

¹³C Solid-State NMR Application to Waste Composting

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¹³C solid-state Nuclear Magnetic Resonance (SSNMR) has often been applied to follow the transformation of organic matter during waste composting to produce soil amendments, as well as to assess the quality of the products and the effectiveness of the treatment.

Keywords: humic substances ; humic acids ; ¹³C 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 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][5][6][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 ^{[8][9]}. 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]}.

Given its ease of implementation and low operational costs, the most commonly used organic waste treatment is composting ^[11]. Composting is the decomposition process of organic matter (OM) performed in aerobic conditions by microorganisms ^[12], 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 OM and oxygen for their metabolism, producing heat, CO₂, H₂O, minerals and stable OM ^[13]. 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 decompose OM very fast, 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

Understanding the transformations of OM that take place during the composting process 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 use 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, CO₂ evolution

rate, seed germination rate, dissolved organic carbon, volatile organic carbon, C/N ratio, and humic acid content (see for instance [15][16][17][18][19][20][21]). 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 (NMR) spectroscopy, 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 compost. Nevertheless, it is generally agreed that a combination of analyses is advised for a thorough characterization.

Among the techniques that can give access to compositional information of 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. [22][23] 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; (v) it allows the investigation of anisotropic interactions, averaged out in solution NMR by the fast isotropic tumbling of molecules; (vi) 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 [18][24].

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

^{13}C has a natural abundance of 1.11% [25][26], 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, expressed as chemical shifts. Experimental techniques providing high-resolution ^{13}C SSNMR spectra are at present routinely available on SSNMR instruments, the most important being Magic Angle Spinning (MAS) and high-power decoupling from abundant ^1H 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 recycle 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), extremely long measurement times are required for acquiring quantitative ^{13}C DE-MAS spectra with an acceptable signal-to-noise ratio. For this reason ^1H - ^{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 ^1H nuclei during a time interval called contact time, 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 ^1H 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. However, it has been reported that in the case of compost ^{13}C CP-MAS NMR spectra recorded with a contact time of about 1 ms and a recycle delay of a few seconds show a similar intensity loss (<10%), with respect to the quantitative spectrum, for signals of all functional groups [27], allowing these conditions to be used for relative quantitation. This also generally applies in the comparison of a series of spectra of samples with similar composition. Therefore, ^{13}C CP-MAS NMR experiments with a contact time of 1 ms and a recycle delay of a few seconds are usually employed in works on OM in composts 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 OM carbons from different functional groups on the basis of the chemical shift. 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. Therefore, spectral regions, rather than individual signals, are usually 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 [28] and long chain aliphatic carbons from plant aliphatic biopolymers (suberin, cutin, waxes, fatty acids) [29] and proteins [30] between 28 and 45 ppm; (ii) O-alkyl and N-alkyl carbon signals, ascribable to lignin, carbohydrates (i.e., cellulose and hemicellulose), and proteins [31], are observed between 45 and 110 ppm; (iii) the 110–160 ppm spectral region is assigned to aromatic carbons

present, for instance, in lignin and polyphenols [32]; (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. Some resolved signals are also assigned to functional groups belonging to specific biomacromolecules present in biomass samples [33], [34].

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	CH_3 and CH_2 in short chain and simple aliphatics	Alkyl C
28-45 ppm	CH_2 and CH in long aliphatic chains	
45-60 ppm	O- CH_3 ; CH-N; aliphatic quaternary C	O-Alkyl C
60-95 ppm ^a	C2-C6 in cellulose and hemicellulose; alcohols; ethers	
95-110 ppm ^b	C1 of cellulose and hemicellulose; anomeric carbon of polysaccharides	
110-145 ppm ^c	Unsubstituted or alkyl-substituted aromatic C	Aromatic C
145-160 ppm ^d	O,N-substituted aromatics	
160-190 ppm	Carboxylic acids; esters; amides	Carboxyl/carbonyl C
190-220 ppm	Ketones; aldehydes	

^aalso referred to as O-alkyl C region; ^balso referred to as di-O-alkyl C region; ^calso referred to as aryl C region; ^dalso referred to as O-aryl C region.

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

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

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

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

^{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 older ones. MAS rotors with diameters of 4 mm, containing few tens of milligrams of sample, and MAS frequencies of 8-13 kHz are usually employed. Few cases are also reported where rotors with 7 mm diameter and spinning rates of 3.5-5 kHz were used. As previously 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) are used. In these experiments, the recycle delay ranges from 0.5 to 5 s and few thousands of 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 better spectral resolution. Considering that only 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 composts, however, instead of investigating the bulk material, focused on extracts. 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 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 [35]. Thanks to their highly aromatic structure, HAs are stable compounds and their amount is typically considered as a measure of compost maturity [36]. In fact, during composting HAs tend to increase whereas FAs tend to decrease [37]. Compost DOM is a mixture of low-molecular weight compounds, such as sugars and free amino acids, and high-molecular weight compounds, among which also HSs [38]. 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 in a hydrophobic fraction (HoDOM) and a hydrophylic fraction (HiDOM) using the Amberlite® XAD-8 or Supelite™ DAX-8 resin [38]. Finally, compost tea is an aqueous extract obtained from the fermentation of compost in water either in forced aerated or non-aerated conditions [39].

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 [40][41][42][43][44][45][46][47][48][49][50][51][52], vegetal residues [10][41][42][52][53][54][55][56][57][58], grape marc [50][59], ground coffee and fresh grass [10][57], cotton gin waste [45], rice husk, bran or straw and wheat straw [44][46][56], domestic organic wastes [55][57][60], and municipal solid wastes (MSW) [27][61][62]. 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 [46]. An example of the evolution of the ^{13}C CP-MAS spectrum of different feedstocks with composting time is shown in **Figure 1**. In most cases the reported experimental data are the integral areas of the characteristic spectral regions described. These data are catalogued in **Table 2**, indicating for each study the feedstock and the composting time.

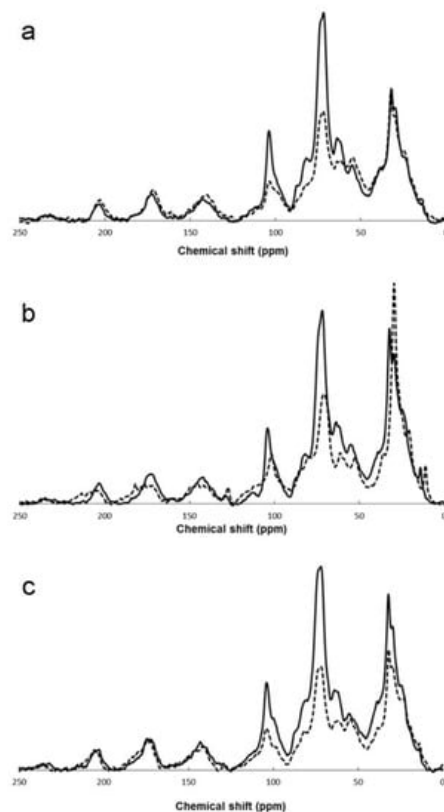


Figure 1. ^{13}C CP-MAS NMR spectra of composts (dashed line) and corresponding feedstocks (solid line). Feedstock compositions are: **(a)** exhausted grape marc and cattle manure 76:24 (w:w); **(b)** grape marc and cattle manure 72:28 (w:w); **(c)** exhausted grape marc and poultry manure 67:33 (w:w). Reproduced from Ref. [50].

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	
[41]	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
[52]	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		

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	
[58]	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
[43]	SM/ rice straw (4:1 w:w)	FS	24.6	57.9		7.7		9.8
		C 40 d	23.2	55.2		9.8		11.8
	SM/ rice straw (8:1 w:w)	FS	28.8	50.5		7.9		12.8
		C 40 d	25.6	48.9		10.6		15.0
[10]	SM/rice straw/biochar (8:1:1 w:w)	FS	24.7	40.9		24.7		9.8
		C 40 d	19.2	28.8		42.5		9.5
	COF/fresh grass/mature compost (18:80:2 w:w)	C 6 d	33.5	15.2	23.3	14.8	3.2	10
[45]	Solid pig slurry/cotton gin waste (4:3 v:v)	FS	13.5	5.9	74.9	3.7	0.9	1.1
		BT	17.5	8.2	62.7	5.3	1.8	4.5
		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
	Solid pig slurry/cotton gin waste (3:4 v:v)	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
	Solid pig slurry/cotton gin waste (3:7 v:v)	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

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	
[51]	BM/CM/maize straw/PT (70:30 w:w)	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
		+bioplastic (1 wt%)	C 108 d	17.6	11.1	47.9	12.3	4.0
		+bioplastic (2 wt%)	C 108 d	18.9	11.0	49.2	11.6	3.6
		SM/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
		CM/pumice	FS	14.6	64.8	12.4		8.3
		C 10 d	9.0	70.4		12.9		7.7
[49]	CM/pumice	C 20 d	10.	69.2		12.7		7.7
		C60 d	11.7	66.6		13.3		8.5
		ChM/pumice	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
	Exhausted grape marc/CM (76:24 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
	Grape marc/CM (72:28 w:w)	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
[50]	Exhausted grape marc/PM (67:33 w:w)	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
	CM	CC	18.2	7.6	48.8	8.9	5.0	11.6
	Broiler litter	CC	17.9	9.1	48	8.0	5.2	11.7
	Green waste	CC	22.4	10.7	40.4	8.2	6.3	12.0
	Nitro-humus	CC	19.6	8.8	43	9.6	6.7	12.4
	MSW	CC	23.0	7.6	48.7	7.3	3.6	9.8
[42]	MSW	CC	23.0	7.6	48.7	7.3	3.6	9.8

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	
[56]	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
[55]	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
[46]	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
		FS	27.2		55.6	9.2		8.0
[44]	SM/wheat straw (95:5 w:w)	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
		FS	15.8		59.5	14.9		7.9
[61]	MSW (composted in spring)	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
	MSW (composted in summer)	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

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]	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	
[62]	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	
[59]	Grape skin	FS	8.7	53.4	17.5	18.7		
		C 160 d	10.9	51.8	18.9	17.0		
	Grape seeds	FS	25.0	41.3	13.1	18.7		
		C 160 d	21.7	38.4	15.7	22.3		
	Grape skin and seeds	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 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. [42] applied the “mixing model” proposed by Nelson and Baldock [63] to several composts for determining the molecular composition. The mixing model is aimed at reproducing the experimental ¹³C 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. [44] for a compost of pig feces and wheat straw. Interestingly, the evolution of the OM composition was followed during 28 days of composting.

In most of the papers reported so far in the literature, ¹³C 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 ¹³C 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 [64], 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. [65] for wood pulp and applied to compost by Albrecht et al. [9]. A "Lignin Ratio" (*LigR*) has been defined by Pane et al. [58] 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. [24] 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. [45][66]), 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. [60] 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. [56], 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. [50] 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. [58] 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. [41] 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 [27][61][62], which generally has a large organic fraction (40%), but, due to its origin, can also show peculiar spectral features. In particular, Pichler et al. [27] 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. [51] by means of different ^{13}C SSNMR measurements. In this case the decomposition of a bio-degradable 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 ^1H longitudinal relaxation time in the rotating frame, $T_{1\rho}$, 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. [67] 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. [40] 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. [43] 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**.

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	O-Alkyl C		Aromatic C		Carboxyl/ Carbonyl C	
				$\text{CH}_3\text{O}/\text{CHN}$	O-/Di-O- Alkyl C	Aryl C	O- Aryl C	Carboxyl C	Carbonyl C
[72]	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	

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
[69]	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	
[87]	CYN/WC (70:30 w:w)	HA 100 d	22.2	13.8	27.5	20.0	5.7	10.9	
[43]	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	
[74]	CYN/corn straw (70:30 w:w)	HS 100 d	16.3	13.8	24.7	28.9	5.6	10.6	
[88]	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	
[68]	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	
[89]	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	
	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		
[83]	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	

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
[78]	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	
		CT 120 d	31.0	9.0	23.1	13.3		23.6	
[85]	OFMSW/GT/foilage R from tobacco (55:30:15)	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	
		OvM/straw	HS FS	18.6	49.7	8.2		23.5	
[70]	Mixture of animal manures	HS 120 d	17.7	25.0		22.3		35.0	
		HS FS	33.7	13.8		11.8		40.7	
	Solid olive mill wastes	HS 120 d	30.4	24.0		9.6		36.0	
		HS FS	23.2	56.4		11.2		9.2	
	Solid wastes of wineries	HS 120 d	22.2	29.3		19.7		28.8	
		HS FS	3.5	46.9		1.5		48.1	
	Domestic wastes	HS 120 d	21.6	13.1		26.8		38.5	
		HS FS	23.7	28.2		20.3		27.8	
	MSW/vegetal wastes (1:1 v:v)	HS 120 d	25.4	34.9		16.4		23.3	
		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
[61]	MSW	HA FS	32	45		13		10	
		HA 49 d	48	26		16		10	
		HA 6 d	38	31		13	5	11	2
		HA 19 d	45	23		15	5	11	1
[82]	MSW	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

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
[81]	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.

As evidenced by the data reported in the table, the chemical composition of compost extracts is strongly dependent on the feedstock. Al-Faiyz [68] applied ¹³C 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 ¹³C CP-MAS NMR spectra, particularly regarding the alkyl C and O-alkyl C spectral regions [69]. Additionally, the data by Fuentes et al. [70] 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 ¹³C CP-MAS NMR data recorded on HSs extracted from compost obtained from artichoke residues mixed with corn straw and wood chips [69] and from the same residues mixed with maize straw [71] 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. [72] 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 ¹³C 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 [73] and favoring carbon and nitrogen retention [74][75]. Liu et al. [43] 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. 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 [76]. 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 [77].

¹³C 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 ^[78] 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. ^[79] studied the transformation of HAs during the composting of MSW with the addition of agricultural wastes. The ¹³C 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. ^[70] 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 were 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. ^[80].

Contrary to the expected trend, the study by Liu et al. ^[43] 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.

González-Vila et al. ^[61] studied HAs extracted from MSW and from its compost obtained after 7 weeks. The ¹³C 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. ^[81], 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. ^[82] 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. ¹³C 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. [83] 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 [18]. The relative enrichment in aromatic C was supposed to be related to the degradation of non-aromatic cell wall compounds [84], while carboxyl and carbonyl groups probably arose from oxidative transformations of lignin [31]. The ^{13}C CP-MAS NMR spectra obtained on DOM extracted from fresh substrates and from the final composts indicated that, as observed for HSSs, 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 HSSs). With the aim of better understanding DOM composition, ^{13}C SSNMR was used on separated hydrophilic (HiDOM) and hydrophobic (HoDOM) fractions [85] and on fractions further separated on the basis of solubility in acid or basic solutions [30]. 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. [38] 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 HSSs. 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 HSSs. Verrillo et al. [71] studied the effects of HSSs 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 HSSs 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 HSSs extracted from composted artichoke and pepper wastes, and from coffee grounds [69]. 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 [86]. 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. [87].

The molecular features responsible for bioactivity were also investigated on compost tea. Verrillo et al. [88] 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 HSSs, 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. [89] 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 [90].

4. Conclusions and Perspectives

^{13}C SSNMR, widely used in the field of materials science, has revealed as a powerful tool also 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 composting of materials of varied nature, from relatively homogeneous garden wastes to widely heterogeneous municipal solid wastes. Most studies have shown that the compositional features of compost 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.

Although not conclusive alone, ^{13}C SSNMR has contributed to the understanding of structure-bioactivity relationship 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 the spectral analysis based on the division of the ^{13}C MAS NMR spectra in regions related to specific functional groups. This is certainly useful for understanding organic matter evolution during composting. However, the results of the different studies indicate that not always the standard decomposition pathway 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 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 as those considered.

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