2500*g*. The supernatant was collected and filtered through a 0.7 μm glass microfiber filter and a 0.45 μm membrane filter to obtain the total DOM extract. The pH of the DOM extract was around 8.2.

The DOM extract was acidified to pH 2 with 0.1 N HCl and passed through the Amberlite XAD-8 and XAD-4 resins. The organic fraction retained by the XAD-8 resin was eluted with 0.1 N NaOH, passed through an AG MP-50 strongly acidic cation exchange resin, and subsequently freeze-dried. This fraction was defined as the hydrophobic fraction (HoDOM). The organic fraction retained by the XAD-4 resin was eluted with a water/acetonitrile (1:3) mixture. The acetonitrile was removed from the eluate by rotary evaporation at 35 °C, and the remaining aqueous solution was freeze-dried. This fraction was defined as the hydrophilic fraction (HiDOM) (*14*). The HoDOM and HiDOM fractions had an organic C content of 499 and 523 mg g^{-1}, respectively.

An aerated compost tea sample (TEA) was obtained by suspending the compost in deionized water (1:5, w/v) and stirred for 10 days at 20 °C. The aerobic conditions were ensured by equipping the fermentation vessel with air diffusers and an oxygen probe to constantly monitor dissolved oxygen. A software-driven control system helped to maintain dissolved oxygen concentrations above 2.0 mg L^{-1} by means of intermittent bubbling of air through the suspension. After 10 days, the compost suspension was filtered through a cellulose filter paper and stored at 4 °C. The organic C concentration of the TEA sample was 1.19 g L^{-1}. A fraction of the TEA sample was freeze-dried to obtain a solid sample for further chemical characterization.

Cross-Polarization Magic-Angle-Spinning−Carbon-13 Nuclear Magnetic Resonance (CPMAS−^{13}C NMR) Spectroscopy. Experiments by CPMAS−^{13}C NMR were carried out on a Bruker AV300 instrument operating on carbon 13. The rotor spin rate was set at 13 000 Hz. A contact time of 1 ms, a recycle time of 1.5 s, and an acquisition time of 20 μs were used. All experiments were conducted with a CP pulse sequence in the ramp mode to take into account the inhomogeneity of the Hartmann–Hahn condition at high rotor spin rates. CPMAS−^{13}C NMR spectra were performed 3 times for each sample. The different chemical-shift regions of the spectra were automatically integrated, and a ratio of hydrophilic (HI) over hydrophobic (HB) carbons (HI/HB) was obtained for each material (**Table 1**).

Chemical Fractionation. *Structurally Unbound Components (SU).* An aliquot of each compost sample (350 mg of compost, TEA, and HoDOM and 150 mg of HiDOM) was oven-dried at 40 °C for 1 h. Structurally unbound compounds were extracted using a mixture of dichloromethane and methanol (2:1, v/v) for 2 h at room temperature. The sample was centrifuged for 25 min at 12000*g*, and the supernatant was removed and preserved. The residue was further re-extracted with a mixture of dichloromethane and methanol (2:1, v/v) for 12 h at room temperature. After centrifugation, the supernatants were combined and rotary-evaporated to complete dryness. The dry extract was redissolved in 20 mL of dichloromethane/isopropanol (2:1, v/v), and 1 mL of this solution was adsorbed on an aminopropyl-bonded solid-phase cartridge column (Strata NH_2 500 mg/3 mL, Phenomenex) previously conditioned with hexane. The column was first eluted with 8 mL of dichloromethane/isopropanol (2:1) to obtain a neutral subfraction and then with 8 mL of 2% (v/v) acetic acid in diethyl ether to obtain an acid subfraction (*15*). Both neutral and acid subfractions were derivatized and analyzed by gas chromatography/mass spectrometry (GC/MS).

Weakly Bound Components (WB). After the extraction of structurally unbound lipids, the air-dried residue was treated with 15 mL of 12% BF_3−CH_3OH complex at 90 °C for 12 h in a polyethylene bottle, to extract the weakly bound compounds. After centrifugation (15 min at 12000*g*), the supernatant was recovered and the residue was treated twice more with 10 mL of 12% BF_3−CH_3OH for 12 h. The combined supernatants were treated with an excess of water to destroy the BF_3−CH_3OH complex and then liquid–liquid-extracted with chloroform. The total extract in chloroform (WB organic fraction) was dehydrated with anhydrous Na_2SO_4 and rotary-evaporated. The resulting dry extract was dissolved in 5 mL of dichloromethane/isopropanol (2:1, v/v), and 1 mL of this solution was loaded into a NH_2-bonded solid-phase cartridge column, to separate the neutral and acid subfractions, as described above. Both neutral and acid subfractions were derivatized and analyzed by GC/MS. The extract in water (WB aqueous fraction) was dialyzed in dialysis tubes (3500 Da cutoff) against distilled water until conductivity was as low as 2 μS and freeze-dried.

Strongly Bound Components (SB). After the BF_3−CH_3OH transesterification, the air-dried residue was suspended in 1 M KOH in CH_3OH and refluxed for 1 h at 70 °C (*15*). After cooling, the reaction mixture was centrifuged (10 min at 5000*g*) and the supernatant was removed. The residue was extracted twice with 15 mL of CH_3OH and twice with 15 mL of dichloromethane. After each step, the suspensions were centrifuged (10 min, 5000*g*) and all supernatants were combined. These were acidified to pH 2 using concentrated HCl (37%) and, after the addition of water, extracted with dichloromethane in a separation funnel. The dichloromethane solution was dehydrated with anhydrous Na_2SO_4 and filtered on a glass microfiber filter (Whatmann) to remove residual salts. The final extract (SB organic fraction) was derivatized and analyzed by GC/MS. The water extract (SB aqueous fraction) was dialyzed in dialysis tubes (3500 Da cutoff) against distilled water until conductivity was as low as 2 μS and freeze-dried.

Derivatization and GC/MS Analysis. Each organic fraction was first methylated, by refluxing for 30 min at 60 °C with 5 mL of MeOH and 0.5 mL of acetyl chloride. The solvent was evaporated to dryness under a gentle stream of nitrogen, and the resulting residue was silylated with 100 μL of *N*,*O*-bis(trimethylsilyl)-trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS), for 1 h at 70 °C, added with 400 μL of hexane, and analyzed by GC/MS.

The GC/MS analyses were conducted on a PerkinElmer Autosystem XL gas chromatograph, equipped with a PerkinElmer Turbomass Gold mass spectrometer. The injector was held at a constant temperature of 250 °C, and a fused-silica capillary column (Restek Rtx-5MS, 30 m length × 0.25 mm i.d. × 0.25 μm film thickness) was used for analytical separation. Helium was the carrier gas, with a flow rate of 1.6 mL/min. The oven was temperature-programmed from 100 to 300 °C, at a rate of 4 °C/min, and held there for 20 min. The mass spectrometer operated in full-scan mode in the range of *m*/*z* 50–600 with an electron impact ionization energy of 70 eV and a cycle time of 1.0 s. Because of the large variety of detected compounds with different chromatographic responses, the quantitative analysis was conducted using calibration curves of different external standards from Aldrich: tridecanoic acid, octadecanol, 16-hydroxy-hexadecanoic acid, docosandioic acid, β-sitosterol, and cinnamic acid. Tridecanoic acid was also used as an internal standard to evaluate derivatization yields and steadiness of the chromatographic response.

Table 2. Yields (μg g^{-1} Dry Weight) and Classes[a] of Main Molecules Freely Released as Structurally Unbound (SU) from Compost and Its Water-Soluble Fractions

compounds	compost	TEA	HoDOM	HiDOM
α,ω-alcanedioic acids	21 100 ± 900[b]			
n-alkanoic acids	8050 ± 550	43 590 ± 380	10 990 ± 1378	27 510 ± 2010
	C_9-C_{30}	C_9-C_{28}	C_9-C_{28}	C_9-C_{28}
	(C_{22}, C_{24})	(C_{16}, C_{18})	(C_{16}, C_{18})	(C_{16}, C_{18})
unsaturated (%)	35	24	29	25
alcohols	6840 ± 250	5950 ± 280		5850 ± 960
	$C_{12}-C_{26}$	$C_{12}-C_{26}$		$C_{12}-C_{26}$
	(C_{24})	(C_{18})		(C_{18})
alkanes	1400 ± 150	8140 ± 190	90 ± 20	2430 ± 460
carbohydrates	10 ± 2	1660 ± 220	19 040 ± 2690	1510 ± 280
di- and trihydroxyacids	410 ± 50		180 ± 30	380 ± 60
ω-hydroxyacids	1750 ± 150		1170 ± 140	
α,β-hydroxyacids	180 ± 20		10 ± 2	30 ± 2
aromatic acids	5120 ± 450	1370 ± 100	1380 ± 260	
sterols	690 ± 20		650 ± 110	660 ± 120
diterpenoids	1490 ± 180	4670 ± 540	490 ± 60	950 ± 180

[a] C_i-C_j indicates the range of variation in carbon atoms in the chain. Compounds in parentheses are the most dominant homologues. [b] Standard deviation calculated over three replicates.

RESULTS AND DISCUSSION

CPMAS−^{13}C NMR Spectroscopy. The CPMAS−^{13}C NMR spectra of different compost samples are shown in Figure S1 in the Supporting Information, while integrations of spectral intervals are reported in **Table 1**. Signals in the 0–50 ppm region are assigned to alkyl carbon, such as in $(CH_2)_n-$ and terminal CH_3 groups of linear plant lipid compounds. However, the peak at 40 ppm could be assigned to branched alkyl groups. This region constitutes about 30% of each compost sample and is the most important signal for all spectra. The 50–60 ppm region, particularly intense for compost, is assigned to CH_3O- substituents on benzene rings of lignin derivatives, as well as to C−N groups in amino acids or peptides. The resonance in the 60–110 ppm interval is attributed to carbohydrate carbon, including the alcoholic C_2-C_6 carbons (60–100 ppm) and anomeric carbons (100–110 ppm). This region was especially visible for both compost and HiDOM while resulted the least intense for HoDOM. Aromatic and carboxylic carbons generate signals in the 110–165 and 165–200 ppm regions, respectively. The spectral areas were chosen according to literature (*16*). It is interesting to point out that a very intense and narrow peak around 163 ppm was visible in the NMR spectrum of TEA. This resonance should be attributed to phenolic C, deriving from the oxidative conditions maintained during the procedure of TEA extraction.

The carbon distribution over the spectral region was used to calculate the HI/HB ratio, an index of hydrophilicity of complex organic materials. This ratio indicated, as expected, that HiDOM was the most hydrophilic fraction, followed by TEA and bulk compost, whereas HoDOM was the most hydrophobic fraction.

Molecular Fractionation. *Structurally Unbound Components (SU).* The extraction yield, dimensional range, and dominant homologue of components that were readily released from the bulk compost and its water-soluble fractions are shown in **Table 2**.

The predominant molecular classes found in compost were alkandioic acids, *n*-fatty acids, *n*-alcohols, *n*-alkanes, and aromatic compounds. Minor amounts of steroids and terpenoids were also detected. Alkandioic acids (**Figure 1**) were the most abundant structurally unbound (SU) compounds, with a clear predominance of long-chain homologues (>C_{18}). These compounds have been identified as major components of the aliphatic domain of most suberins that are dominated by α,ω-dioic and ω-hydroxy acids (*17–20*). Conversely, plant cutins are mainly composed of midchain functionalized hydroxy-alkanoic acids (*17, 18, 21, 22*). Therefore, the dominance of α,ω-dioic acids over dihydroxy-hexadecanoic acids (**Table 2**) suggests that the organic inputs employed in the compost production were richer in suberins than in cutins.

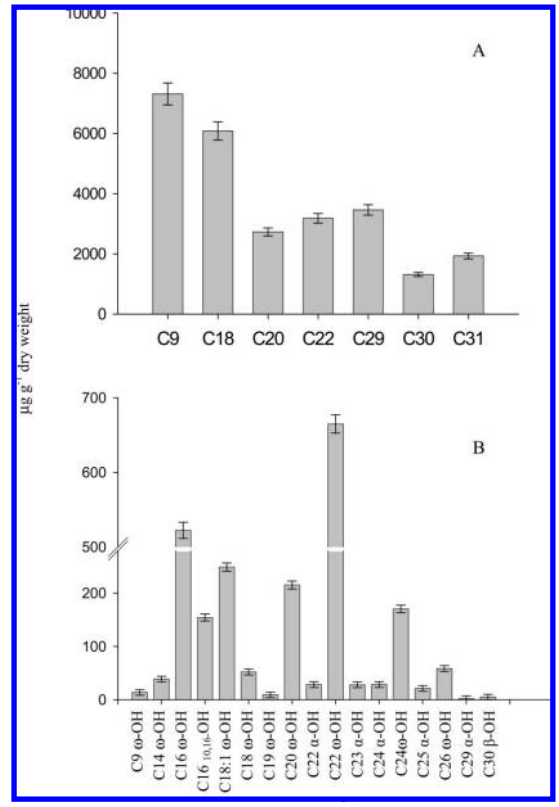

Figure 1. Absolute concentration (μg g^{-1} dry weight) and distribution of (**A**) alkandioic acids and (**B**) hydroxyacids, detected in the organic fraction of structurally unbound (SU) components extracted from the compost.

The ω-hydroxy acids were also found in larger amounts than midchain hydroxy-alkanoic acids. They ranged from C_{16} to C_{26}, showed a maximum intensity for the C_{16} and C_{22} species, and revealed a strong predominance of an even number of carbons in the alkyl chain (**Figure 1**). The ω-hydroxyoctadecenoic acid (ω-$C_{18:1}$) was also found in noticeable amounts together with its degradation product α,ω-alkane (C_9) dioic acid (*15, 23*).

Table 3. Yields (μg g^{-1} Dry Weight) and Classes[a] of Main Molecules Released after Transesterification (WB) and Alkaline Methanolic Hydrolysis (SB) of Compost and Its Water-Soluble Fractions

	compost		TEA		HoDOM		HiDOM
compounds	WB	SB	WB	SB	WB	SB	WB
α,ω-alcanedioic acids	20 110 ± 420[b]		310 ± 30	70 ± 30			530 ± 80
n-alkanoic acids	4510 ± 240 C_9-C_{30} (C_{22}, C_{24})	2200 ± 290 C_9-C_{28} (C_{16}, C_{18})	10 140 ± 540 C_9-C_{28} (C_{16}, C_{18})	470 ± 220 C_9-C_{28} (C_{16}, C_{18})	1350 ± 140 C_9-C_{28} (C_{16}, C_{18})	510 ± 160 C_9-C_{28} (C_{16}, C_{18})	730 ± 140 C_9-C_{28} (C_{16}, C_{18})
unsaturated (%)	35	27	0.02	32	29	51	0.01
alcohols	1020 ± 120 $C_{12}-C_{26}$ (C_{18})	1100 ± 240 $C_{12}-C_{26}$ (C_{18})	390 ± 40 $C_{12}-C_{26}$ (C_{18})	60 ± 10 $C_{12}-C_{26}$ (C_{18})	203 ± 40 $C_{12}-C_{26}$ (C_{18})	80 ± 20 $C_{12}-C_{26}$ (C_{18})	130 ± 20 $C_{12}-C_{26}$ (C_{18})
alkanes	2040 ± 310	100 ± 5	260 ± 50	40 ± 8	210 ± 30	20 ± 5	590 ± 110
carbohydrates	1200 ± 110		470 ± 70	20 ± 5	1350 ± 190	230 ± 50	120 ± 25
di- and trihydroxyacids	2580 ± 330		130 ± 20		20 ± 2		
ω- hydroxyacids	1050 ± 130				50 ± 5		
α,β- hydroxyacids	340 ± 40				540 ± 60		
aromatic acids	81 490 ± 1230	10 700 ± 1370	140 ± 30	20 ± 5	580 ± 70	30 ± 5	60 ± 10
sterols		1200 ± 190			50 ± 10	50 ± 10	160 ± 30
diterpenoids		2600 ± 470	310 ± 35	40 ± 10			

[a] C_i-C_j indicates the range of variation in carbon atoms in the chain. Compounds in parentheses are the most dominant homologues. [b] Standard deviation calculated over three replicates.

Among the di- and trihydroxyacids, only the 9,16- and 10,16-dihydroxyhexadecanoic isomers were identified in small amounts.

The monoacids identified in the organic extract of compost were dominated by a bimodal distribution (Table S1 in the Supporting Information). Short-chain ($<C_{20}$) components, derived from cutins (*21*), were characterized by C_{16} and C_{18} acids, while long-chain ($>C_{20}$) fatty acids, derived from suberins (*17*), were mainly composed by $C_{22}-C_{24}$ members. Long-chain fatty acids were more prevalent than short-chain ones, again suggesting inputs from higher plants and mainly from suberins (*24, 25*). However, other sources can not be excluded. In fact, the highly abundant unsaturated and saturated hexadecanoic (C_{16}) and octadecanoic (C_{18}) homologues are usually found in different substrates (*26*). Straight-chain compounds of fungal origin (*27*) may also be a source of *n*-alkanoic acids, while a contribution from oxidation of *n*-alkane or *n*-alkanols may not be excluded (*24*). Moreover, a direct input from microbial activity was revealed by the detection of branched-chain fatty acids. The most important compounds were the *iso*- and *anteiso*-branched C_{15} and C_{17} alkanoic acids that are typically derived from bacterial production (*24, 28*).

Other compounds found in relatively minor concentration in the SU extract of compost included $C_{10}-C_{28}$ *n*-alkanols, with a strong predominance of even over odd number of carbons and most intense for the C_{22} and C_{24} homologues, typical of higher plants (*26*), and $C_{23}-C_{33}$ *n*-alkanes (Table S1 in the Supporting Information), with a maximum at C_{27} and a relevant predominance of odd over even carbon numbers. The presence of longer chain components in both classes and the homologues distribution again suggested a contribution from higher plants.

In the TEA fraction, it was instead observed a clear disappearance of components derived from suberins. Evidently, these long-chain components are not easy soluble, especially if they do not interact strictly with the compost matrix. In fact, both α,ω-alkandioic and ω-hydroxy acids were not detected (**Table 2**), while *n*-alkanoic acids were detected in the small $C_{10}-C_{24}$ range (Table S1 in the Supporting Information), with a dominance of even over odd carbon numbers, but without the prevalence of long-chain homologues ($>C_{20}$) derived from suberins. Also HoDOM and HiDOM did not release α,ω-alkandioic acids, whereas *n*-alkanoic acids were released in a small range ($C_{10}-C_{24}$), with a prevalence of short-chain homologues. However, some ω-hydroxy acids were found in the organic extracts of these samples, although in minor quantity with respect to the compost. A large amount of alkanes were found in the SU extracts of TEA and HiDOM fractions (Table S1 in the Supporting Information). Contrary to bulk compost, the overall predominance of short-chain compounds with respect to long-chain homologues indicated a preferential microbial origin of these molecules. No alcohols and alkanes were identified in the SU fraction of HoDOM.

Small amounts of hydroxy fatty acids with the hydroxyl group in α or β position were found in bulk compost (**Table 2**), while their concentrations were even lower in HoDOM and HiDOM. These molecules are typical microbial biomarkers in biologically transformed natural organic matter (*29–31*). However, they were found in compost and HoDOM more after transesterification (WB in **Table 3**) than in unbound extracts, suggesting their repartition in inner hydrophobic domains that are only weakly bound to the compost matrix (*9*).

The HoDOM, HiDOM, and TEA samples released large amount of monosaccharides, such as D-ribose, D-glucose, D-mannose, D-xylose, D-galactose, L-arabinose, which were identified as persylylated monosaccharides and glycosides and as trisylylated lactones of deoxyaldonic acids. Although carbohydrates are water-soluble, their release in the organic extract is evidence of their incapsulation among hydrophobic compounds with whom they are co-extracted. Conversely, compost did not release significant quantities of unbound monosaccharides, while a considerable amount was liberated from encapsulation only after transesterification.

Aromatic compounds were found in the SU fraction of compost and, in minor amount, in the same fraction of TEA and HoDOM, whereas no aromatic compounds were identified for HiDOM. However, their release from compost was large after transesterification, whereas they were abundantly released as structurally unbound components from the water-soluble samples.

Small quantities of sterols and terpenes were also detected in most samples too. Steroids (C_{28} and C_{29}) are widely distributed in plants and usually in waxes (*32, 33*), while terpenes are usually found in resins from trees (*34*). Small amounts of β-sitosterol and dehydroabietic acid were found in bulk compost, while a larger quantity of the latter was found in the TEA sample. Dehydroabietic acid is the most oxidized and most soluble among resin acids, possessing three double bonds,

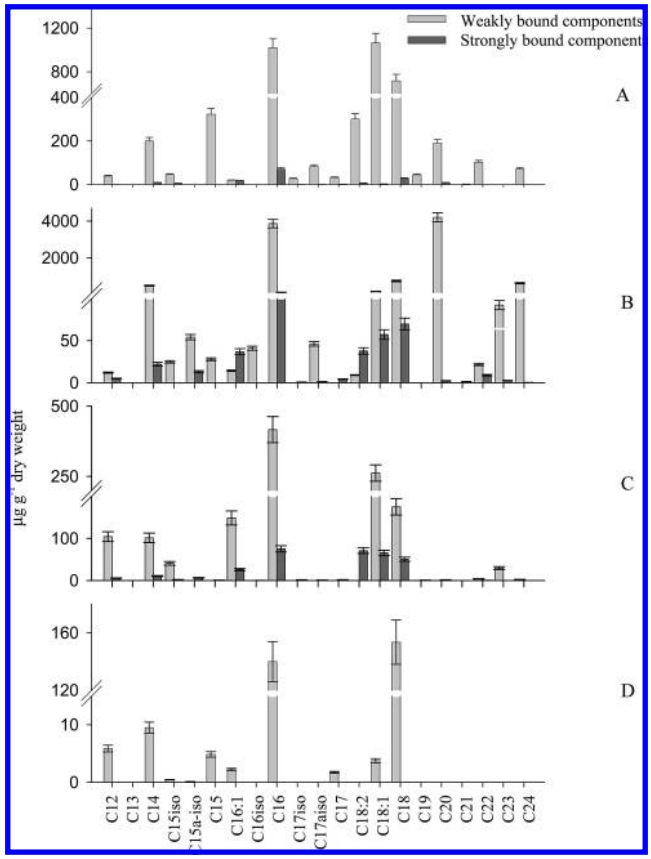

Figure 2. Absolute concentration (μg g^{-1} dry weight) and distribution of alkanoic acids detected in organic fractions of weakly bound (WB) and strongly bound (SB) components extracted from (**A**) compost, (**B**) TEA, (**C**) HoDOM, and (**D**) HiDOM.

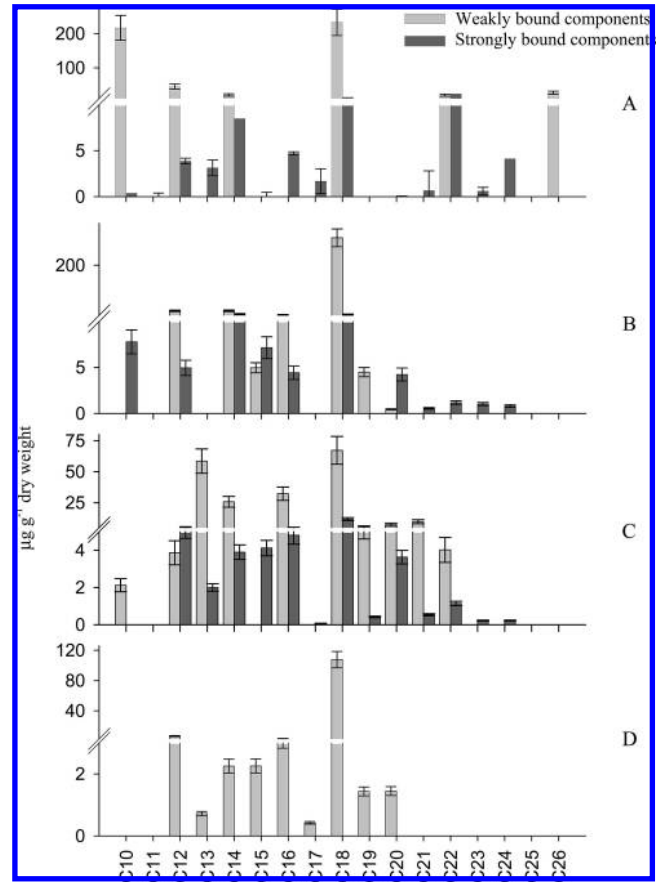

Figure 3. Absolute concentration (μg g^{-1} dry weight) and distribution of alkanols detected in organic fractions of weakly bound (WB) and strongly bound (SB) components extracted from (**A**) compost, (**B**) TEA, (**C**) HoDOM, and (**D**) HiDOM.

while other resin acids have only two (*35*). Again, its high content in the TEA extract may be due to the aerobic conditions used for TEA extraction.

A wider number of steroids and terpenoids was found in the organic extract of HiDOM and HoDOM, although their concentrations were lower than for TEA. Compounds identified on the basis of the National Institute of Standards and Technology (NIST) library were 15-isobutyl-(13α)-isocopalane, 5β-podocarpa-8,11,13-trien-16-oic acid, isopimaric acid, 5β-,9β,10α-labd-8(20)-ene-15,19-dioic acid, stigmasterol, sitosterol, and dehydroabietic acid.

Weakly and Strongly Bound Components Extracted in Organic Fractions (WB-O and SB-O). The weakly and strongly bound compounds released from each sample after transesterification and after methanolysis, their extraction yield, dimensional range, and dominant homologues are shown in **Table 3**.

The most abundant compounds released from compost after both transesterification and methanolysis were aromatic compounds. A lower amount of aromatic compounds were, instead, released from TEA, HoDOM, and HiDOM. The aromatic compounds found in the bulk compost are known to be part of the aromatic domain of suberins (*17*), such as 3-phenylprop-2-enoic acid methyl esters, bearing one or two metoxyl groups (*p*-coumaric and ferulic acid), and mono-, di-, and trimethoxybenzoic acids, which are also reported as lignin derivatives (*36*). The same compounds were found after transesterification of the other samples derived from the bulk compost, but their concentration was much lower and their number was less wide. Generally, alkaline methanolysis released a lesser amount of aromatic compounds for each sample, indicating that lignin and suberin derivatives were linked to the matrix mainly by weak bonds cleaved by transesterification.

Dioic acids were found in a large amount in the bulk compost after transesterification, although a slight decrease of long-chain members was observed with respect to the SU fraction (Table S2 in the Supporting Information). Similarly, ω-hydroxyacids revealed a decrease in long-chain members after transesterification (Table S2 in the Supporting Information). Moreover, the ester-linked ω-$C_{18:1}$ acid homologue was not found, while the C_{16} ω-hydroxyacid was the most important member. Conversely, a larger quantity and a wider number of di- and trihydroxy-functionalized acids were found after transesterification of the bulk compost. Among the di- and trihydroxyacids, the 9,10,16-trihydroxy- and 9- or 10,16-dihydroxyhexadecanoic isomers were the most abundant, followed by the 9,10,18-trihydroxy- and 9,10-epoxy-18-hydroxy-octadecanoic acids (Table S2 in the Supporting Information). The ω-$C_{18:1}$ acid as well as other long-chain ω-hydroxy acids and, to a lesser extent, C_{18}−C_{28} α,ω-dioic acids are more easily released from biopolyesters than other ester-linked aliphatic building blocks, such as the C_{16} ω-hydroxy acid (*12, 15, 23*). They may reside in more accessible portions of the biopolyester and/or be released by natural-occurring hydrolysis, thereby becoming present in the compost matrix as unbound compounds rather than ester-linked. Conversely, it appears that di- and trihydroxy fatty acids, as well as C_{16} ω-hydroxy acid (*37*), are less susceptible to a natural hydrolysis as being part of the core of biopolyester structures (*15*) and become detectable only after transesterification.

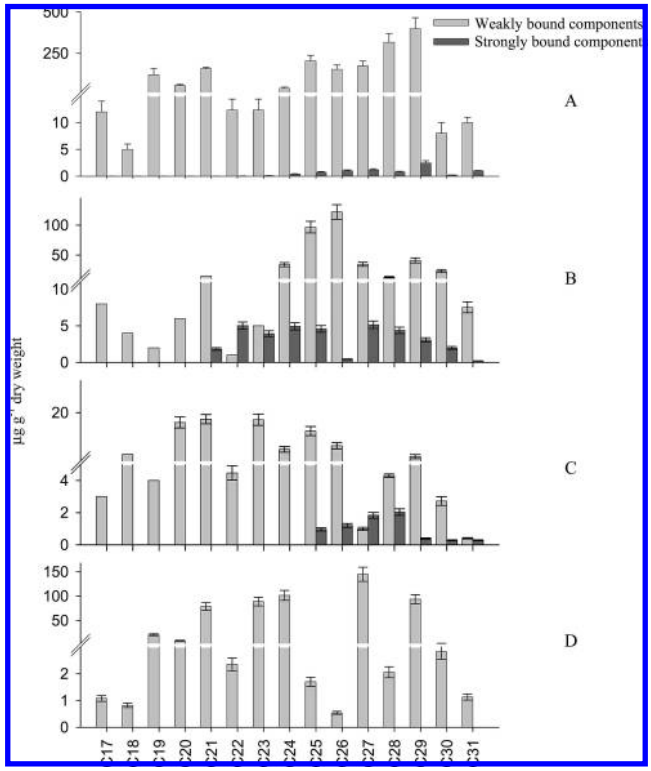

Figure 4. Absolute concentration (μg g^{-1} dry weight) and distribution of alkanes detected in organic fractions of weakly bound (WB) and strongly bound (SB) components extracted from (**A**) compost, (**B**) TEA, (**C**) HoDOM, and (**D**) HiDOM.

Long-chain dioic and hydroxy acids were not released from TEA, HiDOM, and HoDOM. In fact, α,ω-alkanedioic acids were detected in WB organic extracts (WB-O) of TEA and HiDOM (Table S2 in the Supporting Information) but only as short-chain compounds (C < 12), which may derive from the oxidation of unsaturated alkanoic acids and/or dihydroxy fatty acids (*38*). Among the hydroxyacids, only the 9,10,16-trihydroxyhexadecanoic and 9,10-epoxy-18-hydroxyoctadecanoic acids were found in very small amounts in WB-O of HiDOM (Table S2 in the Supporting Information). These compounds were released only in trace amounts after alkaline hydrolysis from all samples.

The distribution of alkanoic acids, alcohols, and alkanes found after both transesterification and alkaline methanolysis from all samples studied here are shown in the **Figures 2**−**4**, respectively. The organic extracts showed a generally larger degree of insaturation for alkanoic acids after alkaline methanolysis and a decrease in the content of long-chain alkanoic acids released from bulk compost after both transesterification and methanolysis. This finding suggests that long-chain components are not extensively ester-linked to the compost matrix. Conversely, short-chain components appear either more ester-linked, or because of their smaller steric hindrance, they may more easily enter the internal voids of the supramolecular compost network, thereby being released from such encapsulation only after transesterification reduces the network complexity. The alkanes distribution (**Figure 4**) revealed an overall bimodal distribution with no evident odd over even predominance, thereby suggesting an additional contribution from fungal and bacterial bioproducts, besides that from plant hydrocarbons.

Low but significant amounts of steroids and terpenoids, similar to those identified in the SU fraction, were released by alkaline methanolysis from bulk compost, while they were not found among the weakly bound components released by transesterification. Spaccini and Piccolo (*9*) had already shown the absolute similarity of steroids and terpenoids in both SU and transesterified extracts. They suggested a direct inheritance of these compounds from the corresponding homologues found among unbound components, because of a progressive repartition of these molecules into the hydrophobic complex matrix of compost. Such hydrophobic protection prevents their direct solubility in an organic solvent, whereas their extraction becomes possible only after the alkaline hydrolysis disrupts the complex protective domains. However, this protection mechanism did not occur to the same extent for the more hydrophilic water-soluble fractions obtained from compost, because some steroids and terpenoids were released from these fractions already after the transterification step, which completely disrupted their hydrophobic domains even without the action of the alkaline hydrolysis (**Table 3**).

Significant differences were found among samples based on the yields of compounds in organic extracts (**Table 4**). The compound distribution showed that not only the composition but also the existing intermolecular interactions may influence the release of molecules from compost and its water-soluble fractions. The SU extract revealed larger amounts of compounds for TEA than for bulk compost, whereas yields resulted similarly lower for both HoDOM and HiDOM. The components in WB-O after transesterification

Table 4. Yields (%) of Total Compounds in (1) Organic Extracts from Structurally Unbound (SU-O), Weakly Bound (WB-O), and Strongly Bound (SB-O) Fractions as Found in GC/MS Chromatograms, (2) Aqueous Fractions (w/w) Released as Weakly Bound (WB-A) and Strongly Bound (SB-A) Fractions, (3) Solid Residues (w/w) Resulting from Extraction of SU, WB, and SB Compounds, and (4) Amount (w/w) of Missing Material[a]

sample	compost	TEA	HoDOM	HiDOM
		Organic Extracts		
SU-O	4.70 ± 2.7[b]	6.59 ± 1.7	3.40 ± 4.7	4.02 ± 4.1
WB-O	11.67 ± 3.1	1.22 ± 0.8	0.43 ± 0.5	0.23 ± 0.6
SB-O	0.18 ± 2.6	0.07 ± 0.3	0.09 ± 0.4	0.0
		Aqueous Fractions		
WB-A	3.4 ± 0.2	0.6 ± 0.1	26.3 ± 0.4	0.5. ± 0.1
SB-A	0.0	0.3 ± 0.0	0.0	0.0
		Solid Residues		
after SU	69 ± 6	68 ± 9	71 ± 8	72 ± 5
after WB	76 ± 9	37 ± 5	3 ± 0.4	3 ± 0.6
after SB	47 ± 5	23 ± 3	2 ± 0.3	0.0
missing material	33	67	68	95

[a] 100 − (SU-O + WB-O + SB-O + WB-A + SB-A + after SB). [b] Standard deviation calculated over three replicates.

were larger for bulk compost and somewhat less for TEA, while they resulted significantly lower for HoDOM and HiDOM. Alkaline hydrolysis further produced a large content of SB-O compounds for bulk compost but much lower for TEA and HoDOM and none for HiDOM.

The amount of matter found in the WB aqueous fraction (WB-A) was large in HoDOM in comparison to the rest of samples, for which only a little amount was retrieved, despite what was expected on the basis of NMR spectra. Because of this mass loss for all samples, the missing material (**Table 4**) must have been lost during the purification in dialysis tubes. The molecular cutoff (3500 Da) of these tubes may have been large enough to allow for the loss of small hydrophilic molecules. Most of the material was lost from aqueous fractions of the largely hydrophilic TEA and HiDOM, while the content of hydrophobic compounds in HoDOM may have formed larger molecular associations with the hydrophilic molecules, thereby favoring a larger retention of matter in the dialysis tubes. It is also to be noted that HiDOM, HoDOM, and TEA were almost completely hydrolyzed after the chemical fractionation (**Table 4**), whereas about 47% of the bulk compost was still unaltered. This result confirms a significant difference in the strength of association of molecular components in the studied samples.

The chemical fractionation applied in this work showed some important differences between bulk compost and its water-soluble fractions, which regarded both the molecular composition and the intermolecular interactions. The bulk compost was richer with components derived from both aromatic (phenolic compounds) and aliphatic (long-chain fatty acids, hydroxy acids, diacids, and alcohols) structures of suberins, whereas components derived from cutins were especially extracted from TEA, HoDOM, and HiDOM. Moreover, the use of the combined Amberlite XAD-8 and XAD-4 resins effectively separated the compost DOM in hydrophobic and hydrophilic fractions. Conversely, the oxygen-enriched procedure of extraction of the TEA sample yielded a significant amount of oxidized products that was dominated by dehydroabietic acids. Significant differences were found among samples with regard to the strength of intermolecular interactions. The water-soluble fractions were almost completely hydrolyzed at the end of the chemical fractionation, whereas a significant part of the bulk compost was still found as a solid residue.

Most of these results suggest that compost is composed by various heterogeneous molecules of relatively small mass that are differently associated in the matrix but readily available, especially in the water-soluble samples, to be released by relatively simple hydrolysis reactions. This understanding substantiates the role that the water-soluble molecules of compost appear to have in soil organic matter dynamics.

Supporting Information Available: CPMAS $-^{13}$C NMR spectra of compost, TEA, HoDOM, and HiDOM (Figure S1); yields (μg g^{-1} dry weight) and distributions of fatty acids, alkanols, and alkanes freely released as structurally unbound (SU) from compost and its water-soluble fractions (Table S1); and yields (μg g^{-1} dry weight) and distributions of dioic and hydroxyl acids released after transesterification (WB) of compost and its water-soluble fractions (Table S2). This material is available free of charge via the Internet at http://pubs.acs.org.

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