Review

Studies of Organic Matter in Composting, Vermicomposting, and Anaerobic Digestion by ^{13}C Solid-State NMR Spectroscopy

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Abstract: Composting, vermicomposting, and anaerobic digestion are three commonly applied processes for the transformation of organic waste into valuable products for soil amendment. The application of compost, vermicompost, and digestate to soil requires specific properties, such as maturity and stability, strongly related to the composition of organic matter. ^{13}C solid-state Nuclear Magnetic Resonance (SSNMR) has often been applied to follow the transformation of organic matter during waste treatment processes, as well as to assess the quality of the produced amendments and the effectiveness of the treatments. Thanks to the possibility of associating the ^{13}C chemical shift to different functional groups of biomacromolecules present in the waste feedstocks and in the final products, thorough characterizations of organic matter have been performed exploiting ^{13}C cross-polarization magic angle spinning experiments, and semiquantitative descriptions of the evolution of the different groups during composting, vermicomposting and anaerobic digestion have been reported. Here, these studies are reviewed with the aim of highlighting the potential of the application of ^{13}C SSNMR to these complex materials, as well as the critical issues and perspectives.

Keywords: compost; vermicompost; digestate; waste; extracts; maturity; stability; humic substances; humic acids; ^{13}C CP-MAS

1. Introduction

Recycling has become a key issue in waste management. In fact, the globally produced waste presently amounts to 2 billion tons/year and, given the continuous population growth and increase in industrial development, it is expected to reach 3.40 billion tons/year by 2050 [1,2]. Considering that the organic fraction represents approximately 45% of the total waste and that, although often considered a waste, it is rich in nutrients and biochemicals, its recycling is certainly of great relevance from a circular economy perspective.

In the past, organic waste was mainly disposed of in landfills with the now well-known environmental impacts [3]; alternatively, it was reused as food for animals or directly applied on agricultural lands as fertilizer. More recently, it has been recognized that organic waste can be used as feedstock for platform chemicals [4–7]. Among the techniques suggested for the conversion of organic waste into valuable products, biological treatments for the production of energy (e.g., biogas from anaerobic digestion) and organic soil improvers or fertilizers (compost from aerobic composting and digestate from anaerobic digestion) are those most commonly used [5,8]. In the case of application to soil, important features of the product are its stability and maturity, the former indicating low biological activity and high resistance to decomposition, the latter referring to the stabilization of the physicochemical characteristics of the material, the disappearance of phytotoxicity, and the ability to favor seed germination and plant growth [9,10].

In recent years, anaerobic digestion (AD) has seen a strong development worldwide [11]. Only in Europe, by 2021 industrial scale biomethane plants numbered 1023, with about 20,000 additional small to large scale anaerobic digesters [12]. Anaerobic digestion is

a complex multi-step process [13] in which the organic matter is broken down by different types of anaerobic microorganisms. The first step is the hydrolysis of lipids, proteins and carbohydrates by extracellular enzymes, yielding oligomers and monomers, which are converted to fatty acids and alcohols in the second step. Oxidation of the fatty acids produces acetic acid, H_2 and CO_2, which are transformed to CH_4 by methanogens in the final step. Thus, AD products are biogases (namely, CH_4, CO_2, and, in some cases, H_2) that are used for renewable energy generation, and a nutrient-rich solid residue, called digestate, which can find application in agriculture, either used directly as a soil amendment or fertilizer or further processed through composting [14–16]. It should be pointed out that generally digestate requires a short composting period to reach full stability and maturity.

Given its ease of implementation and low operational costs, a more commonly used organic waste treatment is composting [17]. Composting is the decomposition process of organic matter performed in aerobic conditions by microorganisms [18], which allows the transformation of organic wastes of different kinds, both vegetal and animal, into a stable organic substance rich in "humus". Humus is constituted by the high molecular weight association of amphiphilic compounds, rich in carboxylic groups, slow to decompose under natural conditions, and is responsible for many physicochemical properties that ensure soil fertility. The composting process is naturally driven by microorganisms, which use organic matter and oxygen for their metabolism, producing heat, CO_2, H_2O, minerals and stable organic matter [19]. This process starts with a bio-oxidative phase characterized by a rising temperature, which reaches 45–70 °C. During this phase, which lasts for days, thermophilic microorganisms ensure very fast organic matter decomposition, using the most easily available substances. The high temperature ensures sanitation, i.e., the inactivation of most pathogens and invasive plant seeds. Afterwards, the temperature progressively decreases to mesophilic conditions, and the fresh compost enters a curing phase, which can last for weeks, in which decomposition continues, involving the more recalcitrant compounds (such as lignin) and bringing to the formation of humic compounds (fulvic and humic acids, and humin) with high maturity and stability. Compost has great value as a soil amendment and fertilizer, with applications in agriculture, horticulture, and gardening, and hence represents a perfect example of a circular economy; in fact, the last decades have seen an increase in compost production. Today, in Europe 42 million tons of waste per annum, that is 59% of the separately collected biowaste, both municipal and commercial/industrial, is composted [20].

An alternative to aerobic composting is vermicomposting. In this case, the transformation and stabilization of organic matter occurs in mesophilic conditions through the combined action of earthworms and microbes. With their movements, the earthworms aerate, mix and grind the substrate, favoring the action of the microbes present in both waste and worms' guts, which are responsible for the biochemical degradation and stabilization of the organic material [21,22]. Thanks to this synergy, vermicomposting gives a compost with better plant growth promoting properties with respect to aerobic compost, containing a considerable amount of organic acids, such as plant growth hormones and humic acids [23], and higher levels of the most soluble nutrients, such as nitrogen, phosphorus, potassium, calcium and magnesium [24].

Whatever the technology used, knowledge of the transformations of organic matter that take place during the stabilization process, as well as of the composition and quality of the feedstock and of the final product, is fundamental for the agricultural application of the stabilized waste and for the optimization of process parameters, also considering that national and international regulations have established stringent requirements for the application of this product as fertilizer or soil amendment in terms of organic carbon content, humic substance content, etc. For these reasons, since the 1990s, many studies have been published in the scientific literature testing and comparing different chemical and instrumental methods for assessing compost stability and/or maturity, as, for example, measurement of pH, electrical conductivity, CO_2 evolution rate, seed germination rate, dissolved organic carbon, volatile organic carbon, C/N ratio, and humic acid content (see

for instance [25–31]). To this end, besides conventional analytical methods, advanced instrumental techniques, such as thermal analyses, Fourier transform infrared spectroscopy (FTIR), UV–Vis and fluorescence spectroscopies, nuclear magnetic resonance spectroscopy (NMR), gas chromatography–mass spectrometry, and scanning electron microscopy, can be used to assess the stabilization of organic matter and the quality of the final product. In fact, these techniques provide detailed information on compositional, functional, and behavioral properties of organic materials, even complex ones such as (vermi) compost and digestate. Nevertheless, it is generally agreed that a combination of analyses is advised for a thorough characterization of these materials.

Among the techniques that can give access to compositional information of organic matter (OM), NMR, and solid-state NMR (SSNMR) in particular, stands out as a powerful method to access comprehensive qualitative and quantitative structural information not available from other techniques. Compared to liquid-state NMR, which is useful for studying soluble components, especially those with low molecular weight (see Refs. [32,33] for a comprehensive review), SSNMR has many advantages when applied to OM: (i) it allows bulk samples to be analyzed without pretreatments or the use of solvents, which may introduce artifacts; (ii) it can be used to investigate insoluble OM samples or fractions; (iii) it allows a much higher sample concentration, enhancing signals and saving instrument time; (iv) it is conservative, i.e., it does not consume sample; (vi) it allows the investigation of anisotropic interactions, averaged out in solution NMR by the fast isotropic tumbling of molecules; (v) it allows domains and heterogeneities to be identified within OM structures. The vast majority of SSNMR studies of OM reported in the literature involve the observation of ^{13}C nuclei, with only a few exploiting ^{15}N and ^{31}P. Indeed, ^{13}C SSNMR yields useful information on OM composition in terms of organic functional groups [28,34].

In the following, the application of ^{13}C SSNMR to the investigation of digestate, compost and vermicompost will be reviewed; most of the scientific literature focused on compost, with publications starting from the late 1980s, while in the last two decades articles were also published for vermicompost and anaerobic digestate. Some of the studies on compost and vermicompost were performed on extracts (i.e., humic substances, dissolved organic matter, compost tea), which have been shown to have important effects in agricultural practice, acting as plant biostimulants or suppressing microbial growth [35–37]. Although ^{13}C SSNMR has been successfully applied for the characterization of organic matter in (vermi)composts and digestates, to the best of our knowledge no reviews have been published on the subject, except for a recent one by Férnandez-Domínguez and co-authors on the use of ^{13}C SSNMR and other spectroscopic techniques to assess the stability of digestate [38].

The review is organized as follows: Section 2 is dedicated to discussing the NMR experiments used, data treatment and preparation of samples for the investigation of these types of materials; in the following sections the literature regarding the application of ^{13}C SSNMR to compost (Section 3), vermicompost (Section 4), and anaerobic digestate (Section 5) is summarized and discussed.

2. ^{13}C Solid-State NMR Spectroscopy for the Investigation of Organic Matter in Compost, Vermicompost, and Digestate

2.1. Experimental Techniques

As already mentioned, most SSNMR studies on OM samples are performed by exploiting the ^{13}C nucleus. ^{13}C has a natural abundance of 1.11% [39,40], which results in a good balance of sensitivity and spectral "simplicity" since complex interactions among ^{13}C nuclei can be neglected. The most important nuclear property used for the identification of functional groups is chemical shielding. On the basis of this property, nuclei in different chemical environments give rise to signals at different resonance frequencies. Experimental techniques providing high-resolution ^{13}C SSNMR spectra, similar to those in solution, are at present routinely available on SSNMR instruments, the most important being Magic Angle Spinning (MAS) and high-power decoupling from abundant ^{1}H nuclei. In the most basic

experiment, usually called direct polarization MAS or direct excitation MAS (DE-MAS), these techniques are combined with a radiofrequency excitation pulse on ^{13}C. The ^{13}C DE-MAS experiment gives the most accurate quantification of the different species in an OM solid sample, provided that it is performed with a sufficiently long delay between consecutive scans (five times the spin-lattice relaxation time, T_1, of the ^{13}C nuclei). Since T_1 values for ^{13}C nuclei in the solid state can be considerably long (even hundreds of seconds), thus requiring a recycle delay of many seconds or even minutes, extremely (and sometimes prohibitively) long measurement times are required for acquiring ^{13}C DE-MAS spectra of OM samples that commonly need thousands of scans to be accumulated for obtaining an acceptable signal-to-noise ratio.

Due to these long measurement times, ^{1}H-^{13}C cross polarization MAS (CP-MAS) experiments are most commonly used in the investigation of OM samples. In CP-MAS experiments, the ^{13}C signal is created by magnetization transfer from the abundant ^{1}H nuclei, with an enhancement by up to a factor of four. Moreover, in CP experiments the recycle delay is determined by the longitudinal relaxation of the ^{1}H nuclei, which is usually much faster than that of ^{13}C. This allows recycle delays of a few seconds to be used, with a substantial reduction in the experimental time. It is worth noting that, while CP-MAS is an excellent technique for investigating the qualitative composition of a sample, the relative peak intensities may not be fully preserved; thus, CP is generally considered a "semiquantitative" technique. In fact, the quantitative reliability of CP depends on several factors related to both the experimental conditions and the nature of the sample. Low CP efficiency of non-protonated and mobile carbons or moieties having short ^{1}H $T_{1\rho}$ (proton spin-lattice relaxation time in the rotating-frame) can indeed make ^{13}C CP-MAS spectra non-quantitative. In fact, in CP experiments the trend of ^{13}C magnetization with contact time (also called CP dynamics) depends on the cross-polarization time, during which there is transfer of magnetization from ^{1}H to ^{13}C spins, and on the ^{1}H $T_{1\rho}$, which determines the loss of ^{1}H magnetization. Examples of CP dynamics profiles in OM samples are reported in [41]. A careful adjustment of the acquisition conditions, and in particular of the contact time between ^{13}C and ^{1}H magnetizations, to the specific requirements of the investigated sample must be performed in order to minimize these problems. For example, Pichler et al. [42], after measuring ^{13}C and ^{1}H T_1's and studying CP dynamics for a compost of municipal solid waste, reported that in the ^{13}C CP-MAS spectrum with a contact time of 1 ms all spectral regions show a similar intensity loss (<10%) with respect to the quantitative spectrum, confirming the acceptability of these CP conditions for relative quantitation. This also generally applies in the comparison of a series of spectra of samples with similar composition. ^{13}C CP-MAS NMR experiments with a contact time of 1 ms and a recycle delay of a few seconds are indeed employed in most of the works on OM in (vermi)composts and digestates (see also Section 2.3). CP efficiency can also be affected by high contents of paramagnetic species (metal impurities and organic radicals) or moisture in the samples; in these cases sample pretreatments are necessary. A thorough discussion of the critical issues connected with ^{13}C CP-MAS experiments in environmental science can be found in refs. [32,41,43,44].

Besides CP-MAS, other ^{13}C SSNMR experiments can aid spectral interpretation. For example, spectral editing techniques that selectively retain certain signals and eliminate others have been applied in order to better distinguish the contribution of different functional groups to the spectra. In this context, in a few works [42,45,46] the standard CP-MAS spectra were complemented by selective dipolar dephased (DD) CP-MAS ones. In a DD CP-MAS experiment, a decoupling delay, usually between 50 and 100 µs, is inserted between CP and acquisition. This implies that the magnetization of ^{13}C nuclei strongly coupled to neighboring ^{1}H nuclei substantially decays, and therefore, in the final spectrum, the corresponding signals are suppressed or strongly depressed. In practice, a DD CP-MAS spectrum almost selectively shows signals of quaternary carbons and of carbons in very mobile environments. An overview of the advanced SSNMR techniques sometimes applied to OM is given in Ref. [43].

2.2. Spectral Analysis

^{13}C CP-MAS NMR experiments on (vermi)compost or digestate samples are usually performed to investigate and compare OM composition in the feedstock (biomasses or wastes) and throughout (or only at the end of) the decomposition process. The spectra are typically analyzed by distinguishing carbons from different functional groups on the basis of the chemical shift, which is directly related to the chemical shielding. Given the complex and heterogeneous chemical nature of OM, spectra span a chemical shift range of over 200 ppm, with a strong overlap of signals (Figure 1). Therefore, spectral regions, rather than individual signals, summarized in Table 1, are identified and assigned to specific organic species: (i) the 0–45 ppm spectral region is ascribed to alkyl carbons, with short chain aliphatic carbons from volatile fatty acids and steroid-like molecules resonating between 0 and 28 ppm [47] and long chain aliphatic carbons from plant aliphatic biopolymers (suberin, cutin, waxes, fatty acids) [48] and proteins [49] between 28 and 45 ppm; (ii) O-alkyl and N-alkyl carbon signals, ascribable to lignin, carbohydrates (i.e., cellulose and hemicellulose), and proteins [50], are observed between 45 and 110 ppm; (iii) the 110–160 spectral region is assigned to aromatic carbons present, for instance, in lignin and polyphenols [51]; (iv) signals of carboxyl carbons in esters, acids, and amides (160–190 ppm) and carbonyl carbons in aldehydes and ketones (190–220 ppm) are observed between 160 and 220 ppm. It must be noted that slightly different spectral region ranges are indicated in the literature by different authors.
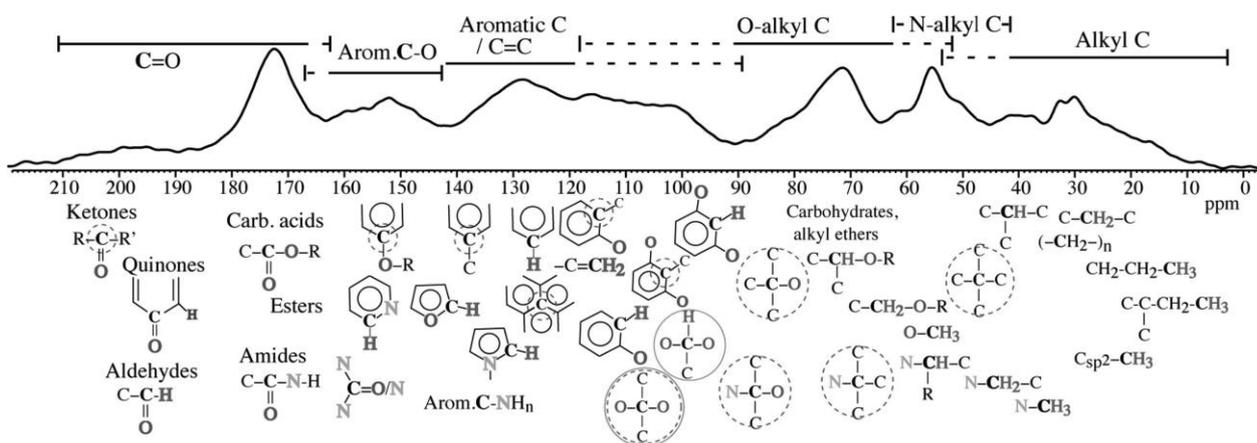
Figure 1. Typical ^{13}C MAS NMR spectrum of OM with division in spectral regions assigned to functional groups (see Table 1) and assignment to specific molecular structures. Reproduced with permission from Ref. [43].

Table 1. Spectral regions used in the analysis of ^{13}C MAS NMR spectra of OM and assignment to carbons in different functional groups.

Chemical Shift Range	Assignment	Conventional Region Name
0–28 ppm 28–45 ppm	CH$_3$ and CH$_2$ in short chain and simple aliphatics CH$_2$ and CH in long aliphatic chains	Alkyl C
45–60 ppm 60–95 ppm [a] 95–110 ppm [b]	O-CH$_3$; CH-N; aliphatic quaternary C C2-C6 in cellulose and hemicellulose; alcohols; ethers C1 of cellulose and hemicellulose; anomeric carbon of polysaccharides	O-Alkyl C
110–145 ppm [c] 145–160 ppm [d]	unsubstituted or alkyl-substituted aromatic C O,N-substituted aromatics	Aromatic C
160–190 ppm 190–220 ppm	Carboxylic acids; esters; amides ketones; aldehydes	Carboxyl/carbonyl C

[a] also referred to as O-alkyl C region; [b] also referred to as di-O-alkyl C region; [c] also referred to as aryl C region; [d] also referred to as O-aryl C region.

Some signals are also assigned to functional groups belonging to specific biomacro-molecules present in biomass samples [52,53]. The peak at about 56 ppm has been assigned to methoxyl groups in lignin. This signal may overlap a contribution from CH-N groups in proteins. Lignin also shows peaks between 60 and 100 ppm, with resonances at 60, 72, and 84 ppm from ether and secondary alcohol groups, as well as minor contributions from fully saturated carbons not linked to oxygen between 15 and 45 ppm. Peaks at about 154 and 148 ppm arise from C-3 and C-5 carbon atoms in syringyl and guaiacyl units of lignin. The spectral region between 60 and 110 ppm is dominated by peaks that are characteristic of polysaccharides and, to a minor extent, proteins. In particular, the peak at 63–65 ppm has been ascribed to C6 and C5 in crystalline hexose and pentose, respectively. Signals around 72–75 ppm may be ascribed to C2, C3, and C5 from cellulose, as well as carbons from xylans. Peaks at 83 and 88 ppm may be due to C4 of non-crystalline and crystalline components of cellulose, and the peak at 105 ppm to dioxygenated anomeric C1. This last peak can overlap the peak of C1 carbons from xylans at 103 ppm.

Although ^{13}C CP-MAS NMR is semiquantitative, the integral values of different spectral regions (expressed as % of total area) have been extensively used as quantitative proxies for the relative C distribution among major OM functional groups in the feedstocks and in the intermediate/final products, thus monitoring the transformation process of the different components. It must be pointed out that, although most authors do not give errors on the integral intensities and some even report integral values with one or two decimal digits, errors on the units digit are to be expected, deriving from sampling and spectral processing and analysis. Moreover, normally, relative integral intensities are compared without taking into account the decrease in the absolute amount of carbon in OM due to decomposition.

Different indices are used to express OM composition, the most common ones being the Alkyl C/O-Alkyl C ratio (*A/OA*), the aromaticity index (*ARM*), and the hydrophobicity index (*HB/HI*), generally defined as:

$$\frac{A}{OA} = \frac{Alkyl\ C}{O - Alkyl\ C} = \frac{Area\ (0 - 45\ \text{ppm})}{Area\ (45 - 110\ \text{ppm})}$$

$$ARM = Aromaticity\ index = \frac{Area\ (110 - 160\ \text{ppm})}{Area\ (0 - 160\ \text{ppm})}$$

$$\frac{HB}{HI} = Hydrophobicity\ index = \frac{Area\ (0 - 45\ \text{ppm}) + Area\ (110 - 160\ \text{ppm})}{Area\ (45 - 110\ \text{ppm}) + Area\ (160 - 220\ \text{ppm})}$$

2.3. Experimental Conditions and Sample Preparation

^{13}C SSNMR experiments on OM samples in waste management are generally carried out on spectrometers working at ^{13}C Larmor frequencies of 75–125 MHz, although a frequency of 150 MHz is used in more recent studies and frequencies as low as 25 MHz were employed in the oldest ones. MAS rotors with diameters of 4 mm, containing a few tens of milligrams of sample, and MAS frequencies of 8–13 kHz were usually employed. Few cases are also reported where rotors with 7 mm diameter and spinning rates of 3.5–5 kHz were used. As said, ^{13}C CP-MAS experiments are usually performed with a contact time of 1 ms, although cases are reported where longer contact times (2–3 ms) were used. In these experiments, the recycle delay ranges from 0.5 to 5 s and a few thousand scans are acquired.

Before ^{13}C SSNMR experiments, OM samples are simply dried (by freeze-drying, oven-drying at 40–110 °C, or air-drying), ground, and possibly sieved to obtain a fine powder; the latter operation is not strictly necessary, but it is useful since it ensures stable sample spinning and, consequently, a good spectral resolution. Considering that only a few tens of milligrams of powder are necessary for the analysis, accurate sampling and homogenization are critical to obtain a representative sample.

Several studies on compost and vermicompost, focused on extracts, instead of investigating the bulk material. Different types of extracts were investigated, that is, humic substances (HSs), dissolved organic matter (DOM), or compost tea (CT). Humic substances are stable compounds with a complex and variable composition, mainly containing aromatic rings linked by methylenic chains and/or oxygen atoms, with carboxyl and hydroxyl groups bonded to the rings and the alkyl chains. On the basis of their solubility, two different extractable HS fractions can be obtained, i.e., fulvic acids (FAs, soluble at any pH value) and humic acids (HAs, soluble at pH > 2). The extraction of HSs is performed by mixing the dry compost in an alkaline solution; HAs can then be separated by acidifying to pH 1.0. HAs have higher molecular weight and degree of aromatization with respect to FAs, which, on the other hand, are richer in carboxyl and hydroxyl groups [54]. Thanks to their highly aromatic structure, HAs are stable compounds and their amount is typically considered as a measure of compost maturity [55]. In fact, during composting HAs tend to increase whereas FAs tend to decrease [56]. Compost DOM is a mixture of low-molecular weight compounds, such as sugars and free amino acids, and high-molecular weight compounds, among which are also HSs [57]. DOM is a very small fraction of the total organic matter present in compost, decreasing as feedstock stabilization proceeds, but it is an important component since many biochemical transformations that occur during composting take place in solution. DOM is extracted from compost by shaking the material with ultrapure water; DOM can be further divided into a hydrophobic fraction (HoDOM) and a hydrophilic fraction (HiDOM) using Amberlite® XAD-8 or Supelite™ DAX-8 resin [57]. Finally, compost tea is an aqueous extract obtained from the fermentation of compost in water either in forced aerated or non-aerated conditions [58].

3. ^{13}C Solid-State NMR Applications to Composting

The literature reports many examples of the application of ^{13}C MAS NMR to the investigation of compost, covering a very broad range of diverse feedstocks, including, often mixed, animal (cattle, swine, chicken, buffalo) manure [59–71], vegetal residues [10,60,61,71–77], grape marc [69,78], ground coffee and fresh grass [10,76], cotton gin waste [64], rice husk, bran or straw and wheat straw [63,65,75], domestic organic wastes [45,74,76], and municipal solid wastes (MSW) [42,79,80]. The composting process has been followed by means of ^{13}C CP-MAS NMR for different times, ranging from a few days, for specifically investigating the initial part of the process [10], up to more than one year [65]. An example of the evolution of the ^{13}C CP-MAS spectrum of different feedstocks with composting time is shown in Figure 2. In most cases the reported experimental data are the integral areas of the characteristic spectral regions described in Section 2.2. These data are catalogued in Table 2, indicating for each study the feedstock and the composting time.

In two cases the intensities of the different spectral regions were exploited to determine the composition of OM in terms of biomolecules. In particular, Eldridge et al. [61] applied the "mixing model" proposed by Nelson and Baldock [81] to several composts for determining the molecular composition. The mixing model is aimed at reproducing the experimental ^{13}C MAS NMR spectrum and N/C ratio of a sample by combining spectral components and N/C ratios of known materials. In particular, four molecular classes were considered: carbohydrates (cellulose, hemicellulose, mucopolysaccharides, smaller molecular weight saccharides), proteins (accounting for proteins, peptides, amino acids), lignin, and an aliphatic component (including cutin, suberin, and membrane components). The experimental spectra were well reproduced and allowed the authors to propose a precise composition of the organic fraction of several composts. A similar estimate of the molecular composition, in terms of polysaccharides, proteins, lignin, and aliphatics, was given by Veeken et al. [63] for a compost of pig feces and wheat straw. Interestingly, the evolution of the composition was followed during 28 days of composting.
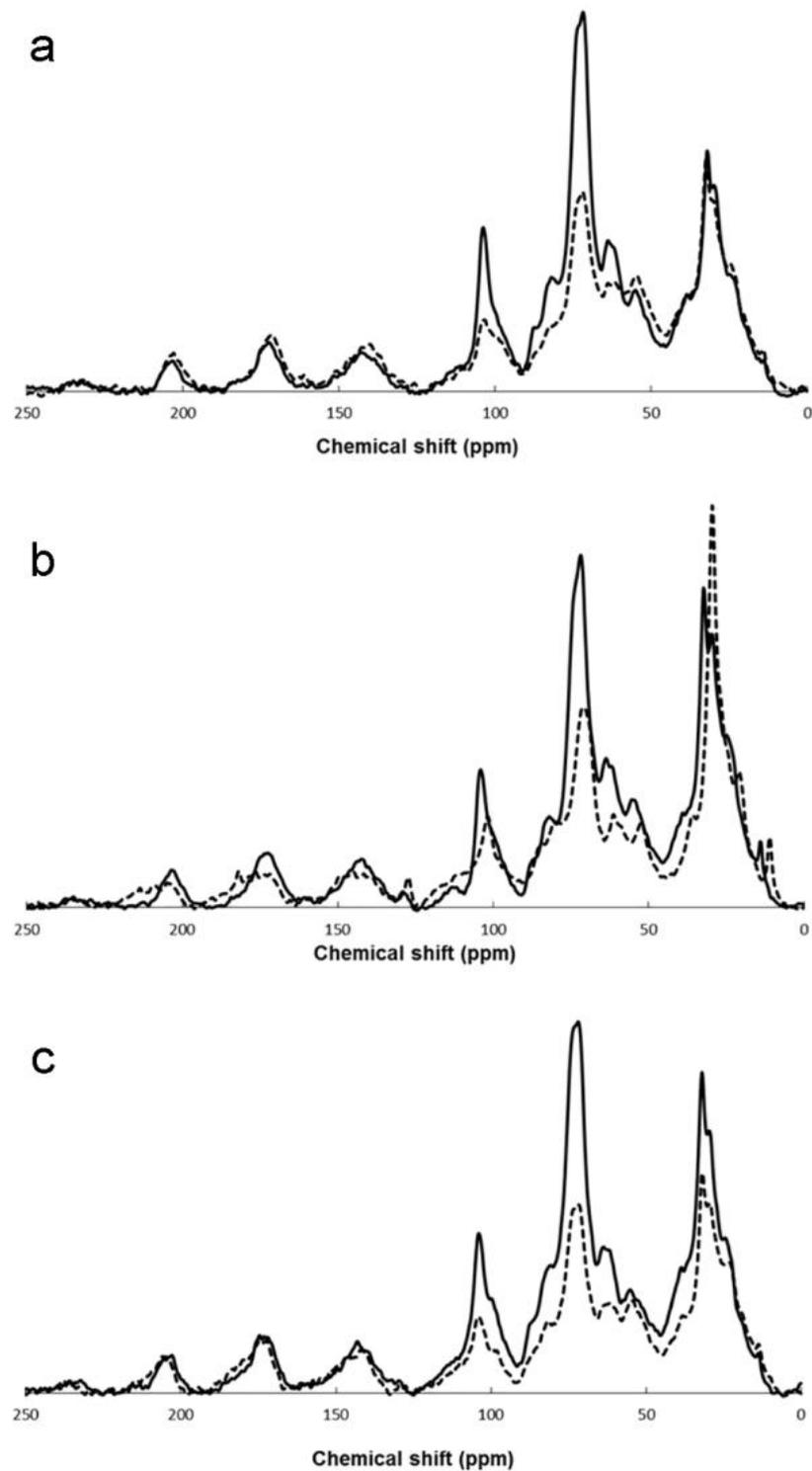
Figure 2. ^{13}C CP-MAS NMR spectra of composts (dashed line) and corresponding feedstocks (solid line). Feedstock compositions are: (**a**) exhausted grape marc and cattle manure 76:24 (*w:w*); (**b**) grape marc and cattle manure 72:28 (*w:w*); (**c**) exhausted grape marc and poultry manure 67:33 (*w:w*). Reproduced with permission from Ref. [69].

Table 2. Relative areas (% of total area) of the different regions of ^{13}C CP-MAS NMR spectra for compost samples and corresponding feedstocks reported in the literature.

Ref. [a]	Feedstock [b]	Sample [c]	Alkyl C	O-Alkyl C		Aromatic C		Carboxyl/ Carbonyl C
				CH$_3$O/CHN	O-/Di-O-alkyl C	Aryl C	O-Aryl C	
[60]	Olive mill waste/orchard pruning residues	C 200 d	17.39	11.63	45.86	12.31	5.75	7.06
	Olive mill waste/animal manure/wool residues	C 200 d	26.82	15.32	38.59	6.93	4.0	8.34
[71]	ChM/saw dust (3:1 *w:w*)	C 56 d	28.38		32.26	16.51		
	CM/saw dust (3:1 *w:w*)	C 56 d	22.13		32.20	10.49		
	SM/saw dust (3:1 *w:w*)	C 56 d	31.62		41.36	17.71		
	Soybean meal/saw dust (3:1 *w:w*)	C 56 d	37.94		43.16	18.40		
	Lemon peel/saw dust (3:1 *w:w*)	C 56 d	36.23		41.10	17.43		
[77]	Wood chips/vegetable R/aromatic plant R	C1	33.9	13.0	31.3	12.6	2.1	7.2
		C2	38.2	12.3	26.8	11.5	2.9	8.2
		C3	23.4	11.7	44.0	12.1	3.7	5.1
		C4	21.0	11.8	41.1	14.3	3.8	8.1
		C5	19.0	11.4	42.4	15.5	3.8	7.9
		C6	24.6	10.9	38.7	15.1	3.3	7.4
		C7	35.7	11.8	31.0	11.8	2.5	7.2
		C8	37.8	12.0	30.1	11.4	2.2	6.5
		C9	43.6	10.5	28.0	9.5	2.2	6.3
		C10	34.7	11.5	30.7	10.4	3.1	9.5
		C11	30.2	11.3	33.6	12.0	5.3	7.7
[62]	SM/ rice straw (4:1 *w:w*)	FS	24.6		57.9		7.7	9.8
	SM/ rice straw (8:1 *w:w*)	C 40 d	23.2		55.2		9.8	11.8
	SM/rice straw/biochar (8:1:1 *w:w*)	FS	28.8		50.5		7.9	12.8
		C 40 d	25.6		48.9		10.6	15.0
		FS	24.7		40.9		24.7	9.8
		C 40 d	19.2		28.8		42.5	9.5
[10]	COF/fresh grass/mature compost (18:80:2 *w:w*)	C 6 d	33.5	15.2	23.3	14.8	3.2	10
[64]	Solid pig slurry/cotton gin waste (4:3 *v:v*)	FS	13.5	5.9	74.9	3.7	0.9	1.1
	Solid pig slurry/cotton gin waste (3:4 *v:v*)	BT	17.5	8.2	62.7	5.3	1.8	4.5
	Solid pig slurry/cotton gin waste (3:7 *v:v*)	AT	26.0	12.3	51.2	4.2	2.5	3.9
		EB	30.1	10.5	48.1	4.4	1.9	5.0
		AM	35.5	13.1	43.8	3.6	0.7	3.4
		FS	19.4	9.3	71.6	0.3	0.0	0.0
		BT	13.6	7.1	71.6	5.5	1.0	1.2
		AT	18.7	4.7	61.9	7.4	3.2	4.1
		EB	26.4	11.4	53.8	5.7	4.5	6.8
		AM	34.1	12.5	44.3	3.8	1.3	4.1
		FS	22.9	7.4	63.6	2.3	1.1	2.7
		BT	24.9	9.3	59.1	0.9	2.0	3.9
		AT	21.0	9.3	65.5	1.1	0.9	2.2
		EB	18.4	8.1	69.7	2.1	1.4	0.3
		AM	21.8	9.0	58.8	2.6	2.6	5.2

Table 2. *Cont.*

Ref. [a]	Feedstock [b]	Sample [c]	Alkyl C	O-Alkyl C		Aromatic C		Carboxyl/ Carbonyl C
				CH$_3$O/CHN	O-/Di-O-alkyl C	Aryl C	O-Aryl C	
[70]	BM/CM/maize straw/PT (70:30 *w:w*) +bioplastic (1 wt%) +bioplastic (2 wt%)	FS	15.4	10.5	58.4	8.6	3.0	4.1
		C 108 d	18.5	11.4	48.0	12.0	3.4	6.7
		C 108 d	17.6	11.1	47.9	12.3	4.0	7.1
		C 108 d	18.9	11.0	49.2	11.6	3.6	5.7
[68]	SM/pumice CM/pumice ChM/pumice	FS	34.6		48.5	7.3		9.7
		C 10 d	21.5		60.4	10.5		7.7
		C 20 d	23.6		57.7	10.9		7.7
		C60 d	26.2		48.3	12.9		12.7
		FS	14.6		64.8	12.4		8.3
		C 10 d	9.0		70.4	12.9		7.7
		C 20 d	10.		69.2	12.7		7.7
		C60 d	11.7		66.6	13.3		8.5
		FS	29.5		52.3	8.2		10.1
		C 10 d	21.5		66.0	6.4		6.1
		C 20 d	21.7		61.6	9.3		7.4
		C60 d	26.8		52.0	10.5		10.7
[69]	Exhausted grape marc/CM (76:24 *w:w*) Grape marc/CM (72:28 *w:w*) Exhausted grape marc/PM (67:33 *w:w*)	FS	30.7	17.0	39.5	3.3	3.5	6.1
		C 28 d	36.4	15.9	31.2	5.7	3.7	7.3
		C105 d	37.9	16.1	28.9	6.3	3.9	6.9
		C 168 d	37.3	14.3	22.4	7.1	4.8	14.2
		FS	33.6	15.3	37.1	2.9	3.7	7.4
		C 28 d	34.3	13.7	37.3	3.6	3.6	7.5
		C105 d	33.3	14.8	38.6	3.8	3.0	6.6
		C 168 d	34.9	15.2	34.4	3.3	4.0	8.2
		FS	30.8	14.2	41.0	3.5	3.5	7.0
		C 28 d	34.4	14.6	35.4	3.2	4.0	8.5
		C105 d	33.9	15.5	35.7	3.7	3.9	7.5
		C 168 d	34.3	15.5	31.5	3.4	5.0	10.2
[61]	CM Broiler litter Green waste Nitro-humus MSW	CC	18.2	7.6	48.8	8.9	5.0	11.6
		CC	17.9	9.1	48	8.0	5.2	11.7
		CC	22.4	10.7	40.4	8.2	6.3	12.0
		CC	19.6	8.8	43	9.6	6.7	12.4
		CC	23.0	7.6	48.7	7.3	3.6	9.8
[75]	Rice husk/rice bran/BEM/molasses	FS	2.15	25.8	67.4	2.98		0.99
		C 13 d	1.98	25.6	68.9	3.07		0.51
		C 34 d	0.66	25.6	70.8	2.98		-
		C 53 d	1.10	22.6	73.1	3.29		-
		C 61 d	1.03	24.0	71.9	3.09		-
		C 116 d	0.88	25.7	69.9	2.63		0.88
[74]	DOW/plant trimming/vegetal R (50:40:10 *w:w*)	C 60 d		37.6	50.8	7.2		4.3
		C 90 d		30.6	56.1	7.3		6.1
		C 150 d		45.3	37.6	9.6		7.4

Table 2. *Cont.*

Ref. [a]	Feedstock [b]	Sample [c]	Alkyl C	O-Alkyl C		Aromatic C		Carboxyl/Carbonyl C
				CH$_3$O/CHN	O-/Di-O-alkyl C	Aryl C	O-Aryl C	
[65]	CM/rice straw	FS	14.9		54.9	20.3		10.0
		C 60 d	17.4		57.4	20.9		10.6
		C 120 d	17.4		48.6	18.6		9.9
		C 240 d	15.2		34.1	16.1		9.2
		C 365 d	13.9		25.7	12.4		6.5
		C 548 d	12.8		22.8	11.3		6.6
		FS	13.5		56.7	20.1		9.7
		C 148 d	15.2		39.6	18.7		10.0
		FS	15.2		56.4	18.8		9.6
		C 60 d	13.4		47.5	16.5		8.4
		C 120 d	12.6		42.0	17.5		8.5
		C 240 d	12.1		37.2	15.9		8.1
		C 365 d	12.6		35.6	15.3		7.7
[63]	SM/wheat straw (95:5 *w:w*)	FS	27.2		55.6	9.2		8.0
		C 7 d	18.6		66.1	9.8		5.5
		C 14 d	15.2		65.3	12.2		7.3
		C 21 d	16.8		65.2	11.5		6.5
		C 28 d	14.7		63.3	13.6		8.3
[79]	MSW (composted in spring) MSW (composted in summer)	FS	15.8		59.5	14.9		7.9
		C 28 d	17.7		59.2	13.6		7.4
		C 42 d	17.5		55.5	16.2		7.2
		C 49 d	17.3		55.5	17.7		5.4
		FS	16.2		60.4	12.1		7.8
		C 28 d	16.8		60.2	13.9		6.9
		C 42 d	18.0		56.1	15.3		6.8
		C 49 d	18.2		56.9	15.8		6.0
[45]	Kitchen waste/garden waste	C1	28.4		45.6	7.4	4.6	14.0
		C2	25.4		48.6	9.8	4.8	11.4
		C3	30.3		32.5	11.1	6.3	19.8
		C4	32.4		38.2	7.9	4.9	16.6
		C5	19.2		53.0	12.8	8.2	6.8
		C6	25.5		40.8	12.6	6.6	14.5
		C7	27.4		42.8	11.3	6.3	12.2
		C8	26.7		42.6	9.0	7.9	13.8
		C9	14.1		43.6	8.1	12.0	22.2

Table 2. *Cont*.

Ref. [a]	Feedstock [b]	Sample [c]	Alkyl C	O-Alkyl C		Aromatic C		Carboxyl/ Carbonyl C
				CH$_3$O/CHN	O-/Di-O-alkyl C	Aryl C	O-Aryl C	
[80]	MSW	FS	26.9	47.9		11.6	4.3	8.3
		C 34 d	25.5	52.1		10.5	3.6	8.2
		C 76 d	24.7	46.5		13.6	4.9	10.3
		C 90 d	23.6	42.4		19.0	6.6	11.4
		C132 d	23.6	40.4		16.9	7.6	11.4
[78]	Grape skin Grape seeds Grape skin and seeds	FS	8.7	53.4		17.5		18.7
		C 160 d	10.9	51.8		18.9		17.0
		FS	25.0	41.3		13.1		18.7
		C 160 d	21.7	38.4		15.7		22.3
		FS	16.2	49.7		13.2		18.8
		C 160 d	18.6	43.7		15.9		20.3

[a] References are reported in reverse chronological order. [b] BEM = beneficial effective microorganisms; BM = buffalo manure; ChM = chicken manure; CM = cattle manure; COF = coffee grounds; DOW = domestic organic waste; MSW = municipal solid waste; PM = poultry manure; PT = poplar trimmings; R = residues; SM = swine manure. [c] AM = after maturation; AT = advanced thermophilic phase; BT = beginning of thermophilic phase; C = compost; CC = commercial compost; d = days; EB = end of biooxidative phase; FS = feedstock.

In most of the papers reported so far in the literature, ^{13}C SSNMR spectroscopy has been used either for monitoring and investigating the composting process up to maturation or for relating the composition of mature compost to its functional properties for specific applications.

As far as the investigation of the composting process is concerned, the analysis of simple ^{13}C CP-MAS NMR spectra recorded on samples taken at different times has allowed the evolution of the characteristic spectral regions and of the corresponding functional groups to be highlighted. Even if there is an intrinsic variability, due to differences in the process conditions (volumes, moisture level, aeration) and, most of all, to the wide variety of feedstocks used [82], in most of the reported cases composting manifests its progress with a decrease in the relative intensity of the O-alkyl region due to the decomposition of easily biodegradable carbohydrates, mainly cellulose and hemicellulose. Moreover, the relative intensity of the aromatic region is often found to increase as an effect of the humification process and of the accumulation of lignin, which is quite recalcitrant to decomposition. It is worth mentioning that a successful method for quantifying lignin from ^{13}C MAS NMR spectra has been proposed by Haw et al. [83] for wood pulp and applied to compost by Albrecht et al. [9]. A "Lignin Ratio" (*LigR*) has been defined by Pane et al. [77] as the ratio between the spectral intensity in the 45–60 ppm region, where the methoxyl groups of lignin resonate together with CH-N groups of peptides, and that in the 145–160 ppm region, to which lignin aromatic and other phenolic carbons contribute. *LigR* has been used in several works of the same research group to discriminate between lignin signals and those of either other phenolic compounds (giving lower *LigR*) or peptidic moieties (giving higher *LigR*). As far as the alkyl region is concerned, the trend of the intensity is variable since many different compounds contribute to it. Often, an increase with composting progress is observed, due to the transformation of carbohydrates to compounds with longer aliphatic chains, but also to the accumulation of recalcitrant compounds, such as suberin and cutin molecules, lipids, and waxes. The intensity of the carboxyl region often increases due to the oxidation processes occurring during composting and this is generally related to the increase in the compost cation exchange capacity, of which carboxyl groups are mainly responsible.

Efforts have been made to identify correlations between ^{13}C SSNMR observables and other maturity indices and to define an NMR parameter that could assess compost maturity. Baldock et al. [34] indicated the *A/OA* ratio as a sensitive index of the extent of OM decomposition provided that its use is limited to OM derived from a common source. As mentioned before, the contents of alkyl and O-alkyl components often, but not always (see for instance refs. [46,64]), move in opposite directions as decomposition proceeds, thus their ratio may provide an index of decomposition more sensitive than their individual values.

In many cases ^{13}C CP-MAS NMR spectra of mature compost have been used to try to find correlations between OM molecular composition and final applicative properties. Preston et al. [45] analyzed compost from backyard sources in Canada to be used as self-produced soil fertilizer in gardens, also reducing residential waste at the source. They observed that backyard operations produced a substantial peak broadening and a marked tendency to increase of carboxyl C signals; these findings, together with other chemical evidence, were attributed to vigorous biological activity, confirming the traditional knowledge of the efficacy of backyard composting.

Anda et al. [75], in contrast to most of the literature, observed that composting of rice husk resulted in a high O-alkyl C content, followed by N-alkyl/methoxyl C, and di-O-alkyl C, attributed to cellulose, with trace amounts of phenolic, carboxyl, and alkyl C, indicating that rice husk is quite resistant to microbial degradation. Such a compost applied to an Oxisol significantly increased soil pH and the Ca, Mg, K, Na, and Si content of in situ soil solution, but decreased the amount of toxic ions (Al, Mn, and Fe). Moreover, this compost was found to increase cocoa growth up to 37%. It was hypothesized that the high content of organic C of the composted rice husk and the chemical resistance to microbial

decomposition could provide special benefit in sustaining an organic C reserve if used as a soil ameliorant, especially in tropical soils where organic matter decomposes rapidly.

Torres-Climent et al. [69] examined the composting process of winery-distillery waste, i.e., grape marc (the primary waste of wine production obtained after the pressing process) and exhausted grape marc (the solid waste of alcohol distillation), showing the importance of combining thermal analysis, and FTIR and ^{13}C SSNMR spectroscopies with classical analytical techniques, the latter being insufficient to study the humification processes of these kinds of feedstocks. Areas of the ^{13}C CP-MAS NMR spectra, evaluated at the beginning and after two months of composting, showed a relative decrease in the carbohydrates and an increase in the alkyl groups. Interestingly, the obtained compost showed good properties for agronomic uses.

Pane et al. [77] used ^{13}C CP-MAS NMR spectra to characterize the OM composition of eleven different composts obtained from waste derived from aromatic plant production and compared the NMR results with the suppressive properties against *Rhizoctonia solani* and *Sclerotinia sclerotiorum* on garden cress, a vegetable crop appreciated for its nutraceutical and taste-giving components, but threatened by soil-borne pathologies due to its very short life-cycle. The study demonstrated good potential for several of the investigated composts as sustainable remedies, substituting for synthetic chemicals. The analysis of ^{13}C CP-MAS NMR spectra showed correlations between the best performing composts and the higher humification level. In particular, the removal of essential oils resulted in an improved humification, a preservation of apolar compounds, and a decrease in *LigR*. This beneficial effect was attributed to the known antioxidant and antimicrobial properties of essential oils, which could have hampered the microbial activity in the processed biomass.

Iacomino et al. [60] investigated the effect on soil fertility and crop yields in a volcanic Mediterranean soil of two different composts, one made from olive mill waste and orchard pruning residues, and one from olive mill waste, animal manure and wool residues, as well as of a biochar and of mixtures of the biochar and the composts. ^{13}C CP-MAS NMR spectra were recorded for the two composts and the biochar, and the areas of the characteristic spectral regions showed that biochar had the lowest content of O-alkyl, methoxyl and alkyl C and the highest content of aromatic species. Overall, the authors concluded that the effects of these organic amendments on crop yield are largely variable and influenced by the interaction with soil chemistry.

^{13}C SSNMR has been applied in a few cases to study compost from MSW [42,79,80], which generally has a large organic fraction (40%), but, due to its origin, can also show peculiar spectral features. In particular, Pichler et al. [42] applied a complete set of quantitative and selective ^{13}C SSNMR experiments to MSW after various composting times and, besides observing the expected decomposition of carbohydrates, the presence of plastics (especially polystyrene, but also polyethylene and polypropylene) was recognized from characteristic signals in both the aliphatic and aromatic regions. Both aromatic and aliphatic signals increased with composting time because of an accumulation of these non-biodegradable materials.

The effect of composting on plastics was also investigated by Spaccini et al. [70] by means of different ^{13}C SSNMR measurements. In this case the decomposition of a biodegradable starch-based thermoplastic mulching film for horticultural crops was followed in a real on-farm composting process. By recording variable contact time ^{13}C CP-MAS NMR spectra of the film before and after composting, the authors detected changes in the ^{1}H $T_{1\rho}$ values that were ascribed to an alteration of the inter-molecular linkages among the organic components of the plastic, due to the progressive decomposition of its constituents.

The effect of specific additives on different composts was also investigated. Skene et al. [84] studied the decomposition of straw in the presence and absence of inorganic matrices, such as sand, kaolin, and loamy sand, and found that straw incubated in inorganic matrices decomposes faster than straw alone and that sand seems to preserve alkyl groups. Considering that biochar in ruminant diets is being assessed as a method for simultaneously improving animal production and reducing enteric CH_4 emissions, Romero et al. [59]

examined the effects of feeding a pine-based biochar to beef cattle on manure composting. ^{13}C CP-MAS NMR showed that biochar increases compost aromaticity, without altering the bulk C speciation of manure. Liu et al. [62] investigated the effect of adding biochar to a swine manure compost, and found a promoting influence of biochar on the aromatization process.

^{13}C SSNMR has also been applied to freeze-dried extracts of compost to aid the understanding of the chemical structure of this complex material, as well as the changes occurring in OM during composting. Results on the contribution of the different functional groups to the ^{13}C CP-MAS NMR spectra of different types of extracts obtained from various composts and feedstocks are reported in Table 3.

As evidenced by the data reported in the table, the chemical composition of compost extracts is strongly dependent on the feedstock. Al-Faiyz [85] applied ^{13}C CP-MAS NMR spectroscopy to characterize HAs extracted from three types of composted feedstocks: agricultural crop plants, date palm fronds, and animal waste. For all samples, the most intense spectral region was the alkyl C one, with HAs of compost from agricultural crop plants showing the highest contribution, counterbalanced by a lower amount of carbohydrate and aromatic carbons. On the other hand, HAs from animal waste compost had the lowest alkyl C content but the highest aromatic C content, while HAs of compost from date palms had the lowest amount of carboxyl carbons. None of the samples showed intensity in the carbonyl region.

Similarly, HSs extracted from composts obtained from artichoke, pepper or coffee wastes showed significantly different ^{13}C CP-MAS NMR spectra, particularly regarding the alkyl C and O-alkyl C spectral regions [86]. Additionally, the data by Fuentes et al. [87] on HSs extracted from composts of different origin (animal manure, olive mill and winery wastes, domestic wastes) showed significant differences among the spectral intensities of the different regions.

Bulking agents were also found to affect the composition of compost extracts. The ^{13}C CP-MAS NMR data recorded on HSs extracted from compost obtained from artichoke residues mixed with corn straw and wood chips [86] and from the same residues mixed with maize straw [37] showed major differences, particularly in the aromatic spectral region.

Even the use of additives was found to influence HA composition after composting. Zheng et al. [88] studied compost obtained from pig manure and sawdust with the addition of different amounts of sepiolite, a clay mineral, which is indeed expected to affect the composting process due to its high absorption capacity and cation exchange ability, possibly favoring the humification process. The ^{13}C CP-MAS NMR spectra of HAs extracted from compost obtained from samples with increasing amounts of sepiolite confirmed the increasing extent of humification, as highlighted by the higher relative amount of aromatic C and concomitant reduction in the contribution of alkyl and O-alkyl C. Sepiolite also induced the formation of carbonyl and carboxyl groups. The authors suggested that the reduction in bioavailability of toxic metals, such as Cu and Zn, abundant in pig manure, in sepiolite-amended compost might be related to the higher amount of phenolic and oxygen-containing functional groups of the resulting HAs.

The effect of biochar on compost HAs was also investigated. Biochar has been demonstrated to have positive effects on compost, for example improving compost porosity [89] and favoring carbon and nitrogen retention [90,91]. Liu et al. [62] studied the effect of adding biochar to swine manure and rice straw on the composition of HAs, as well as on compost, as previously mentioned. A comparison of ^{13}C CP-MAS NMR spectra and carbon species distribution of compost and HA samples during composting is shown in Figure 3. Three mixtures of manure, straw as bulking agent, and biochar in different proportions, i.e., 4:1:0, 8:1:0 and 8:1:1 by wet weight, were investigated. In Table 3 the data relative to the different NMR spectral regions for HAs obtained for the three different samples before and after composting are reported. The authors observed a positive effect of biochar on HA formation on the basis of the higher overall spectral intensity. In all cases, the alkyl C contribution sensibly decreased after 40 days of composting, while the aromatic and

O-alkyl C contents increased. This was taken as an indication of the decomposition of macromolecules, such as lignin, hemicellulose and cellulose, contributing to the formation of HAs [92]. The amount of carboxyl C in the HA sample with the biochar added was higher with respect to the other samples before composting and also remained high after composting, with a value similar to that of the sample with a higher amount of bulking agent. A high proportion of both aromatic and carboxyl groups in compost HAs, as observed in the presence of biochar and with a higher amount of bulking agent, has been suggested to have positive agronomic effects [93].
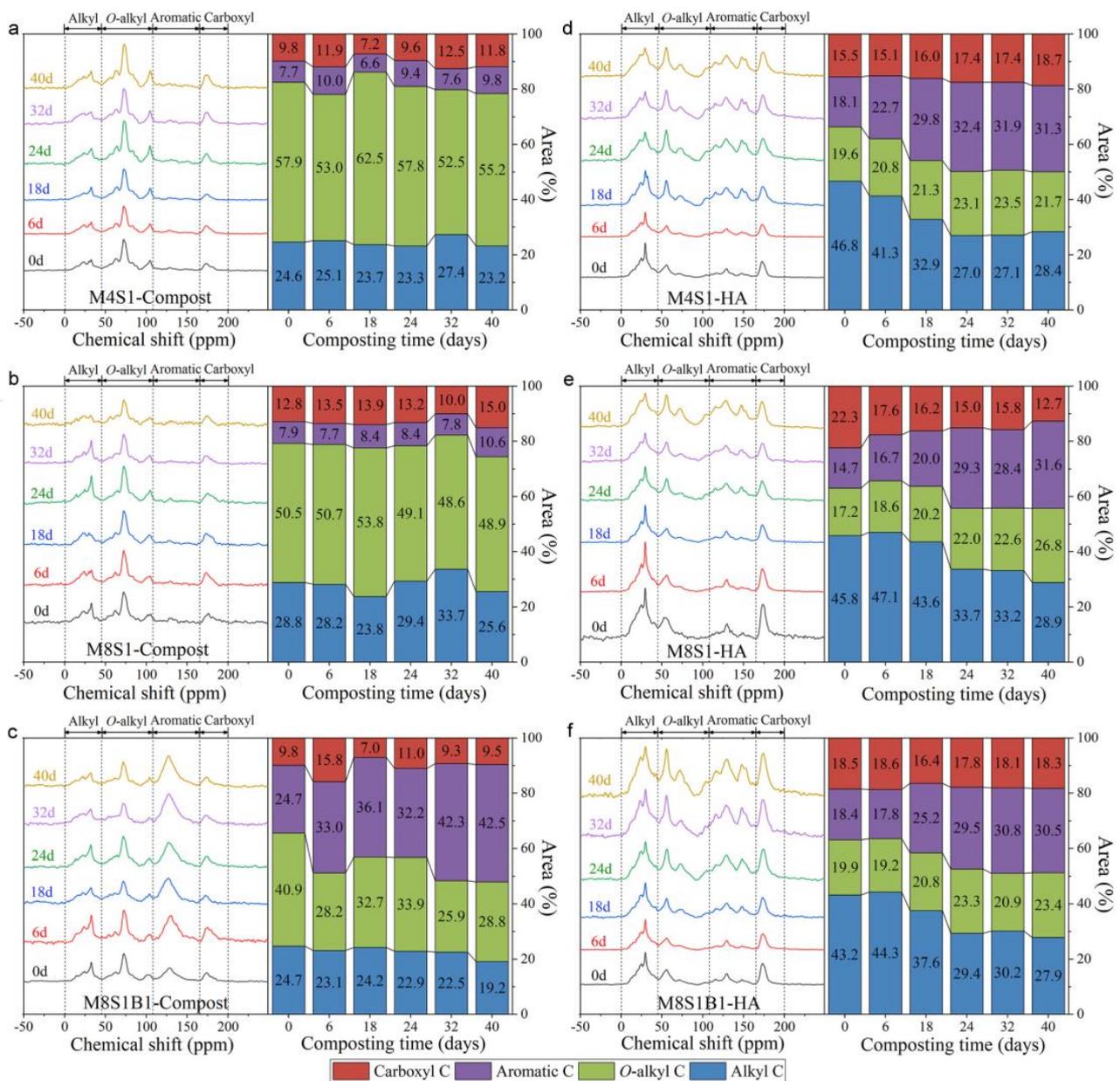

Figure 3. ^{13}C CP-MAS NMR spectra of composts (**a**–**c**) and HA samples (**d**–**f**) and relative contributions of the different spectral regions. Feedstocks were constituted of mixtures of swine manure/rice straw/biochar in the ratios: 4:1:0 (M4S1), 8:1:0 (M8S1), and 8:1:1 (M8S1B1). Reproduced with permission from Ref. [62].

^{13}C SSNMR was more extensively applied to monitor the changes occurring in HAs during composting, although, again, the changes observed were strongly dependent on the feedstock. In fact, although it is generally stated that, during composting, carbohydrates

are decomposed and new aromatic groups are formed, no fixed trends were reported in the case of compost HAs.

Data in agreement with the expected trend, were reported by Spaccini and Piccolo [94] on a mixture of domestic waste, plant trimmings and tobacco residues. After 2 months of composting, the HA spectrum was dominated by alkyl and O-alkyl signals, the former probably deriving from lipids, waxes, plant polyesters, and peptidic compounds, the latter from sugars, but also from ether and epoxy groups of plant polyesters. The aromatic signals were mainly associated with H- or C-substituted carbons, with a low content of O-aryl carbons. Dipolar dephasing NMR experiments showed a low contribution from lignin. On the other hand, there was a large contribution from carboxyl groups in aliphatic acids and/or amino acid amide groups. After 5 months of composting, the extracted HAs showed a significantly lower carbohydrate content and a higher proportion of alkyl C due to the incorporation of stable lipids. Observable, but minor increases were found for the aromatic and carboxyl C signal intensities. The results suggested a preferential preservation in the HA fraction of hydrophobic alkyl components, as well as the inclusion of labile peptidic compounds.

Castaldi et al. [95] studied the transformation of HAs during the composting of MSW with the addition of agricultural wastes. The ^{13}C CP-MAS NMR spectra of HAs, extracted at the beginning and after 100 days of composting, showed that the composting process caused a reduction in carbohydrates and aliphatic structures, and an increase in the aromatic, carboxyl and carbonyl carbons, which suggested a partial neo-formation of humified material.

Fuentes et al. [87] followed the composting process by analyzing HSs in five composted materials of diverse origin, that is, solid wastes of wineries, solid olive mill wastes, domestic wastes, ovine manure with the addition of straw, and a mixture of animal manures. Except for domestic wastes and the mixture of animal manures, humification was somewhat as expected, that is, there was an increase in the number of aromatic carbons, with a large contribution of phenolic groups, and a reduction in O-alkyl groups, while the changes in carboxyl/carbonyl C contents at variance. On the contrary, in the case of domestic wastes and mixed manures, the distribution of functional groups did not undergo significant changes, but there was an unexpected increase in O-alkyl C and a slight decrease in aromatic and carbonyl C. The authors ascribed this unusual behavior to the presence of non-aromatic recalcitrant material and of tannins, these exerting a limiting effect on the decomposition of aliphatic biomolecules, as observed by Almendros et al. [96].

Contrary to the expected trend, the study by Liu et al. [62] on the composting of swine manure with straw and biochar, discussed above, showed in all HA samples a significant decrease in the contribution of alkyl C, and an increase in the contribution of all the other carbon types (except for the carboxyl signal in the pig manure/straw with an 8:1 ratio), the major increase involving the aromatic region. As already stated, this trend was explained by assuming the formation of new aromatic structures contributing to HAs resulting from the decomposition of other macromolecular components.

Gonzáles-Vila et al. [79] studied HAs extracted from MSW and from its compost obtained after 7 weeks. The ^{13}C CP-MAS NMR spectra of the HAs isolated before and after the composting process showed a significant reduction in carbohydrate content with a large increase in alkyl carbon upon composting, which indicated a concentration of recalcitrant polymethylenic structures (i.e., waxes and polyesters), while no major increase in aromatic structures or carboxyl groups was observed. The authors pointed out that, given the type of feedstock, most of the signals in the aromatic region were probably due to unsaturated fatty acids, physically occluded and/or esterified with other aliphatic macromolecules. At variance with those results are those obtained by García et al. [97], who investigated HA extracts from the organic fraction of MSW and from its compost. In this case, the only changes observed were a reduction in alkyl C and an increase in aromatic C content upon composting, while the intensity in the carbohydrate region and in the carboxyl/carbonyl C region did not change.

With the aim of better understanding the transformations of HSs during the composting of MSW, Chefetz et al. [98] extracted two types of HAs, i.e., HAs and core-HAs, the latter obtained by organic solvent and sulfuric acid extractions prior to the alkaline extraction. Core-HAs represent a recalcitrant fraction, whereas HAs also contain labile components, some of which coat core-HA structures. ^{13}C CP-MAS NMR spectra indicated that, while core-HAs, being more stable, practically did not change during the process, HAs showed a decrease in carbohydrates and an increase in aromatic structures. Essentially, the "coating" materials (polysaccharides, peptides, and lipids) bonded to the core-HA structure degraded during composting, leaving HA structures of higher aromaticity. In fact, as composting proceeded, HAs tended to resemble core-HAs. Besides relative intensities, the authors also determined absolute intensities, taking into account the carbon content reduction in the process. Considering the absolute data, the authors noted that, besides degradation of coating compounds, new HAs were formed. Thus, they concluded that during composting carbohydrates were lost by degradation while the residual HAs were enriched with aromatic structures.

Changes of OM in wastes were also followed by observing the changes in DOM. Caricasole et al. [99] compared the spectra of DOM extracted from different mixtures of domestic and garden wastes and their composts. In all cases, composting gave rise to a decrease in O-alkyl C, counterbalanced by an increase in all other functional groups. The increase in alkyl C was attributed to the breakdown of polysaccharides yielding simple alkyl chains and/or to the accumulation of highly aliphatic biopolymers occurring in cuticles of higher plants and/or some cell wall products highly resistant to decomposition [28]. The relative enrichment in aromatic C was supposed to be related to the degradation of non-aromatic cell wall compounds [100], while carboxyl and carbonyl groups probably arose from oxidative transformations of lignin [50]. The ^{13}C CP-MAS NMR spectra obtained on DOM extracted from fresh substrates and from the final composts indicated that, as observed for HSs, DOM composition depends on the initial feedstock. The highest increase in alkyl C associated with the lowest increase in aromatic C was observed in the compost containing hardly degradable pine needles. The highest carbohydrates reduction associated with the highest increase in the *A/OA* ratio and to the lowest carboxyl C increase was observed for the sample originating from the mixture containing spent yeast from beer production, which is a highly degradable material.

Comparison of DOM spectra among different samples and composts can indeed give important information on the composting process. However, the determination of DOM composition is difficult since it is a mixture of low-molecular weight compounds (free amino acids and sugars) and chemically heterogeneous macromolecules (enzymes, amino-sugar complexes, polyphenols, and HSs). With the aim of better understanding DOM composition, ^{13}C SSNMR was used on separated hydrophilic (HiDOM) and hydrophobic (HoDOM) fractions [101] and on fractions further separated on the basis of solubility in acid or basic solutions [57]. The two studies highlighted the composition of the two main fractions confirming the hydrophilic nature of HiDOM, richer in oxygenated functional groups, and the hydrophobic nature of HoDOM, richer in alkyl and aromatic carbons. Chefetz et al. [57] followed the fate of three fractions of HoDOM (Ho-acid, Ho-base and Ho-neutral) and three fractions of HiDOM (Hi-acid, Hi-base and Hi-neutral) through the composting process. The Ho-acid fraction, characterized by a fulvic acid composition, was stable throughout the process and became the dominant DOM component in the final compost. The amount of the Ho-neutral fraction, constituted of highly apolar compounds, was considered to represent an intermediate stage in the transformation of DOM compounds to HSs. On the other hand, the Hi-neutral fraction, mainly containing poly- and oligosaccharides, significantly decreased during composting, and was of relevance in OM degradation. On the basis of their findings, the authors suggested possible reasons for the beneficial effects of compost on plant growth: (i) higher amounts of Ho-neutral and Ho-acid in the compost imply an increase in the concentration of functional groups which are able

to chelate metals, thus favoring their uptake by plants; (ii) the reduced concentration of Hi-neutral results in the removal of compounds toxic to plants.

Several studies focused on the relationship between structure and bioactivity of compost HSs. Verrillo et al. [37] studied the effects of HSs extracted from a compost obtained from artichoke biomass. The use of ^{13}C CP-MAS NMR, combined with pyrolysis gas chromatography mass spectrometry, indicated that the biostimulation activity was related to the presence of hydrophobic domains containing numerous bioactive molecules, such as lignin-derived molecules and saccharidic and aminoacidic components. Of relevance for understanding the structure–activity relationship were the *ARM*, *A/OA*, *HB/HI*, and *LigR* indices. The relatively high *HB/HI* index (=0.90) observed for HSs from artichoke residues and the relatively high aromatic content revealed a significant incorporation of hydrophobic molecules in the humic supramolecular structure, mainly represented by aromatic compounds. The *A/OA* ratio of 0.65 indicated the predominance of bioavailable polar O-alkyl groups with respect to apolar alkyl components, and the high *LigR* value (=2.5) indicated that most of the intensity in the 45–60 ppm region was due to lignin units and, hence, confirmed their incorporation in HAs.

Similar results were found for HSs extracted from composted artichoke and pepper wastes, and from coffee grounds [86]. These extracts were also investigated for their antibacterial activity and antioxidant properties, the former being related to the aromatic and phenolic content and the latter only to the phenolic content [102]. The anti-inflammatory properties of HAs were also shown to be related to the content of aromatic, phenolic and lignin components in a study by Verrillo et al. [103].

The molecular features responsible for bioactivity were also investigated on compost tea. Verrillo et al. [104] studied CTs from different composts of recycled agricultural biomasses (artichoke and pepper with maize straw, coffee husks with lettuce residues). Germination tests performed using basil seeds showed that CT from coffee husks and pepper caused an increase in both root and epicotyl length, while artichoke CT showed positive effects only on root development. As in the case of HSs, this biostimulation effect was ascribed by the authors to a higher amount of phenolic and aromatic units, as observed by ^{13}C CP-MAS NMR.

Pane et al. [35] studied three CTs, produced from agricultural wastes (tomato and escarole residues) with the addition of different proportions of woodchips, and a CT from a commercial biowaste compost, investigating their effects in a processing tomato cropping system. The ^{13}C CP-MAS NMR spectra of CTs obtained from recycled agricultural biomasses were mainly characterized by prominent signals attributable to the incorporation of lignin molecules and polyphenol derivatives, while that of commercial compost showed a composition dominated by carbohydrates. Notwithstanding the chemical differences, the general bioactivity observed for all CT treatments, independently from the sources, was attributed to a correct balance of molecular components giving rise to an equilibrium between the hydrophobic and the hydrophilic domains [105].

Table 3. Relative areas (% of total area) of the different regions of ^{13}C CP-MAS NMR spectra for extracts of composts and corresponding feedstocks reported in the literature.

Ref. [a]	Feedstock [b]	Sample [c]	Alkyl C	CH$_3$O/CHN	O-/Di-O-Alkyl C	Aryl C	O-Aryl C	Carboxyl C	Carbonyl C
				O-Alkyl C		Aromatic C		Carboxyl/ Carbonyl C	
[88]	SM/poplar sawdust (5:3 *w:w*)	HA 60 d	30.3		33.9	16.8	7.1	11.9	
	+sepiolite 3 wt%	HA 60 d	26.7		29.9	18.3	8.4	16.8	
	+sepiolite 6 wt%	HA 60 d	34.7		22.6	17.4	7.9	17.4	
	+sepiolite 9 wt%	HA 60 d	24.3		29.4	18.3	10.4	17.6	
	+sepiolite 12 wt%	HA 60 d	24.0		29.9	20.8	8.7	16.5	
[86]	CYN/corn straw and WC (70:30 *w:w*)	HS 100 d	20.93	15.98	29.78	16.26	5.51	11.54	
	COF/corn straw and WC (70:30 *w:w*)	HS 100 d	30.63	13.07	24.69	15.33	3.42	12.85	
	PEP/corn straw and WC (70:30 *w:w*)	HS 100 d	20.06	14.22	35.75	14.40	4.49	11.08	
[103]	CYN/WC (70:30 *w:w*)	HA 100 d	22.2	13.8	27.5	20.0	5.7	10.9	
[62]	SM/rice straw 4:1	HA FS	46.8		19.6		18.1	15.5	
		HA 40d	28.4		21.7		31.3	18.7	
	SM/rice straw 8:1	HA FS	45.8		17.2		14.7	22.3	
		HA 40d	28.9		26.8		31.6	12.7	
	SM/rice straw/biochar 8:1:1	HA FS	43.2		19.9		18.4	18.5	
		HA 40d	27.9		23.4		30.5	18.3	
[37]	CYN/corn straw (70:30 *w:w*)	HS 100 d	16.3	13.8	24.7	28.9	5.6	10.6	
[104]	Coffee husks/lettuce residues at (60:40 *w:w*)	CT 100 d	26.9	11.9	26.4	16.6	4.3	14	
	CYN with maize straw/WC (70:30 *w:w*)	CT 100 d	27.6	12.1	27.3	15.0	4.2	13.9	
	PEP with maize straw/WC (70:30 *w:w*)	CT 100 d	17.4	14.3	31.1	19.2	5.8	12.3	
[85]	Agricultural crop plants/NH4NO3 (66:34 *w:w*)	HA 90 d	41.63		24.89		19.31	14.16	
	Date palm fronds/NH4NO3 (66:34 *w:w*)	HA 90 d	36.39		29.05		23.85	10.70	
	Animal waste/NH4NO3 (66:34 *w:w*)	HA 90 d	31.39		29.0		25.45	14.33	
[35]	Tomato R/escarole R/WC/CS (17.5:20.5:60:2)	CT 105 d	23.50	15.60	28.65	16.80	5.25	10.20	
	Tomato R/escarole R/WC/CS (37:11:50:2)	CT 105 d	22.55	14.95	26.65	18.25	5.90	11.75	
	Tomato R/escarole R/WC/CS (50:0:48:2)	CT 105 d	23.40	14.70	26.80	17.10	6.90	11.15	
	Commercial compost from biowaste	CT 105 d	26.95	12.85	32.70	10.35	2.90	14.25	
[99]	DOW/COF/pine needles and WT (1:1:1)	DOM FS	26.0	6.3	42.1	9.5	4.9	11.1	
		DOM 90 d	30.8	7.4	27.1	13.4	7.3	14.0	
	DOW/GT/FR (2:1:1)	DOM FS	31.8	6.2	38.5	7.9	4.3	11.4	
		DOM 90 d	34.6	7.3	25.9	13.1	4.9	14.2	
	GT/COF/spent yeast (1:1:1)	DOM FS	30.8	5.3	41.2	6.2	2.8	13.6	
		DOM 90 d	35.2	8.4	22.7	11.8	5.8	16.2	
	GT/COF/FR/sewage sludge (4:2:2.5:0.25)	DOM FS	30.8	6.2	38.2	8.6	4.9	11.3	
		DO 90d	33.9	7.8	25.0	13.2	5.4	14.7	

Table 3. *Cont.*

Ref. [a]	Feedstock [b]	Sample [c]	Alkyl C	O-Alkyl C		Aromatic C		Carboxyl/Carbonyl C	
				CH$_3$O/CHN	O-/Di-O-Alkyl C	Aryl C	O-Aryl C	Carboxyl C	Carbonyl C
[94]	DOW/GT/vegetal R from tobacco (50:30:20)	HA 60 d	28.0	11.3	32.4	16.8		11.5	
		HA 90 d	34.9	10.8	28.6	15.7		10.0	
		HA 150 d	34.5	9.3	23.1	19.8		13.4	
[101]	OFMSW/GT/foliage R from tobacco (55:30:15)	CT 120 d	31.0	9.0	23.1	13.3		23.6	
		HoDOM 120 d	34.6	12.6	19.5	19.3		14.0	
		HiDOM 120 d	30.3	9.8	36.8	9.1		14.0	
[87]	OvM/straw Mixture of animal manures Solid olive mill wastes Solid wastes of wineries Domestic wastes	HS FS	18.6		49.7	8.2		23.5	
		HS 120 d	17.7		25.0	22.3		35.0	
		HS FS	33.7		13.8	11.8		40.7	
		HS 120 d	30.4		24.0	9.6		36.0	
		HS FS	23.2		56.4	11.2		9.2	
		HS 120 d	22.2		29.3	19.7		28.8	
		HS FS	3.5		46.9	1.5		48.1	
		HS 120 d	21.6		13.1	26.8		38.5	
		HS FS	23.7		28.2	20.3		27.8	
		HS 120 d	25.4		34.9	16.4		23.3	
[95]	MSW/vegetal wastes (1:1 *v:v*)	HA FS	43.4		25.9	10.3		16.4	4.0
		HA 28 d	44.7		22.1	11.0		17.9	4.3
		HA 100 d	42.9		20.3	11.4		17.9	7.5
[79]	MSW	HA FS	32		45	13		10	
		HA 49 d	48		26	16		10	
[98]	MSW	HA 6 d	38		31	13	5	11	2
		HA 19 d	45		23	15	5	11	1
		HA 33 d	44		23	16	6	11	1
		HA 62 d	42		24	15	6	12	2
		HA 105 d	39		25	16	6	12	2
		HA 187 d	38		26	17	6	12	3
		Core-HA 6 d	34		24	21	8	12	2
		Core-HA 19 d	33		23	23	9	11	2
		Core-HA 33 d	34		23	23	9	11	1
		Core-HA 62 d	38		23	19	7	11	3
		Core-HA 105 d	43		21	19	7	10	0
		Core-HA 187 d	35		22	20	8	13	3
[97]	OFMSW	HA FS	37.20		34.34	16.28		12.23	
		HA C	30.07		34.71	22.67		12.58	

[a] References are reported in reverse chronological order. [b] COF = coffee grounds; CS = compost starters; CYN = artichoke residues; DOW = domestic organic waste; FR = fresh green vegetable residues; GT = garden trimmings; MSW = municipal solid waste; OFMSW = organic fraction of MSW; OvM = ovine manure; PEP = pepper waste; R = residues; SM= swine manure; WC = wood chips; WT = wood trimmings. [c] CT = compost tea; d = days; DOM = dissolved organic matter; FS = feedstock; HA = humic acid; HiDOM = hydrophilic fraction of DOM; HoDOM = hydrophobic fraction of DOM; HS = humic substance.

4. ^{13}C Solid-State NMR Applications to Vermicomposting

Several studies applied ^{13}C CP-MAS NMR to characterize vermicomposts obtained from different substrates [106–111]; the relative areas of the spectral regions of the vermicomposts together with details on the feedstocks and on the process are reported in Table 4. The hydrophobic index *HB/HI* is also reported, when available. Most of the studies used feedstocks containing cattle manure (CM), either alone or mixed with other organic wastes, like flock cotton residues, garden green wastes, sugar cane bagasse, and sunflower cake. In fact, CM has been demonstrated to be a good habitat for earthworms. Only in two cases the feedstock did not contain CM, but it was either a mixture of lettuces, apples, residual maize, ground spent coffee and pieces of cardboard [106], or it was made of maple pruning wastes [111]. In two independent studies [108,110], CM alone was vermicomposted in apparently very similar conditions (5 kg of worms per m^3 of organic residue, precomposting time of 1 month, vermicomposting time of 4 months), but the ^{13}C CP-MAS NMR spectra of vermicomposts were quite dissimilar, displaying different integral intensities especially for alkyl C and O-aryl C. The areas of the spectral regions, taken from Table 4, are shown in Figure 4, where data on humic extracts are also shown. The observed variability in the compost composition can be due to differences in unstated process parameters or, more probably, to the different origin of the feedstock.
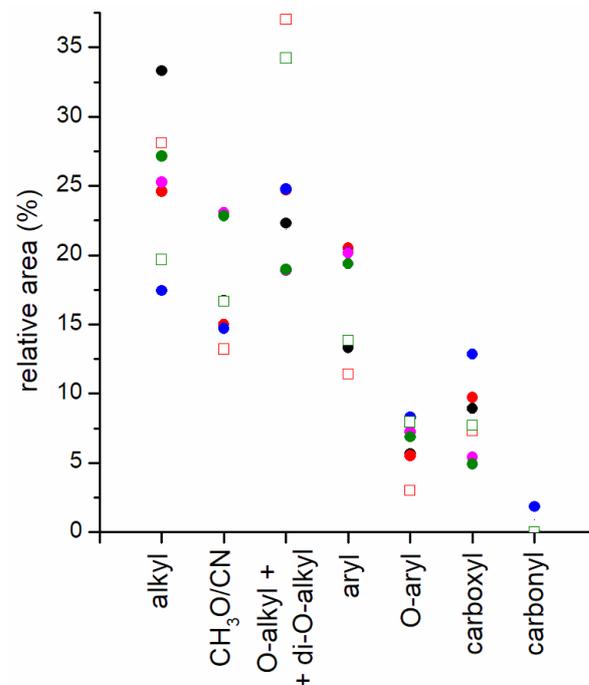

Figure 4. Relative areas of the ^{13}C NMR spectral regions recorded for CM vermicomposts (squares) and humic extracts of CM vermicomposts (circles) in the following studies: (●,□) [108], (●) [110], (●) [112], (●) [113], (●) [114], (□) [115].

In fact, the data of Table 4 indicate that vermicomposts tend to inherit the molecular features of the starting feedstocks. For example, when CM is progressively replaced with lignocellulosic residues from cotton [108], the integral intensities of the O-alkyl C and aromatic C regions tend to increase, whereas the area of the alkyl C region decreases. Indeed, the fractions of O-alkyl C and aromatic C show values of about 44% and 18% in vermicompost entirely produced from cotton, whereas they are 37% and 14% in CM vermicompost; on the other hand, the fraction of alkyl carbons is about 19% in the first case and about 28% in the second one. The ^{13}C CP-MAS NMR spectra of vermicomposts containing CM and different amounts of cotton residues are shown in Figure 5.

Table 4. Relative areas (% of total area) of the regions of ^{13}C CP-MAS NMR spectra of vermicomposts and corresponding feedstocks reported in the literature; when available, the hydrophobic index, *HB/HI*, is also reported.

Ref. [a]	Feedstock [b]	Sample [c]	Alkyl C	CH$_3$O/CHN	O-Alkyl C	Di-O-Alkyl C	Aryl C	O-Aryl C	Carboxyl/ Carbonyl C	*HB/HI*
[106]	OMx	VC 196 d	24.1		51.8		12.4	3.1	8.6	
		C 196 d	27.6		48.2		11.7	2.7	9.8	
	OMx/montmorillonite (7:3 *w:w*)	VC 196 d	19.9		57.0		11.8	3.0	8.3	
		C 196 d	21.6		56.7		10.0	2.5	9.1	
	OMx/kaolinite (7:3 *w:w*)	VC 196 d	22.6		47.2		14.6	3.8	11.8	
		C 196 d	18.4		58.2		11.5	3.0	9.0	
	OMx/goethite (8.5:1.5 *w:w*)	VC 196 d	22.5		42.2		15.3	5.2	14.7	
		C 196 d	19.2		55.0		11.7	3.6	10.5	
[107]	OMx/montmorillonite (7:3 *w:w*)	FS	9.1		76.9		7.0	1.7	5.3	
		F1 VC 196 d	17.6		56.7		14.8	3.9	7.0	
		F1 C 196 d	28.0		47.4		12.0	3.0	9.7	
		F2 VC 196 d	21.9		53.4		12.1	3.2	9.3	
		F2 C 196 d	22.9		51.8		12.0	3.1	10.2	
		F3 VC 196 d	19.0		57.5		10.7	3.0	9.8	
		F3 C 196 d	21.1		58.8		9.0	2.3	8.8	
[108]	CM [d]	VC 120 d	28.1	13.2	37.0		11.4	3.0	7.3	1.0
	CM/flock cotton residues [d] (75:25 *v:v*)	VC 120 d	29.5	11.8	35.8		11.3	3.7	7.8	1.0
	CM/flock cotton residues [d] (50:50 *v:v*)	VC 120 d	24.1	11.9	39.0		13.7	4.2	7.1	0.9
	CM/flock cotton residues [d] (25:75 *v:v*)	VC 120 d	20.1	11.2	44.5		13.3	4.0	7.0	0.8
	Flock cotton residues [d]	VC 120 d	19.4	11.5	44.4		13.7	4.5	6.6	0.8
[109]	CM/garden green waste [e]	VC 70 d	17.8	11.1	31.1	8.9	13.3	7.8	10.0	
[110]	CM [d]	FS	23.16	17.70	24.97	9.99	11.45	6.39	6.34	0.69
		VC 120 d	19.67	16.64	23.70	10.51	13.85	7.94	7.69	0.71
	CM/SCB [d] (1:1 *w:w*)	FS	14.46	16.58	31.90	12.39	12.00	6.94	5.73	0.50
		VC 120 d	19.22	16.68	24.49	10.91	13.46	7.82	7.42	0.68
	CM/SC [d] (1:1 *w:w*)	FS	15.04	17.29	33.35	12.48	10.50	5.36	5.99	0.45
		VC 120 d	13.88	17.13	29.80	12.19	13.44	7.95	5.60	0.54
	CM/SCB/SC [d] (1:1:1 *w:w*)	FS	15.85	17.65	32.74	11.26	9.93	5.33	7.25	0.45
		VC 120 d	15.77	16.53	25.23	11.10	14.94	9.16	7.26	0.66
[111]	Maple pruning waste	FS			79.26		8.28	4.75	6.04	
		VC 30 d			76.87		9.83	5.24	6.54	
		VC 150 d			75.45		10.07	5.73	6.79	
		VC 180 d			74.62		10.25	5.72	7.80	
		VC 240 d			74.43		10.43	5.65	7.56	
		VC 300 d			75.40		12.12	5.45	5.90	
		C 30 d			77.94		10.56	4.84	5.76	
		C 150 d			72.11		12.37	5.98	7.51	
		C 180 d			72.19		11.63	6.21	7.80	
		C 240 d			73.10		11.59	6.03	7.85	
		C 300 d			72.54		11.88	6.21	7.79	

[a] References are reported in reverse chronological order. [b] CM = cattle manure; OMx = organic mixture (lettuce/apples/residual maize/spent coffee ground/cardboard 1.2:19.6:10.1:39.1:27.9 *w:w*); SC = sunflower cake; SCB = sugar cane bagasse. [c] C = compost; d = days; F1, F2, F3 are VC and C fractions with increasing density (<1.6 g/cc, in the range 1.6–1.8 g/cc, >1.8 g/cc); FS = feedstock; VC = vermicompost. [d] Precomposting for 30 days. [e] Precomposting for 60 days.
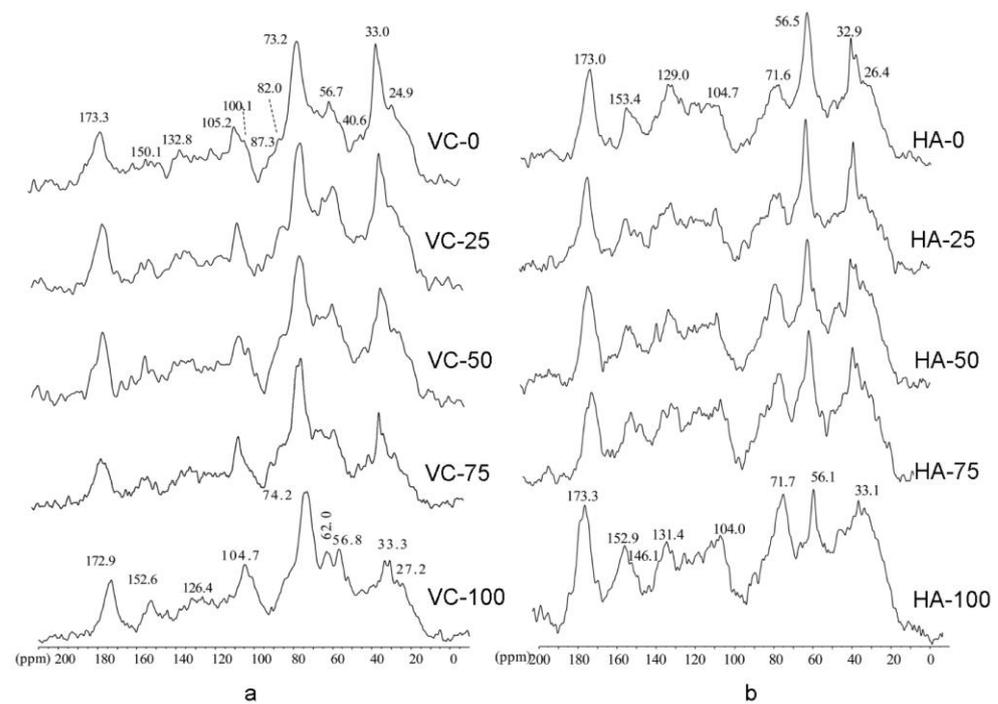
Figure 5. ^{13}C CP-MAS NMR spectra of (**a**) vermicomposts (VC) and (**b**) HAs extracted from vermicomposts at increasing rate of CM replacement (0, 25, 50, 75 and 100 are the percentages of cotton residues). Adapted from Ref. [108].

The effect of vermicomposting time on the composition of the resulting vermicompost was studied by Aguiar et al. [110] and Vinceslas-Akpa et al. [111]. Aguiar et al. monitored vermicomposts produced from different substrates (i.e., CM; CM and sugar cane bagasse; CM and sunflower cake; CM, sugar cane bagasse and sunflower cake) and observed a decrease in carbohydrates after 120 days of vermicomposting together with an increase in aryl and O-aryl groups. The decrease was larger when sugar cane bagasse was used. The intensities of the spectral regions for the feedstocks and vermicomposts after 120 days of treatment are reported in Table 4. The described trends resulted in an increase in the hydrophobic index in the mature vermicomposts compared to the starting materials and suggested the mineralization of carbohydrates and the selective preservation of the aromatic components. The carboxyl C fraction tended to increase for vermicomposts obtained from CM and from CM and sugar cane bagasse, whereas no significant changes were reported for vermicompost from the other feedstocks. The increase in the carboxyl C fraction was tentatively attributed to side-chain oxidation of plant derived lignin and phenolic compounds, which are relatively less abundant in sunflower cake. The trend of the integral intensity of the alkyl C region depended on the substrate. In fact, it decreased for vermicomposts derived from CM and from CM and sunflower cake, whereas an increase was observed for vermicompost from CM and sugar cane bagasse and no changes were detected for that produced from CM, sugar cane bagasse and sunflower cake. The authors did not make conclusive statements regarding this behavior.

A larger number of studies aimed to determine the composition of vermicompost extracts, mainly HAs [105,108,112–122], by means of ^{13}C CP-MAS NMR spectroscopy. In most of these studies, the goal was to evaluate the bioactivity of the extracts and possibly find structure–activity relationships. The composition of the extracts was monitored for different feedstocks and sometimes at different maturation stages of vermicomposting. The relative areas of the regions of ^{13}C CP-MAS spectra of vermicompost extracts are reported in Table 5 together with details on the feedstocks used and on the process. The hydrophobic index *HB/HI* is also reported, when available.

Table 5. Relative areas (% of total area) of the regions of ^{13}C CP-MAS NMR spectra of extracts of vermicomposts and corresponding feedstocks reported in the literature. The hydrophobic index, *HB/HI*, is also reported when available.

Ref. [a]	Feedstock [b]	Sample [c]	Alkyl C	O-Alkyl C			Aromatic C		Carboxyl/Carbonyl C		HB/HI
				CH$_3$O/CHN	O-Alkyl C	Di-O-Alkyl C	Aryl C	O-Aryl C	Carboxyl C	Carbonyl C	
[112]	CM	HA 120 d	33.3	16.7	16.7	5.6	13.3	5.6	8.9		0.98
[108]	CM	HA 120 d	24.6	15.0	24.7		20.5	5.5	9.7		1.4 [h]
	CM/flock cotton residues [d] (25:75 *v:v*)	HA 120 d	24.2	13.8	25.6		18.4	6.8	11.3		1.3 [h]
	CM/flock cotton residues [d] (50:50 *v:v*)	HA 120 d	26.8	13.1	25.7		18.1	6.5	9.9		1.4 [h]
	CM/flock cotton residues [d] (75:25 *v:v*)	HA 120 d	24.1	12.9	28.1		19.0	7.0	9.0		1.3 [h]
	Flock cotton residues [d]	HA 120 d	22.1	12.2	29.4		17.6	7.6	11.1		1.2 [h]
[114]	CM	HAw 120 d	37.63	11.82	10.75	4.3	11.82	3.22	12.90	7.52	1.32
		HA 120 d	17.43	14.68	15.60	9.17	20.18	8.26	12.84	1.83	0.88
		HR 120 d	33.68	11.57	26.31	7.36	8.42	2.10	8.42	2.10	0.82
[119]	CM	HS	21.21	15.15	14.14	9.09	16.16	10.10	14.14		
[116]	CM/SD (85:15 *v:v*)	HA 0–30 d	20.38		47.94		21.06		10.61		
		HA 45–60 d	20.58		49.75		19.50		10.17		
		HA 90–135 d	19.93		49.22		20.45		10.39		
	CM/SD/TWS (72.5:12.5:15 *v:v*)	HA 0–30 d	21.54		47.69		20.64		10.12		
		HA 45–60 d	21.33		47.81		20.44		10.42		
		HA 90–135 d	20.54		47.85		21.53		10.08		
	CM/SD/TWC (70:10:20 *v:v*)	HA 0–30 d	21.57		48.18		19.90		10.35		
		HA 45–60 d	23.76		46.65		19.62		9.97		
		HA 90–135 d	20.74		49.11		20.14		10.00		
	CM/SD/TWS/TWC (70:10:7.5:12.5 *v:v*)	HA 0–30 d	20.33		46.61		21.23		11.83		
		HA 45–60 d	22.07		48.41		19.41		10.11		
		HA 90–135 d	20.31		47.80		21.15		10.74		
[117]	Tomato plant /PMS (2:1 *w:w*)	HA FS	49.4		26.5		11.1		13.0		
		HA 180 d	33.1		39.3		15.9		11.7		
	Tomato plant/PMS (1:1 *w:w*)	HA FS	50.9		21.1		13.1		14.9		
		HA 180 d	29.1		38.2		19.3		13.4		
[113]	CM [d]	HA 90 d	25.25	23.07	12.38	6.53	20.15	7.22	5.41		1.11
	CM/SCB [d] (1:1 *w:w*)	HA 90 d	20.77	21.31	19.61	9.53	17.62	5.86	5.30		0.79
	CM/SC [d] (1:1 *w:w*)	HA 90 d	22.68	22.24	18.96	8.87	17.31	5.13	4.81		0.82
	CM/SCB/SC [d] (1:1:1 *w:w*)	HA 90 d	19.08	22.81	20.46	8.96	18.53	5.51	4.65		0.76
	Sugar cane filter cake residue [d]	HA 90 d	21.91	17.94	14.64	9.35	20.39	7.48	8.30		0.99

Table 5. *Cont.*

Ref. [a]	Feedstock [b]	Sample [c]	Alkyl C	O-Alkyl C			Aromatic C		Carboxyl/ Carbonyl C	HB/HI
				CH$_3$O/CHN	O-Alkyl C	Di-O-Alkyl C	Aryl C	O-Aryl C	Carboxyl C Carbonyl C	
[115]	CM [e]	HA FS	28.41	21.29	15.74	8.58	16.62	4.97	4.39	1.00
		HA 60 d	25.55	19.45	14.28	7.53	18.42	7.54	7.23	1.06
		HA 120 d	27.12	22.82	12.40	6.55	19.36	6.87	4.88	1.14
	CM/SCB [e] (1:1 *w:w*)	HA FS	23.79	19.9	22.56	10.99	13.17	5.31	4.29	0.73
		HA 60 d	25.40	21.36	17.52	8.94	16.23	5.11	5.43	0.87
		HA 120 d	25.01	22.37	18.20	9.68	16.37	5.39	4.98	0.88
	CM/SC [e] (1:1 *w:w*)	HA FS	42.22	18.76	12.57	5.4	10.39	4.16	6.49	1.31
		HA 60 d	27.00	21.34	17.93	8.73	16.12	4.34	4.54	0.90
		HA 120 d	21.83	21.64	17.22	7.90	18.57	6.83	6.01	0.90
	CM/SCB/SC [e] (1:1:1 *w:w*)	HA FS	37.27	18.56	12.66	6.67	14.25	4.88	5.71	1.29
		HA 60 d	21.24	22.68	21.27	9.80	16.12	4.36	4.52	0.71
		HA 120 d	26.23	22.15	17.25	8.71	16.95	4.51	4.19	0.91
[121]	CM/plant residues (1:5 *v:v*)	HS 45 d [g]	23.20		42.90		23.90		10.00	0.89
[105]	CM/plant residues (1:5 *v:v*)	HA 90 d	26.3		26.0	6.0	30.6		11.2	
		SF1	26.9		41.8	5.4	18.4		7.5	
		SF2	25.1		38.9	5.8	21.1		9.0	
		SF3	28.0		47.9	4.3	13.0		6.9	
		SF4	31.3		52.0	4.2	9.0		3.5	
		SF5	36.2		49.6	2.2	5.2		6.8	
		SF6	35.8		48.6	3.7	6.8		5.2	
[122]	CM/pressmud/trash/bagasse [f] (1:7:1:1 *w:w*)	HA FS	17.0		71.7		1.3		10.0	
		HA 20 d	34.1		47.3		7.1		11.3	
		HA 40 d	14.8		50.4		21.6		12.8	
		HA 60 d	26.9		60.1		6.1		6.6	

[a] References are reported in reverse chronological order. [b] CM = cattle manure; PMS = paper mill sludge; SC = sunflower cake; SCB = sugar cane bagasse; SD = sawdust; TWC = tannery waste as chips; TWS = tannery waste as sludge. [c] d = days; FS = feedstock; HA = humic acid; HAw = humic fraction obtained by extraction in water and successive acidification of the supernatant; HR = humified solid residue after alkaline extraction of HS; HS = humic substance; SF = size fraction. [d] Precomposting for 30 days. [e] Precomposting for 60 days. [f] Precomposting until the thermophilic phase. [g] Vermicomposting time was 45 days according to a private communication. [h] Here the hydrophobic index is defined as:

$$\frac{HB}{HI} = \frac{Area\ (0-45\ ppm) + \frac{Area\ (45-60\ ppm)}{2} + Area\ (110-160\ ppm)}{\frac{Area\ (45-60\ ppm)}{2} + Area\ (60-110\ ppm) + Area\ (160-190\ ppm)}.$$

The data on HAs extracted from vermicomposts produced by progressively replacing CM with residues of flock cotton production [108] and on HAs extracted from vermicomposts produced from a variety of feedstocks, i.e., CM, CM and sugar cane bagasse, CM and sunflower cake, and CM and a mixture of sugar cane bagasse and sunflower cake [110,115], show that HAs are characterized by larger fractions of aryl C and smaller fractions of O-alkyl and di-O-alkyl C, compared to the original vermicomposts (Tables 4 and 5). These trends are reflected in a larger *HB/HI* index exhibited by HAs. Figure 5 compares the spectra of vermicomposts with those of the corresponding Has, exemplifying the described trends.

As noticed for vermicomposts, the spectral intensities of HAs remarkably depend on the feedstock and on the maturation stage of the vermicompost. Even when the feedstock is simply constituted by CM [108,112–115,119], HAs composition shows some variability, as highlighted by the spread of the relative areas shown in Figure 4.

HAs extracted from mature vermicomposts derived from CM and sugar industry waste [113,115] showed relatively larger areas over the region including CH_3O/CHN, O-alkyl, and di-O-alkyl C compared to HAs extracted from mature CM vermicomposts. The relative areas were equal to 50% in the former case and to 40% in the latter. The larger values are due to the larger amount of carbohydrates in HAs, thus reflecting the composition of the feedstock. The difference in composition of the two HAs results in a slightly lower *HB/HI* index exhibited by the HAs originating from CM and sugar industry waste. Analogously, the progressive replacement of CM with lignocellulosic residues of flock cotton production [108] resulted in an increase in the integral intensity in the O-alkyl and di-O-alkyl C regions, where cellulose signals are observed, and in an increase in the O-aryl C region due to lignin, reflected in the lowering of the *HB/HI* index.

As expected, the dependence of the ^{13}C CP-MAS NMR spectra of extracts on feedstock is generally less pronounced for extracts obtained from mature vermicomposts. This was clearly shown by Aguiar et al. [115], who studied HAs extracted from vermicomposts produced from different feedstocks at three different maturation stages. The integral intensities over the different spectral regions for HAs extracted from the feedstocks and the vermicomposts after 120 days of maturation (also reported in Table 5) are shown in Figure 6. The integral intensities are spread over larger intervals for HAs extracted from feedstocks compared to those extracted from vermicomposts, in particular for the alkyl, O-alkyl, and aromatic C regions. Significant changes of the integral intensity over these regions were also observed in HAs extracted from vermicompost produced with CM and plant residues [105,121] at two different maturation stages (45 and 90 days), and in HAs extracted from vermicomposts produced with tomato plant debris and paper mill sludge [117] at the beginning and at the end of the process (180 days). The reported data suggest that vermicomposting leads to a general standardization of the Has' composition, although some specificities remain inherent to the composition of the feedstock.

In an interesting study, García et al. [114] compared the composition of HAs with that of the vermicompost fraction obtained using only water as extraction medium and of the solid residue after alkaline extraction. The authors found that aromatic structures were more abundant in HAs (20.18%) than in the aqueous extracts (11.82%) and in the solid residue (8.42%). The aqueous extracts showed the largest presence of carbonylic groups and were found to be the most hydrophobic ones according to the *HB/HI* index values.

Sen et al. [122] studied the composition of HAs extracted from a vermicompost made with CM, bagasse, pressmud and trash at different maturation stages up to 60 days after the thermophilic phase. In this case, monitoring the areas of the spectral regions allowed extensive mineralization in the final stages of the process to be revealed, as indicated by an initial increase in the intensities of the aromatic and carboxyl C regions followed by a decrease after about 40 days.
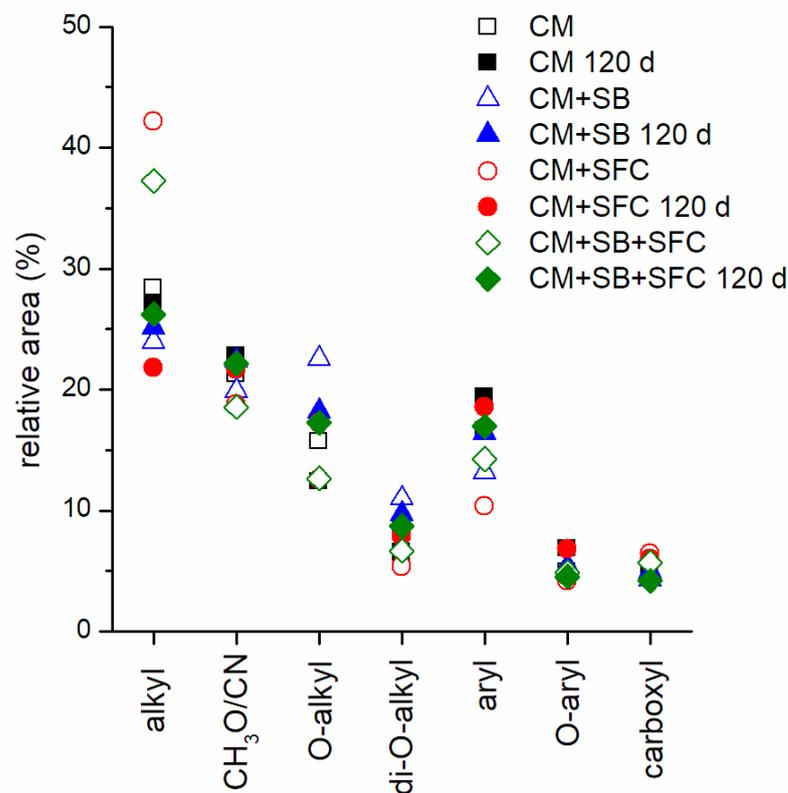
Figure 6. Relative areas of the ^{13}C NMR spectral regions recorded for HAs extracted from feedstocks (CM, CM+sugar cane bagasse (SB), CM+sunflower cake (SFC), and CM+SB+SFC) and vermicomposts matured for 120 days (Data taken from Ref. [115]).

As an isolated case, Scaglia et al. [116] did not detect any significant change in the composition of HAs from vermicomposts produced with CM, saw dust, and tannery waste over a 135 days vermicomposting time. The absence of significant changes seems rather extraordinary, considering that the feedstock is mainly constituted of CM, for which changes were observed by other authors [115], and that some degradation does occur, as revealed by a decrease in the total organic carbon in the vermicompost at different composting times.

HA fractions characterized by different molecular dimensions were investigated by Canellas at al. [105]. Size-exclusion chromatography with an aqueous solution as the mobile phase was used and the composition of the different size-fractions (SFs) was investigated. The authors found that the larger molecular size fractions, SF1 and SF2, contained a higher relative amount of aryl C and a lower relative amount of carbohydrate C compared to the smaller molecular size fractions SF3-SF6. This suggests that aryl carbon atoms constitute hydrophobic humic components that self-associate in water forming large supramolecular aggregates, while carbohydrates are hydrophilic components that are more easily solvated and occur in smaller-sized associations.

5. ^{13}C Solid-State NMR Applications to Anaerobic Digestion

^{13}C CP-MAS NMR experiments have been used for monitoring the transformation of OM in anaerobic digestion (AD) of different feedstocks with the aim of evaluating digestate stability in view of its possible applications as soil amendment or fertilizer. Amendment properties are related to the presence in digestate of organic fractions that can contribute to soil organic matter turnover, as this process influences biological, chemical, and physical soil characteristics. On the other hand, fertilizer properties come from the content of nutrient elements in the digestate organic matter and their chemical forms.

During AD, starting organic matrices are transformed, with a high degradation of polysaccharide components and preservation of recalcitrant molecules (lignin-like molecules and complex lipids and steroids), which are concentrated. These molecules were found to be precursors of humus in the soil [123]; therefore, macromolecular composition of OM in digestate determined by ^{13}C CP-MAS NMR spectra is particularly useful for evaluating amendment properties. The relative integral intensities over the spectral regions defined in Table 1, reported in the reviewed studies, are listed in Table 6, while values of the *A/OA* and *ARM* indices are given in Table 7. These data are used in the following to describe the conversion of OM during AD and OM composition in digestate in relation to application properties.

Gómez et al. [124] studied the AD of CM and poultry manure (PM), also containing straw as residual bedding material, to obtain digestate to be used as a soil amendment. AD was performed as a solid-state process in a pilot plant (3 L) under mesophilic conditions (34 °C) in batch operation mode. The digestion process was concluded when the daily gas production was negligible (45 days for CM and 35 days for PM). For both feedstocks, digestion removed a considerable part of the polysaccharides, and an increase in the relative content of aromatic and carboxylic, amidic and ester structures was observed (Figure 7), the aromatic structures mostly arising from lignin. Stability was also evaluated by investigating the thermal behavior of feedstocks and digestates under inert atmosphere. A correlation could be drawn between the higher aromaticity degree (see *ARM* values in Table 7) and the lower degree of volatilization in pyrolysis, indicating a higher stability of digestate.
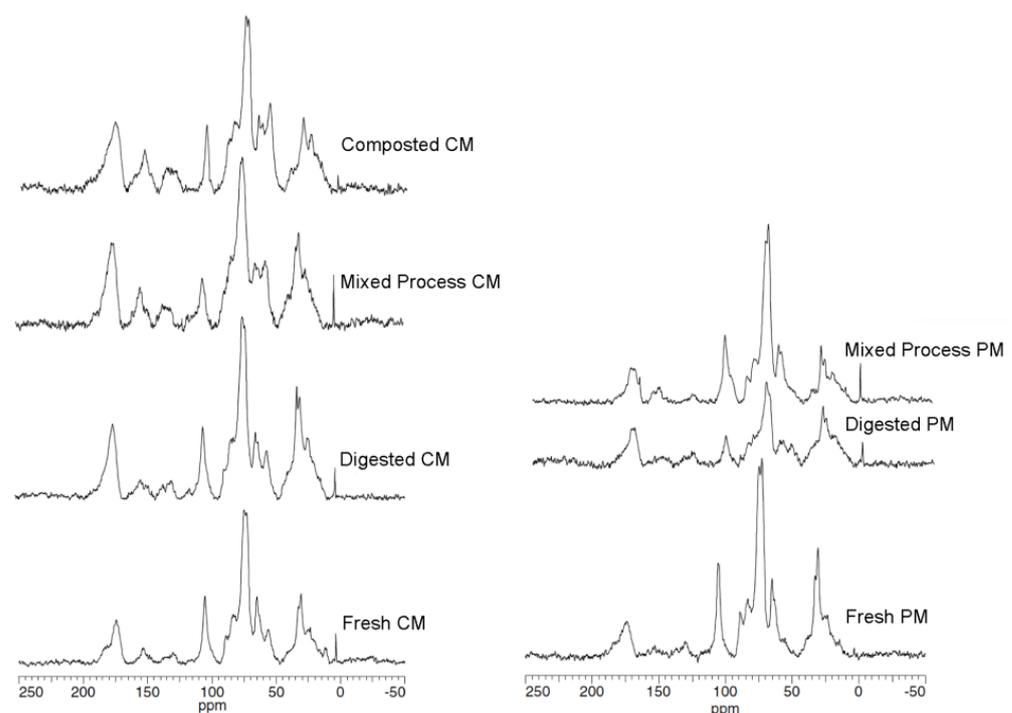

Figure 7. ^{13}C CP-MAS NMR spectra of CM (**left**) and PM (**right**) before and after anaerobic digestion, composting, and a mixed aerobic–anaerobic process. Adapted from Ref. [124].

Tambone et al. [125–127] studied the OM composition of different biomasses (mixtures of energetic crops, cow slurry, agro-industrial waste and organic fraction of municipal solid waste; mixtures of pig slurry, energy crops and agro-industrial residues; mixtures of sorghum silage, beef cattle slurry and agro-industrial residues; mixtures of sorghum silage, olive mill wastewater, olive residues and agro-industrial residues; pig slurry) and of the corresponding digestates obtained by AD under mesophilic conditions in full-scale biogas production plants located in Italy (Lombardia [125,126], Emilia Romagna [127], and Umbria [127] regions). The aim of their work was to investigate the quality and fertilizer

value of digestates in view of their use in agriculture. The analysis of ^{13}C CP-MAS NMR spectra indicated that anaerobic digestion proceeds through preferential degradation of carbohydrates, such as cellulose and hemicellulose, and, as a consequence, concentration of more recalcitrant aliphatic molecules (non-hydrolysable lipids and steroid-like molecules) occurs. The modifications of OM occurring during AD were found to determine a higher degree of biological stability of digestate, measured for instance through the cumulative oxygen uptake rate and anaerobic potential biogas production [125], with respect to the starting mixture.

Laera et al. [128] applied ^{13}C CP-MAS NMR to study organic matter in feedstock (mixture of household waste (50%), slaughterhouse waste (25%) and industrial waste (25%)) and digestate from a full-scale AD plant in Sweden, as well as in fractions of feedstock and digestate obtained by sequential extraction (DOM; extractable soluble from particulate organic matter, SPOM; readily extractable organic matter, REOM; slowly extractable organic matter, SEOM; poorly extractable organic matter, PEOM). The study intended to evaluate OM composition and trace element bioaccessibility in the different fractions of feedstock and digestate. A higher contribution of aliphatic, aromatic and carbonyl C resonances, arising from lipid and/or protein-like structures, was observed in the spectra of digestate solid residues compared to feedstock solid residues, while carbohydrate signals had higher intensities in the feedstock spectra. NMR analysis of solid pellets after each step of the sequential extraction showed a similar distribution of C among different organic groups in both feedstock and digestate samples. Only a slight reduction in carbohydrates was observed in SEOM solid residue in the feedstock. On the other hand, a reduction in carbohydrates was found in solid residues of feedstock and digestate after PEOM extraction. As far as trace elements are concerned, a much lower bioaccessibility was found for all elements in digestates with respect to feedstocks.

Pigoli et al. [129] performed thermophilic high-solid anaerobic digestion (THSAD) of a mixture of municipal sewage sludge (85%), sewage sludge from the agro-food industry (5%) and liquid fraction of food waste from separate collection (10%) in a plant located in Lombardia region (Italy) to obtain fertilizers, besides biogas for electrical and thermal energy production. The process was monitored for 42 months and three digestate samples were collected at almost one year intervals and investigated by ^{13}C CP-MAS NMR experiments. A quite uniform composition of digestate in time was found, with spectra dominated by signals from polysaccharides and proteins (54.8 ± 10% of total area), followed by hydrocarbons (29.2 ± 6.7%), and low amounts of aromatic carbons (7.6 ± 2.5%) from lignin and lignin-derived molecules, and carbonyl carbons (8.4 ± 2.9%) from amino acid moieties and lipids. When compared to digestates of different origin, the spectroscopic characteristics of the investigated digestates resembled those originating from animal manure [126,127], because of the similar presence of fecal material and vegetal matter. On the contrary, they showed a lower content of aliphatic structures than digestates from the organic fraction of MSW [125,126], due to the lower content of vegetable waste and oil/fat from food in the feedstock. Interestingly, ^{13}C CP-MAS NMR spectra of the investigated digestates resembled those of composts [126], apart from the lower content of aromatic C and higher content of alkyl C fractions due to biomass origin (fecal origin material in AD vs. lignocellulosic material in composting). Considering the total organic carbon content and biological stability, determined through substrate oxygen rate uptake and potential biogas production tests, NMR data also allowed to state that thermophilic digestate from sewage sludge is a valuable product to be used as amendment in soil.

Stability is also important, particularly when digestate has to be disposed in landfills. In fact, anaerobic digestion of waste, especially MSW, is used as pretreatment before landfilling to reduce odorous emissions. In this case, parameters are needed that describe the stability of the treatment product with respect to further biodegradation in the landfill. Since the chemical composition is a major controlling factor for the biodegradability and stability of pretreated waste, ^{13}C CP-MAS NMR spectroscopy is particularly useful in this context. Pichler et al. [130] applied this technique, in combination with chemolytic

analyses and determination of the respiration rate, to investigate the stabilization of MSW in composting and AD pretreatments used before landfilling. High correlations were found between carbohydrate content, integral of the O-alkyl C region in ^{13}C NMR spectra and respiration rate, indicating that MSW stabilization is mainly due to carbohydrate degradation during pretreatments and that carbohydrates are the most important components of MSW as far as emission potential in landfills is concerned. Proteins, aromatic C, and the *A/OA* ratio were found to be correlated with the respiration rate, but only exhibited a low correlation coefficient. A detailed description of OM changes upon different aerobic, anaerobic, and combined processes was reported, following the trends of integral intensities of different functional groups. The concentration of plastics in the final products was also highlighted by the trend of the intensities of specific signals in the ^{13}C CP-MAS spectra.

A dependence of digestate composition and stability on the composition of the corresponding waste was reported by several authors [124,125,127]. Feedstocks with different compositions were considered in Ref. [127] in order to verify possible chemical differences among the corresponding digestates and finally ascertain if AD leads to stabilized final products regardless of the initial composition of biomass. In particular, very different trends of variations of C types were observed using pig slurry as ingestate with respect to feedstocks made of mixtures of energetic crops, agro-industrial residues and beef cattle slurry. Alkyl C content was found to decrease in the pig slurry digestion, whereas it increased in all the other cases. Moreover, aromatic C slightly decreased for pig slurry but increased for the other feedstocks. Digestate from pig slurry showed a distinct aliphatic nature related to the much higher content of aliphatic structures in pig slurry with respect to other organic matrices. In conclusion, this study highlighted that alkyl C and O-alkyl C contents in digestates are correlated with those in feedstocks. Feedstock degradation resulted in a reduction in O-alkyl C and an increase in alkyl C, whereas the increase in aromatic C observed for some digestates was not statistically significant. In all cases, concentration of more recalcitrant aliphatic molecules occurred during AD, regardless of feedstock composition.

Corno et al. [131] reported the feedstock and digestate compositions derived from ^{13}C CP-MAS NMR spectra analysis in a study where the performance of giant cane as a new energy crop in biogas production was compared to that of corn, taken as an example of a traditional energy crop. Digestate was obtained in a continuously stirred lab scale reactor after mixing crops with pig slurry. As shown in Table 6, a decreasing intensity in the spectral region of O-alkyl C was found for both giant cane and corn mixtures upon digestion, indicating that AD mainly proceeds by degradation of carbohydrate-like molecules; correspondingly, a relative increase in intensity was observed for the alkyl C region where carbons from recalcitrant aliphatic structures resonate. The degradation of carbohydrates was more marked for corn compared to giant cane mixtures because the former contained more easily degradable starch while the latter contained fibers mainly made of cellulose.

Shakeri Yekta et al. [132] investigated the molecular properties of particulate organic matter (POM) in feedstock and digestate samples of seven full-scale AD plants present in Sweden for the production of biogas (three agricultural waste and four food waste digesters). Six different substrates were used and ^{13}C CP-MAS NMR spectra, analyzed by principal component analysis (PCA), revealed in most cases an enrichment of protein structures with respect to carbohydrates, implying a preferential degradation of the carbohydrates over proteins and/or increase in microbial biomass upon AD of agricultural and food wastes. Moreover, the composition of digestate was found to be mainly determined by that of the feedstock.

A study was carried out by Fernandez-Bayo et al. [133] to compare the impact of initial feedstock properties on the stabilization rate by AD and composting and soil amendment characteristics of the final products. Green and food wastes were considered as the main components of municipal organic waste. ^{13}C CP-MAS NMR spectroscopy demonstrated that both treatments resulted in a reduction in aliphatic chain content, while an opposite

trend was observed for aromatic moieties. In fact, the aromaticity increased for all treated samples and was higher for the composted materials (Table 7). The composted samples always showed a greater change than samples produced by AD. The carboxyl groups were reduced in all the samples, except for the samples with a C/N ratio of 34 from AD. Moreover, data suggested that for the same feedstock composting gave more stable and humified products than AD.

The evolution of insoluble organic matter upon AD of sewage sludge and swine manure, pretreated with a low temperature hydrothermal (HT) treatment at 125 or 225 °C, was investigated by Fang et al. [134] using ^{13}C CP-MAS NMR experiments in a study aimed at investigating the effect of the HT treatment on the efficiency of AD in producing biogas. The HT pretreatment enhanced the release of organics in sludge and improved the degradation of organics in manure during the AD process. After both HT and AD treatments of sludge samples, more O-alkyl C was converted into carboxyl, carbonyl, and soluble organics. For manure samples, the combined processes converted soluble organics and O-alkyl C partly into carboxyl and carbonyl and partly into alkyl C (Table 6). HT pretreatment was found to enhance biogas production for sewage sludge, while a minimal effect of HT on gas production was observed for swine manure.

The ability of AD to transform organic matter was also compared to those of aerobic (composting) and mixed aerobic–anaerobic processes. Gomez et al. [124] applied composting in two stages and a mixed process in three phases (aerobic hydrolysis, gas production under mesophilic anaerobic conditions at 30 °C, aerobic maturation) to CM and PM and compared the OM composition of the products to those of the corresponding digestates (see Figure 7). For CM, compost and the product of mixed aerobic–anaerobic treatment showed a higher relative content of aromatic and carboxyl functional groups with respect to digestate, with a corresponding lower content of alkyl groups, indicating a lower degradation of lignin and the generation of carboxyl groups in oxidative degradation. For PM, the mixed process gave only a slight increment in the aromatic and carboxyl/carbonyl structures, yielding a low value of the aromaticity index when compared to the stabilized samples obtained by the other processes.

Differences and similarities of OM composition among digestates, composts and digested slurries, taken as references for organic amendments and fertilizers, respectively, were also investigated by Tambone et al. [126]. Digested slurries and digestates showed similar composition, whereas higher O-alkyl C and aromatic C contents and lower alkyl C content were found in composts with respect to the other organic matrices studied (Table 6). These results were ascribed to both the different biological process (aerobic vs. anaerobic) that compost has undergone and the use of lignocellulosic materials in the starting mixture as bulking agents, which determine higher lignin-like and O-alkyl C concentrations in composts. This study also demonstrated that macromolecular composition of OM, as determined by ^{13}C CP-MAS NMR spectra analysis, is not a suitable approach to determine recalcitrance/biological stability of OM in digestate by comparison to compost. In fact, digestates with macromolecular compositions different from that of composts showed high biological stability, similar to that of composts, as measured by the anaerobic potential biogas production. Digestates were also compared to composts and products of mixed aerobic–anaerobic processes in Ref. [130]. However, a direct comparison of OM composition for the products of the different processes was not possible because of considerable differences among the initial compositions of substrates.

Table 6. Relative areas (% of total area) of the different regions of ^{13}C CP-MAS NMR spectra for digestate samples and corresponding feedstocks reported in the literature.

Ref. [a]	Feedstock [b]	Sample [c]	Alkyl C	O-Alkyl C	Aromatic C	Carboxyl/Carbonyl C
[129]	Sewage sludge	D	29.2 ± 6.7	54.8 ± 10	7.63 ± 2.5	8.37 ± 2.9
[134]	Sludge	FS	44.4	28.9	0.5	26.1
		D	38.7	19.0	4.5	37.7
	Sludge hydrochar 125 °C	FS	41.8	18.0	0.4	39.8
		D	38.7	10.0	3.9	47.4
	Sludge hydrochar 225 °C	FS	43.8	10.1	2.9	43.2
		D	44.5	21.1	6.5	27.8
	SM	FS	33.1	45.4	4.3	17.0
		D	41.1	29.9	2.4	26.6
	SM hydrochar 125 °C	FS	32.7	44.6	4.9	17.5
		D	39.8	32.4	6.8	20.9
	SM hydrochar 225 °C	FS	58.4	16.6	8.2	16.8
		D	45.7	14.1	17.4	22.8
[128]	Organic fraction of household W/SW/industrial W (50:25:25)	FS	25	60	8	7
		D	33	42	13	9
[132]	Starch/cereals/forage/corn silage	FS	8–16	70–75	5–13	4–7
		D	11–15	66–68	13–16	5–6
		Post-D	14–15	64–65	14–15	6–7
	Cereals/sugar beets/crop/corn silage	FS	8	81	9	2
		D	17–23	55–62	15	7–8
		Post-D	19–24	52–57	17	7–8
	Pig slurry/ChM/HM/deep litter/cereals	FS	16–27	55–65	9–12	7–8
		D	19–20	57–58	15–16	7
	MFW/SW/ food industry W	FS	22	63	8	7
		D	38	39	11	12
		Post-D	37	38	13	12
	MFW/vegetables/food package/SW/food industry W/SM/GSS	FS	38–39	42–45	8–9	10
		D	30–35	47–49	11–13	8–9
		Post-D	29–33	47–50	11–13	8–10
	MFW	FS	19–22	61–64	9–10	7–8
		D	34–37	38–42	12–14	11–12
		Post-D	35–38	37–40	13–14	11–12
	MFW	FS	16–18	64–69	10–11	6–7
		D	21–22	52–54	17–18	7–8
[133]	Green waste/food waste	FS	21.19	63.6	7.49	7.72
		C	17.48	65.8	13.63	3.09
		D	16.74	63.06	14.27	5.93
		FS	15.5	67.44	11.43	5.63
		C	14.88	70.37	12.14	2.61
		D	12.19	67.79	14.49	5.53
		FS	12.35	69.12	14.05	4.48
		C	10.27	71.11	14.97	3.65
		D	12.24	65.17	15.99	6.59

Table 6. *Cont.*

Ref. [a]	Feedstock [b]	Sample [c]	Alkyl C	O-Alkyl C	Aromatic C	Carboxyl/Carbonyl C
[131]	Corn/pig slurry (36.3:63.7)	FS	12.4	75.6	6.9	5.1
		D	22.3	58.5	10.3	8.9
	Giant cane/pig slurry (32.8:67.2)	FS	9.7	78.4	7.9	4.0
		D	11.7	74.6	9.6	4.1
[127]	Sorghum silage/OMW/organic R/agro-industrial R (7:1:1:1)	FS	16.2	68.5	9.1	6.2
		D	21.2	57.3	12.7	8.8
	Sorghum silage/beef cattle slurry/agro-industrial R (7:1:2)	FS	18.4	65.6	8.2	7.8
		D	21.3	60.4	10.9	7.4
	Pig slurry	FS	38.5	44.4	7.0	10.1
		D	29.5	38.5	6.9	25.1
		DSF	16.2	71.1	7.4	5.3
[126]	OFMSW/pig slurry (80:20)	FS	25.9	59.1	5.1	9.8
		D	44.5	35.4	7.9	12.2
	Pig slurry/milk serum/cow slurry/maize silage/rice R (48:24:10:4)	FS	18.6	66.9	7.0	7.5
		D	26.8	57.4	8.0	7.8
	Pig slurry/blood industry residues/maize silage (65:20:15)	FS	19.8	65.8	8.0	6.3
		D	32.5	51.2	8.1	8.1
	Slurries from municipal wastewater	D	37.3	43.8	6.9	12.1
	Ligno-cellulosic residues	C	25.0	53.4	12.8	8.8
	Ligno-cellulosic residues/OFMSW (33:67)	C	20.1	56.5	14.4	9.0
[125]	Energetic crops/cow slurry/agro-industrial waste/OFMSW	FS	21.77	65.5	4.81	7.92
		D	39.08	42.01	9.07	9.84
		Post-D	43.76	36.75	8.17	11.32
[9]	Green waste/pine bark/DMSS (1:1:1 *v:v*)	C 4 d	22.4	54.8	14.5	8.3
		C 18 d	19.0	58.7	14.1	8.3
		C 31 d	22.2	56.6	13.3	7.8
		C 40 d	19.8	57.9	14.3	8.1
		C 57 d	21.0	54.9	15.4	8.7
		C 67 d	19.7	57.6	14.3	8.4
		C 84 d	19.4	55.8	16.0	8.7
		C 101 d	18.5	56.9	15.8	8.9
		C 114 d	17.9	56.5	16.4	9.3
		C 128 d	19.6	57.1	14.8	8.5
		C 146 d	18.5	57.4	16.3	7.8
[124]	CM (+straw)	FS	20.3	61.1	5.6	11.1
		D	24.7	52.6	7.2	14.4
		C	18.0	53.6	10.0	16.8
		Mixed	20.4	51.8	10.2	15.7
	PM (+straw)	FS	21.9	62.0	4.8	8.6
		D	28.2	45.8	7.6	14.1
		Mixed	17.7	65.6	5.2	10.4

Table 6. *Cont.*

Ref. [a]	Feedstock [b]	Sample [c]	Alkyl C	O-Alkyl C	Aromatic C	Carboxyl/Carbonyl C
[130]	Municipal solid waste	FS	50	34	7	9
		D	51	26	13	10
		FS	30	56	11	3
		C	39	36	20	5
		FS	23	59	13	5
		C	32	43	20	5
		FS	23	62	10	4
		C	36	34	24	6
		FS	23	62	10	4
		C	28	41	24	7
		FS	23	62	10	4
		C	32	37	25	7
		FS	30	55	12	3
		Alt	46	35	15	5
		FS	19	53	20	8
		Alt	26	45	22	7
		FS	23	62	10	5
		Mixed	34	38	24	5

[a] References are reported in reverse chronological order. [b] ChM = chicken manure; CM = cow manure; DMSS: digested sewage sludge; GSS = grease separator sludge; HM = horse manure; MFW = municipal food waste; OFMSW = organic fraction of municipal solid waste; OMW = olive mill wastewater; PM= poultry manure; R = residues; SM = swine manure; SW = slaughterhouse waste; W = waste. [c] Alt =product of alternate aerobic/anaerobic processes; C = compost; d = days; D = digestate; DSF = digestate solid fraction; FS = feedstock; Mixed = product of mixed aerobic/anaerobic processes; Post-D = product of post-digestion.

Table 7. *A/OA* and *ARM* indices determined from ^{13}C CP-MAS NMR spectra for digestate samples and corresponding feedstocks reported in the literature.

Ref. [a]	Feedstock [b]	Sample [c]	A/OA	ARM
[132]	Starch/cereals/forage/corn silage	FS	0.1–0.3	
		D	0.2–0.3	
		Post-D	0.3	
	Cereals/sugar beets/crop/corn silage	FS	0.1	
		D	0.3–0.5	
		Post-D	0.4–0.6	
	Pig slurry/ChM/HM/deep litter/cereals	FS	0.3–0.6	
		D	0.4	
	MFW/SW/food industry W	FS	0.4	
		D	1.1	
		Post-D	1.1	
	MFW/vegetables/food package/SW/food industry W/SM/GSS	FS	0.9–1.1	
		D	0.7–0.9	
		Post-D	0.7–0.8	
	Municipal food waste	FS	0.4	
		D	0.9–1.1	
		Post-D	1.1–1.2	
	Municipal food waste	FS	0.3	
		D	0.5	
[133]	Green waste/food waste	FS		8.12
		C		14.07
		D		15.17
		FS		12.11
		C		12.46
		D		15.34
		FS		14.71
		C		15.54
		D		17.12
[9]	Green waste/pine bark/DMSS (1:1:1 *v:v*)	C 4 d		15.8
		C 18 d		15.4
		C 31 d		14.5
		C 40 d		15.5
		C 57 d		16.9
		C 67 d		15.6
		C 84 d		17.6
		C 101 d		17.3
		C 114 d		18.1
		C 128 d		16.2
		C 146 d		17.6
[124]	CM (+straw)	FS		6.2
		D		9.5
		C		13.0
		Mixed		15.2
	PM (+straw)	FS		6.3
		D		13.8
		Mixed		6.9
[130]	Municipal solid waste	FS	1.45	
		D	1.93	
		FS	0.53	
		C	1.08	
		FS	0.39	
		C	0.75	
		FS	0.37	
		C	1.05	
		FS	0.37	
		C	0.68	
		FS	0.37	
		C	0.85	
		FS	0.55	
		Alt	1.53	
		FS	0.37	
		Alt	0.57	
		FS	0.36	
		Mixed	0.89	

[a,b,c] See Table 6.

In some cases, digestate was subjected to post-treatments to improve its properties as soil amendment and fertilizer. Compost of digestate solid fraction (DSF) is sufficiently stable for nuisance-free storage and handling and mature enough to be safely used in agriculture. However, the beneficial effects of digestate composting on OM stabilization are still under debate. To better understand this point, ^{13}C CP-MAS NMR spectroscopy was applied to

investigate the composition of DSF separated from digestates by a screw press separator and their composts [135]. Digestates were obtained from mixtures of pig slurry, energy crops, and agro-industrial residues in different ratios, while the composting process was applied to mixtures of DSF and lignocellulosic bulking agent in different proportions. It was found that AD itself was able to transform OM to obtain digestate with high biological stability. As a consequence, composting led to only a slight modification of digestate DSF, except for ammonia content, which significantly decreased because of its volatilization. ^{13}C CP-MAS NMR spectra of composts were dominated by signals from carbohydrate molecules and also showed signals from lignin. These components had higher concentration than in non-composted digestates, indicating that lignocellulosic materials used as bulking agents were only partially degraded and strongly affected compost composition.

In a study by Albrecht et al. [9], the transformation of OM during composting (from 4 to 146 days) of digested municipal sewage sludge mixed with green waste and pine bark was investigated. A detailed analysis of the ^{13}C CP-MAS NMR spectra recorded on 4 replicates of samples collected at 11 different times, including a deconvolution procedure and a statistical analysis by PCA, revealed an increase in the relative content of aromatic structures with composting duration and a concomitant decrease in alkyl structures arising from lipids, proteins and hemicellulose, correlated with an increased humification of organic matter during composting. The analysis also indicated a progressive increase in the content of crystalline cellulose with respect to hemicellulose and amorphous cellulose, the latter being preferentially degraded in the microbial process. The transformation of lignin was also observed through the increase in phenolic and carbonyl and carboxyl structures, and a preferential attack of syringyl units with respect to guaiacyl ones was highlighted. The observed structural changes were associated with an increase in organic matter stability and maturity, which renders the produced compost suitable for soil amendment. The acquired ^{13}C NMR data were employed in a subsequent work to draw correlations between functional group contents in OM and indices from Rock–Eval pyrolysis [136].

6. Conclusions and Perspectives

^{13}C SSNMR, widely used in the field of materials science, has revealed as a powerful tool for the investigation of organic natural matter of relevance in agriculture and environmental science. In fact, ^{13}C SSNMR gives access to information on the composition of complex organic materials that cannot be obtained otherwise, and which can be of great relevance for the optimization of waste recycling processes and the exploitation of waste materials that presently are directly disposed of in landfills.

^{13}C SSNMR has been applied to investigate the (vermi)composting or anaerobic digestion of materials of varied nature, from relatively homogeneous garden wastes to widely heterogenous municipal solid wastes. Most studies have shown that the compositional features of compost or digestate depend on the starting feedstock and this is important for the application of these materials, which are often intended to be used as fertilizers or amendments in agricultural soils. Nevertheless, ^{13}C SSNMR has highlighted the degradation of labile OM components (mainly carbohydrates) and the preservation of the recalcitrant ones (lipids, waxes, lignin). The relative amounts of the different components obtained from NMR data have been correlated with stability and maturity indices determined by analytical methods. In some cases, the results indicate that waste pretreatments may be required, as for example in the case of municipal solid waste where non-biodegradable plastic materials remain in the compost or digestate.

Although not conclusive alone, ^{13}C SSNMR has contributed to the understanding of structure–bioactivity relationships of composts, highlighting the role of hydrophobic and polar chemical functionalities in determining the biostimulation properties of compost of interest in agriculture and the antibacterial, anti-inflammatory and antioxidant properties of extracts of relevance for their application in pharmacology.

Most of the studies cited relied on spectral analysis based on the division of the ^{13}C MAS NMR spectra in regions related to specific functional groups. This is certainly use-

ful for understanding organic matter evolution during (vermi)composting or anaerobic digestion. However, the results of the different studies indicate that it is not always the standard decomposition pathway, in which carbohydrates are decomposed faster and recalcitrant lignin and lipids are concentrated, that is followed and no univocal indices of humification and maturity, as those typically used in soil science, can be defined. This is probably due to the variable and complex composition of waste materials, especially in the case of municipal solid waste. Nonetheless, comparison of ^{13}C MAS NMR spectra before and after the composting or digestion process yields detailed information on the transformations that have occurred, which is important for process optimization. To this end, molecular models can be of help, especially for the characterization and quality assessment of the final products and would certainly deserve more consideration and possibly further refinement. Moreover, given the complex nature of OM in waste transformation, a combined ^{13}C SSNMR investigation of the whole sample and different extracts is advisable for a better comprehension of the degradation processes and the chemical structure of the final products.

As evidenced by the growing literature in the field and by the results therein reported, ^{13}C SSNMR is an important instrument for the investigation of organic waste transformation within a circular economy model. It can be easily foreseen that research in this field will experience a significant growth given the increased public awareness of the importance of waste recycling, and that ^{13}C SSNMR will become even more important in the future in this field thanks to the rapid hardware and software advancements that NMR technology is experiencing, which will extend the information that can be obtained on complex materials such as those considered in this review.

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