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Analysis of Carbon and Nitrogen Forms in Soil Fractions after the Addition of ^{15}N -Compost by ^{13}C and ^{15}N Nuclear Magnetic Resonance

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A quantitative laboratory assessment of the different C and N forms in soil humus fractions was carried out by incubation of a mineral substrate after the addition of ^{15}N -labeled compost. The experimental design included (i) preparation of the ^{15}N -labeled organic matter (city refuse compost, 640 g kg⁻¹ wheat straw and K¹⁵NO₃ composted for 80 days), (ii) a further 80 day incubation of a mixture of the labeled compost with a mineral soil (32 g kg⁻¹), (iii) measurement of stable isotope ratios, and (iv) isolation and structural comparison by ^{13}C and ^{15}N cross-polarization, magic-angle spinning nuclear magnetic resonance (NMR) of different organic fractions, i.e., soluble, colloidal (humic and fulvic type), and particulate (free organic matter and humin), from both the compost and the compost-treated soil. The results showed that the amide forms dominated in all of the newly formed N compounds, but an increased amount of alkali insoluble organic fractions was observed after incubation of the soil. The analysis of the insoluble, particulate fractions shows that nonextractable amides constitute the major pool of newly formed N compounds. The particulate soil fraction isolated by flotation in CHBr₃-MeOH contained 16.8% of the total soil N and 26% of the ^{15}N . The ^{13}C NMR spectra showed that the fulvic acid-like fraction (7.6% of the soil N, 8.8% of ^{15}N) consisted almost completely of a C=O-containing carbohydrate material, whereas the humic acid-like fraction (20.3% of the total soil N, 8.6% of ^{15}N) resembled an oxidized lignoproteic fraction containing the most significant aromatic domain. The water soluble fraction was, in both soil and compost, the one with the highest isotopic abundance of ^{15}N (96%), but the ^{15}N NMR spectrum revealed minor amounts of soluble mineral N in this fraction and the remainder consisting of amide compounds.

KEYWORDS: Soluble organic matter; particulate organic matter; labeled compost; humic acid; fulvic acid; humin

INTRODUCTION

No extensive information exists on the fate of the different organic fractions of compost in soil, even though such materials are extensively used in agricultural practices focused on the enhancement of soil fertility in sustainable systems based on periodic organic inputs (1).

In particular, it is still unclear whether most compost types can be considered as a material suitable to contribute to the pool of humic-like soil stable substances or, on the contrary, whether most of the compost is consumed by soil microorganisms and released as CO₂ (2, 3). The knowledge of the evolution and stabilization in soil of external inputs of organic matter is

of prime importance in order to calculate the compost application rates and to forecast the element balance and the residual effect of the organic matter added (4). The structural assessment of the C and N forms in soil is of special interest in the case of composted materials, where it is well-established that the presence of humic substances [viz., humic acid (HA), fulvic acid (FA), and humin] cannot be defined by operative laboratory methods used in soil organic matter studies but requires the application of molecular characterization techniques (5).

Laboratory incubation experiments using ^{15}N as a tracer isotope represent a valuable strategy in the study of the transformations of N-bearing substances incorporated into the soil since they allow quantitative monitoring of exogenous N forms and also permit more rapid ^{15}N nuclear magnetic resonance (NMR) analyses than with natural abundance ^{15}N NMR (6).

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In this work, we have intended to assess the fate and the speciation of the compost C and N forms after their transformation in the soil. The experimental approach was based on two successive incubation experiments and included four steps: (i) previous ^{15}N compost labeling in the course of an 80 day composting period, (ii) a further 80 day incubation of the compost mixed with a mineral soil, (iii) wet chemical fractionation of soil and compost organic fractions, and (iv) a comparative molecular characterization of these fractions by analyzing the stable isotope ratios and the ^{13}C and ^{15}N cross-polarization, magic-angle spinning (CPMAS) NMR spectra.

EXPERIMENTAL PROCEDURES

Labeling of the Compost. The ^{15}N -labeled compost was prepared by incubating a mixture, homogenized to 2 mm, of city refuse compost (295.5 mg g^{-1} , oven dry weight, Valdemingómez landfill, Madrid), wheat straw (640 mg g^{-1}), and 60.5 mg g^{-1} K^{15}NO_3 (99% ^{15}N) for 80 days (36 wt % loss by biodegradation). These proportions were calculated to obtain a C/N starting ratio equal to 20, where just 50% of the N was as ^{15}N (25% ^{15}N from straw and 25% from urban waste). The water-holding capacity of the mixture was measured, and the moisture was adjusted to 54.44 dry weight percentage. After 80 days of incubation at 27 °C, a portion of the labeled compost was added to an organic matter-lacking soil substrate in order to study its further transformation, and another portion was freeze-dried for chemical characterization.

Soil Incubation. The original soil material used in the experiment was collected from the upper 20 cm of a Calcic Luvisol in the experimental farm "La Higuera" (CSIC, Toledo, Spain), with a pH of 7.4; CO_3^{2-} , 10 g kg^{-1} ; cation exchange capacity (pH 7), 125 mmol kg^{-1} ; sand, 780 g kg^{-1} ; silt, 80 g kg^{-1} ; and clay, 140 g kg^{-1} . The soil material used contained a negligible concentration of organic matter (<0.5%) consisting of [in g C (kg soil $^{-1}$)] free organic matter (FOM), 0.4; HA, 0.7; and FA, 1.8. The soil had a C/N ratio of 7.8 and a concentration of available nutrients in mg (kg soil $^{-1}$) of P_2O_5 , 195; K, 245; Ca, 1185; and Mg, 142.

The mixture of ^{15}N compost (1.6 g) and mineral soil (50 g) was moistened to 66% of its water-holding capacity and incubated for 80 days at 27 °C. Two parallel replications of the experiments with compost and soil (incubations, standard chemical analyses, and ^{13}C NMR) were carried out but using the same doses of K^{14}NO_3 .

Fractionation Protocols. A series of organic fractions in compost and compost-treated soil was isolated by physical and chemical procedures. An extraction with water at room temperature was carried out after 2 h of shaking and repeated twice. Further separation by flotation in 2 M H_3PO_4 (7) was performed in order to achieve the total isolation of a particulate organic matter fraction not yet incorporated into the soil organo-mineral matrix (FOM). Some yellowish, FA-like organic fraction was removed with this treatment, and the acid supernatant solution was not discarded but aggregated to the total humic extract obtained in further stages of the fractionation protocol. The soil residue was then extracted with 0.1 M $\text{Na}_4\text{P}_2\text{O}_7$ followed by repeated extractions with 0.1 M NaOH, and the extract was used to separate the HA fraction (precipitation with HCl) from the FA (soluble) fraction. Finally, the extraction residue was washed with water, dried, and homogenized to <0.25 mm. In this residue, two humin (insoluble) fractions with different degrees of association to the mineral fraction were considered. The former humin fraction considered was that slightly associated to the mineral fraction, the isolation of which by physical methods was still possible. This humin subfraction was removed by flotation in a dense liquid (CHBr_3 -MeOH mixture of $\rho = 1.8 \text{ g cm}^{-3}$) after vigorous shaking with a rotary stirrer for 5 min. Because of the low contact time and the previous treatments removing organic fractions of wide range of polarity, no optical evidence of a substantial loss of extractive fractions was observed in the supernatant liquid after centrifugation. The particulate floating fraction was analogous to the so-called soil "inherited humin" (8) and presumably consisted of particulate organic matter that was originally encapsulated in stable soil aggregates and turned out to be a free particulate fraction after the removal of organic

and inorganic cementing agents and the mechanical disruption of the soil heavy residue carried out in the present fractionation protocol. The second humin fraction was composed of the organic matter strongly associated with the mineral fraction and could not be isolated without previous drastic chemical treatments, which simultaneously degraded a portion of its organic constituent. This fraction was studied as a whole, including the inorganic soil residue. Except for the water soluble (WS) fraction, which was directly lyophilized, the other soil fractions (including the FA extract) were dialyzed in cellophane bags to remove the soluble salts added during the extraction process and lyophilized.

Analysis of Stable Isotope Ratios in Compost and Soil. The ^{15}N abundance in the whole soil and compost and their different fractions was determined with a Finnigan MAT delta S spectrometer (Finnigan, Bremen, Germany) operating on-line in a continuous flow with a CHN elemental analyzer (SCA CNRS, Vernaison, France).

Solid State CPMAS NMR Spectroscopy. The solid state ^{13}C NMR spectra were acquired at 25.1 MHz with a Bruker MSL 100 spectrometer (2.3 T) with the CPMAS. For each spectrum, 1000 free induction decays were accumulated. The pulse repetition rate was set to 5 s, and the contact time was 1 ms. The sweep width was 37.5 kHz, and the acquisition time was adjusted to 0.016 s. The MAS was performed at 4 kHz. The chemical shift range of the NMR spectra was referred to tetramethylsilane (= 0 ppm). Under these conditions, the NMR technique provides quantitative integration values in the different spectral regions (9). The whole ^{13}C spectra were divided into standard ranges: 0–50 ppm = alkyl (13 = methyl, 21 = acetate, and 30 = polymethylene); 50–110 ppm = *O*-alkyl (56 = methoxyl-to- α -amino, 73 = major carbohydrate signal, 103–105 = anomeric C in carbohydrate, and 105 = quaternary aromatic carbons in tannins); 110–160 ppm = aromatic/unsaturated; 160–220 ppm = carbonyl (172 = carboxyl/amide and 198 = ketone/aldehyde) (10–13).

On the other hand, the solid state ^{15}N CPMAS NMR spectra were obtained on a Bruker MSL-300 spectrometer operating at 7.05 T (^{15}N resonance frequency 30.4 MHz) using a probe similar to that used for the solid state ^{13}C NMR studies. The rotation frequency was set between 4 and 4.5 kHz. A contact time of 0.7 ms and a pulse delay of 4 s were used. The chemical shifts were referred to ammonium chloride (= 0 ppm).

In the case of the ^{15}N NMR spectra, up to five different regions were considered for peak area measurements corresponding, respectively, to nitro groups, nitrate (370–320), nitrile, oximes (320–200), indole, imidazole, pyrrole (200–110), amide, pyrrole, lactame (110–50), $-\text{NH}_2$, $-\text{NR}_2$ (50–20), and terminal amino groups in aliphatics, NH_2 [20–(–29)] (14).

RESULTS AND DISCUSSION

Organic Fractions Isolated from Soil and Compost. The distribution of the organic C in the different fractions of both the compost and the compost-treated soil is shown in **Table 1**. The compost contained a significant amount of WS compounds, representing 53 mg kg^{-1} of the total compost. As a whole, the colloidal fractions (HA + FA) amounted to roughly one-third of the total C. The most significant changes observed after incubation of the soil-compost mixture were the decrease (degradation, insolubilization, or both) of soluble and colloidal fractions, leading to a relative accumulation of particulate organic matter. Of the latter, only about 5.6% of the organic matter added to soil remained as low-density particulate organic fractions (FOM), whereas about 18% of the total compost-treated soil corresponded to the CHBr_3 -MeOH floating fraction.

Stable Isotope Analysis. **Table 2** indicated that a considerable ^{15}N accumulation was found in the colloidal fractions, mainly HA. In both the soil and the compost fractions, the highest ^{15}N abundance corresponds to ^{15}N in the WS fraction, but when calculated as a percentage of the total N, a substantial amount (about 40% of the total compost N) was stabilized as ^{15}N in particulate organic fractions.

Carbon and Nitrogen Forms in Soil Fractions

Table 1. Distribution of the Organic Soil and Compost C into Different Organic Fractions after 80 Days of Incubation^a

	¹⁵ N-labeled compost		soil treated with ¹⁵ N compost			
	mg C g ⁻¹	percentage of the total ¹⁵ N	mg C g ⁻¹	percentage of the total ¹⁵ N		
total compost/soil	340	100	10.2	100		
water-soluble fraction	18 ± 0.1	soluble fraction	0.2 ± 0.00	1.9 ± 0.0		
		5.3 ± 0.0				
HA	70 ± 0.8	colloidal fractions	1.5 ± 0.00	15.0 ± 0.0		
		20.6 ± 0.2				
FA	39 ± 0.3	11.5 ± 0.0	0.6 ± 0.00	5.5 ± 0.0		
FOM	ND	particulate fractions	0.6 ± 0.04	5.6 ± 0.4		
		ND				
		humin (CHBr ₃ -MeOH floating)			1.9 ± 0.02	18.6 ± 0.2
		humin (alkali insoluble residue)			213 ± 0.6	62.7 ± 0.18

^a ND refers to operationally defined soil fractions not isolated from compost samples.

Table 2. Nitrogen Isotopic Abundances and Distribution of the N Isotopes in the Different Organic Fractions from Compost and from Soil Amended with Compost

	isotopic abundance		nitrogen, mg g ⁻¹ of the different soil or compost fractions					
	(mg ¹⁵ N g ⁻¹ fraction)	¹⁵ N (atom %)	¹⁵ N	¹⁴ N	total N			
initial compost	17.7 ± 0.1	21.8 ± 4.2	3.85 ± 0.02	13.80 ± 0.07	17.65 ± 0.1			
water soluble	3.4 ± 0.4	soluble fraction	0.53 ± 0.06	0.02 ± 0.00	0.55 ± 0.06			
		95.7 ± 5.0						
HA	42.0 ± 0.0	colloidal fractions	0.73 ± 0.0	4.29 ± 0.0	5.02 ± 0.0			
		14.5 ± 2.9						
FA	19.2 ± 0.0	12.7 ± 1.1	0.27 ± 0.0	1.87 ± 0.0	2.14 ± 0.0			
humin (alkali insoluble residue)	16.5 ± 0.2	particulate fractions	2.98 ± 0.03	7.03 ± 0.09	10.01 ± 0.12			
		29.7 ± 4.4						
compost-treated soil								
water soluble	2.6 ± 0.5	soluble fraction	0.01 ± 0.00	0.00 ± 0.00	0.01 ± 0.00			
		96.4 ± 2.8						
HA	35.5 ± 0.1	colloidal fractions	0.01 ± 0.00	0.10 ± 0.00	0.11 ± 0.00			
		8.6 ± 1.0						
FA	21.5 ± 1.8	8.8 ± 1.4	0.00 ± 0.00	0.04 ± 0.00	0.04 ± 0.00			
FOM	15.6 ± 0.0	particulate fractions	0.01 ± 0.00	0.02 ± 0.00	0.02 ± 0.00			
		27.9 ± 1.0						
		CHBr ₃ -MeOH floating				0.02 ± 0.00	0.07 ± 0.00	0.09 ± 0.00
		humin (alkali insoluble residue)				ND	ND	0.26

195 The N values calculated as percentages of each stable isotope
196 in the different fractions provide an insight on the extent of the
197 microbial turnover in the soil and compost fractions. In both
198 compost and soil, more than half of the total N was in the form
199 of particulate fractions, but about a third part of the total N
200 consisted of colloidal substances (HA + FA). The stable isotope
201 distribution (**Table 2**) showed changes in the composted organic
202 matter before and after its transformation in the soil. In
203 particular, the ¹⁵N pool in the WS fraction was lower in soil
204 than in compost, suggesting rapid incorporation of N forms into
205 insoluble fractions in the soil. In fact, the tendency observed in
206 soil was toward insolubilization, i.e., the highest accumulation
207 of ¹⁵N in soil was found in insoluble fractions.

208 Both in compost as in soil, the considerable amount of ¹⁵N
209 accumulated in the HA fraction agrees with the fact that in the
210 case of organic matter from lignocellulosic wastes, this fraction
211 frequently includes most N in nonhydrolyzable forms and is
212 often considered to consist of lignoprotein material (15). A large
213 portion of the remainder ¹⁵N was stabilized in noncolloidal,
214 particulate organic fractions as it could be the case with
215 microbial biomass-derived material containing altered protein
216 stabilized in the presence of lignin or aromatic extractives and
217 chitin derived from, e.g., soil fungi.

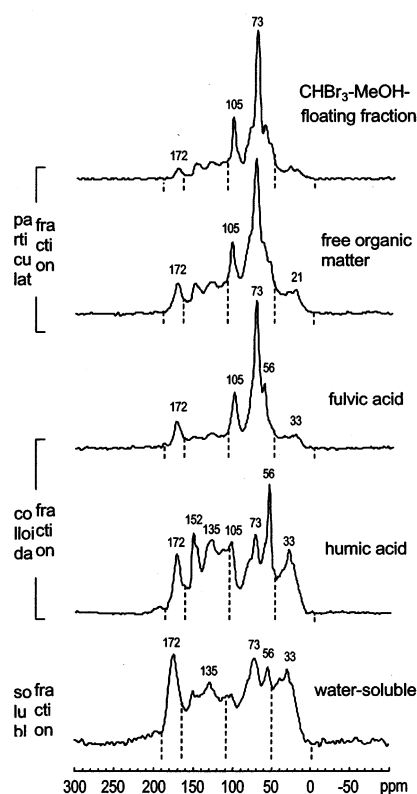
Analyses by ¹³C NMR Spectrometry. The ¹³C NMR spectra
218 of the whole compost and its different fractions are shown in
219 **Figure 1**. The spectral region with a maximum at ca. 135 ppm
220 may be attributed to C₁ and C₄ in S lignin and to C₁ in G units
221 (16). In this region, also consider that the major lignin signals
222 at ca. 153 and 145 ppm may be overlapped with those of tannins
223 (17). The alkyl region (50–0 ppm) showed a maximum at ca.
224 21 ppm, which is frequently attributed to acetate groups in
225 hemicellulose (18), but the most typical signal in this region,
226 with a maximum at ca. 30 ppm, corresponds to a large extent
227 to protein-like structures and polymethylene carbons in lipids
228 and lipid macromolecules. The signal ca. 172 ppm, in the soil
229 humic substances frequently attributed to carboxyl groups,
230 showed the highest intensity in the most oxidized soil fractions
231 (FAs and to a lesser extent HAs). However, the area of this
232 peak in the composts under study (**Table 3**) is similar in
233 colloidal and particulate fractions and has an even greater
234 intensity in the case of HAs than of FAs. These facts could be
235 interpreted that the intensity in the carbonyl region was in part
236 due to aliphatic esters, which could be the case with hemicel-
237 lulose esters (18). In addition, one may also take into account
238 that the amides also show a typical signal in this region: in
239 fact, protein-like compounds in compost produce diagnostic
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Table 3. ^{13}C NMR Peak Area Measurements^a in the Different Organic Fractions from Compost and from Soil Amended with Compost

	carbonyl		aromatic		O-alkyl				alkyl
	220–190	190–160	160–140	140–110	110–100	100–90	90–60	60–50	50–0
	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm
	keto, aldehyde	carboxyl	O,N-aromatic	H-aromatic	C ₁ in sugars, tannins	C ₂ , C ₃ , C ₅ in sugars	C ₂ –C ₆ in sugars	OCH ₃ + amino	
initial compost	0.5	6.7	6.8	13.3	8.3	6.1	39.5	7.1	11.6
water soluble	1.9	15.5	9.0	15.8	4.9	3.7	20.5	8.3	20.5
HA	0.7	7.0	11.4	21.2	8.5	4.0	22.7	10.6	13.8
FA	1.1	4.1	3.2	5.7	10.9	6.3	58.9	3.9	5.9
humins (alkali insoluble residue)	0.3	4.1	5.4	12.1	9.5	6.2	48.1	6.7	7.5
compost-treated soil									
water soluble	2.4	16.0	8.9	15.5	4.7	4.1	19.8	7.1	21.4
HA	0.9	9.3	11.5	21.6	7.2	3.5	18.7	10.0	17.3
FA	0.9	8.4	4.7	9.0	8.8	6.2	47.4	5.4	9.1
FOM	0.5	7.1	5.4	11.3	11.1	5.1	40.9	6.2	12.2
CHBr ₃ -MeOH floating	0.0	3.2	5.2	11.8	10.0	5.3	49.2	5.3	7.9

^a Percentage of the total area.**Figure 1.** ^{13}C CPMAS spectra of soluble, colloidal, and particulate organic fractions from mineral soil treated with ^{15}N -labeled compost soil after 80 days of incubation.

NMR signals in the 50–45 ppm range ($\text{C}\alpha$ in amino acids), which overlap with the typical methoxyl (56 ppm) signal but also with the alkyl (0–60 ppm region) and with the above-mentioned carbonyl region. In the case of the whole compost (Figure 1 and Table 3), the ^{13}C spectrum and peak area measurement suggest a predominantly lignocellulosic material (19, 20). It showed the prominent 73 ppm signal corresponding to carbohydrate (overlapped resonance of C₂, C₃, and C₅ of pyranoside rings in sugar constituents of celluloses and hemicelluloses). In addition, some ill-resolved resonances for the sugar ring carbon at the C₆ position (shoulder at ca. 63 ppm), for C₄ in amorphous cellulose (shoulder at 84 ppm), and for C₁ in carbohydrate (anomeric C at 105 ppm) (18) are also characteristic for polysaccharides. In the case of the signal at

ca. 103 ppm, there could be a certain contribution of nonprotonated carbon arising from tannins (17, 21).

The aromatic region (160–110 ppm) shows two fairly differentiated regions corresponding, respectively, to the region between 160 and 140 ppm, mainly produced by aromatic carbons linked to O or N, and that between 140 and 110 ppm for H-substituted and C-substituted aromatic carbons. Assuming that altered plant lignin could be the major source of aromatic carbons in the compost under study, the maximum at ca. 153 ppm could correspond to C₃ and C₅ in etherified syringil (S) structures and also to C₃ and C₄ in guaiacyl (G) units (22). The spectra also showed low-intensity carbonyl signals (220–160 ppm).

In the case of the WS fraction, the ^{13}C NMR spectra showed a very heterogeneous composition, with a large variety of C types, pointing to the presence of aromatic extractives, carbohydrate, alkyl structures, and carboxyl groups.

The spectrum of the HA suggested a substantial aromatic domain and showed relatively sharp peaks mainly in the methoxyl/ α -amino region, an intense signal at 73 and 105 ppm, and minor peaks or shoulders at ca. 64 and 84 ppm. This ^{13}C NMR profile was quite different with regards to the humin fractions; the spectra suggested the dominance of lignin and protein. In particular, the signal intensity at 105 ppm is higher than expected when compared with the other carbohydrate peaks, which may be interpreted as if the carbohydrate NMR region of the HA fractions was to some extent reflecting the presence of tannins or cinnamic acid-containing oligomer fractions, including fragments of lignin–hemicellulose complexes typical from grass lignins.

When comparing the ^{13}C NMR spectra of the compost fractions with the homologous fractions isolated from the incubated, compost-treated mineral substrate, it was observed that they were qualitatively similar. Only quantitative differences were found in the relative intensity of the peaks (Table 3). This may be interpreted as nonselective biodegradation of the different C and N forms, which dominate the formation of humic substances. The partition of the compost fractions into the different soil aggregate fractions has led to speciation patterns resulting in ^{13}C NMR spectra very similar to that of the whole compost. This suggests that decomposition of the added compost in soil resembles early humification stages, a process that has been described as little affected by organo–mineral interactions characteristic of different soil types (23).

Carbon and Nitrogen Forms in Soil Fractions

Table 4. ^{15}N NMR Peak Area Measurements^a of Different Organic Fractions from Compost and from Soil Amended with Compost

	370–320 ppm	320–200 ppm	200–110 ppm	110–50 ppm	50–20 ppm	20–(-29) ppm
	nitro groups, nitrate	nitrile, oximes	indole, imidazole, pyrrole	amide, pyrrole, lactame	-NH ₂ , -NR ₂	terminal amino groups in aliphatics, NH ₄
initial compost						
water soluble	12.7	1.2		69.0	6.9	5.6
HA	6.9	3.3	6.9	67.6	8.4	6.9
FA	0.2	2.3	5.0	77.8	6.8	7.9
humin (alkali insoluble residue)	1.4	2.4	4.8	79.0	4.8	7.6
compost-treated soil	0.6	3.4	5.2	80.0	6.5	4.3
water soluble	20.8	4.0	8.0	53.8	5.2	8.2
HA	0.4	2.0	5.4	78.8	6.1	7.3
FA	0.7	2.5	4.2	81.5	4.7	6.3
FOM	0.3	2.3	4.3	79.2	7.3	6.5
CHBr ₃ -MeOH floating	0.1	2.0	5.7	78.7	5.9	7.8

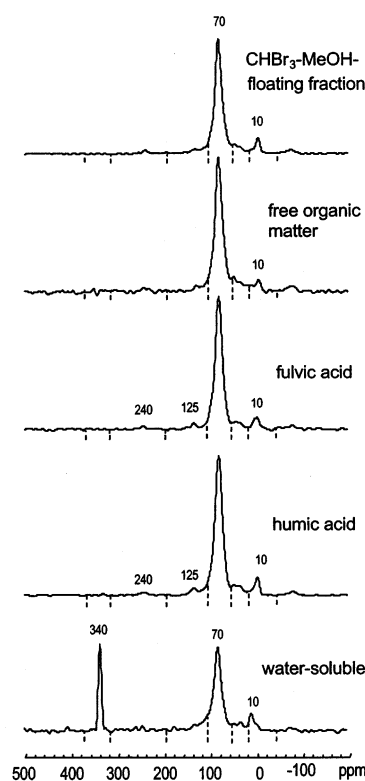
^a Percentage of the total area.

299 In the case of the particulate fractions, the ^{13}C NMR spectra
300 suggest a predominant lignocellulosic nature of these insoluble
301 fractions of organic matter being similar to that of the humin
302 fraction, the latter showing a lower signal-to-noise ratio
303 (spectrum not shown). When compared to other soil humin
304 preparations (24), this compost-derived material shows a very
305 low amount of alkyl carbons and the signal intensity in the
306 aromatic and carboxyl regions was also comparatively low.
307 Despite the appreciable losses of weight and carbon occurring
308 during the sequential incubation experiments, the compost-
309 derived particulate fractions showed a large holocellulose
310 content. It seems as if aromatic or alkyl structures observed in
311 the soils organic matter require a longer decomposition time
312 (20).

313 The FA showed a ^{13}C NMR profile strikingly different from
314 the other fractions. The spectrum almost completely consisted
315 of the main peaks of sugars with no signal intensity in the
316 aromatic/unsaturated region. The very low intensity of the 56
317 ppm peak, the lack of a substantial signal intensity in the 0–50
318 ppm region, and the presence of a clear but low intensity 174
319 ppm peak can be interpreted as colloidal hemicellulose or a
320 polyuronid moiety conforming to most of the FA fractions under
321 study. Concerning the CHBr₃-MeOH floating humin fraction,
322 the ^{13}C NMR spectra also point to a composition comparable
323 to that of the whole labeled compost but with a higher relative
324 intensity of the peaks in the *O*-alkyl region.

325 **^{15}N NMR Spectra.** As usual in most types of sedimentary
326 organic matter (25), the prominent spectral peak with a
327 maximum at ca. 70 ppm (**Figure 2**) is assigned to amide
328 structures such as those in amino acid or peptide-derived
329 substances.

330 In the case of the WS fraction and after considering the large
331 percentage of N in this fraction (**Table 2**) and the ^{15}N NMR
332 spectrum, it seems reasonable to assume that amino acids and/
333 or peptides were responsible for a substantial proportion of the
334 intensity in the 160–220 and the 0–50 ppm ^{13}C NMR ranges
335 but mainly in the 56 ppm signal, which coincides with that of
336 methoxyl groups. On the other hand, several studies have shown
337 that the prominent 70 ppm amide peak is also observed after
338 the samples have been subjected to acid or enzymatic degrada-
339 tion (20, 26). This has been interpreted as if most of the
340 recalcitrant, “unknown” N forms in soils and composts did not
341 largely consist of the heterocyclic rings postulated in classical
342 literature (low signal intensity in the 200–110 range) but to
343 amine and amide structures whose stabilities could depend on
344 a stable physical occlusion and/or covalent bonding in complex

**Figure 2.** ^{15}N CPMAS spectra of soluble, colloidal, and particulate organic fractions from mineral soil treated with ^{15}N -labeled compost soil after 80 days of incubation.

345 macromolecular domains in which these N compounds behave
346 as recalcitrant against chemical or biological degradation. In
347 any case, the fact that no major changes on organic N
348 functionality occur as a result of the compost application to soil
349 is frequently reported in the literature (27).

350 The ^{15}N NMR spectra of the composts clearly show a small
351 peak ca. 340 ppm, corresponding to the available soluble mineral
352 pool, which suggests that most of the ^{15}N added has turned into
353 microbial N-containing metabolites. In general, the ^{15}N spectra
354 of the colloidal and particulate soil and compost fractions were
355 similar both qualitatively and quantitatively, with no evidence
356 of additional, new N forms (e.g., heterocyclic) being formed in
357 the course of the transformation in soil. These results coincide
358 with those by other authors (25, 28) and indicate a successful
359 stabilization of amino acid-containing domains in humic-like,

360 lignin-derived macromolecules in a way that would not probably
361 require the collapse of the preexisting peptidic structure.

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