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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 K^{15}NO$_3$ 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 CHBr$_3$−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 CO_2 (*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% ^{14}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 Higueruela" (CSIC, Toledo, Spain), with a pH of 7.4; CO_3^{2-}, 10 g kg^{-1}; cation exchange capacity (pH 7), 125 $mmol_c kg^{-1}$; 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 surnatant 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 $Na_4P_2O_7$ 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 $\rho = 1.8$ 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 surnatant 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), $-NH_2$, $-NR_2$ (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.

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
		soluble fraction		
water-soluble fraction	18 ± 0.1	5.3 ± 0.0	0.2 ± 0.00	1.9 ± 0.0
		colloidal fractions		
HA	70 ± 0.8	20.6 ± 0.2	1.5 ± 0.00	15.0 ± 0.0
FA	39 ± 0.3	11.5 ± 0.0	0.6 ± 0.00	5.5 ± 0.0
		particulate fractions		
FOM	ND	ND	0.6 ± 0.04	5.6 ± 0.4
humin ($CHBr_3$–MeOH floating)	ND	ND	1.9 ± 0.02	18.6 ± 0.2
humin (alkali insoluble residue)	213 ± 0.6	62.7 ± 0.18	5.5 ± 0.00	53.9 ± 0.0

[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
		soluble fraction			
water soluble	3.4 ± 0.4	95.7 ± 5.0	0.53 ± 0.06	0.02 ± 0.00	0.55 ± 0.06
		colloidal fractions			
HA	42.0 ± 0.0	14.5 ± 2.9	0.73 ± 0.00	4.29 ± 0.00	5.02 ± 0.00
FA	19.2 ± 0.0	12.7 ± 1.1	0.27 ± 0.00	1.87 ± 0.00	2.14 ± 0.00
		particulate fractions			
humin (alkali insoluble residue)	16.5 ± 0.2	29.7 ± 4.4	2.98 ± 0.03	7.03 ± 0.09	10.01 ± 0.12
compost-treated soil					
		soluble fraction			
water soluble	2.6 ± 0.5	96.4 ± 2.8	0.01 ± 0.00	0.00 ± 0.00	0.01 ± 0.00
		colloidal fractions			
HA	35.5 ± 0.1	8.6 ± 1.0	0.01 ± 0.00	0.10 ± 0.00	0.11 ± 0.00
FA	21.5 ± 1.8	8.8 ± 1.4	0.00 ± 0.00	0.04 ± 0.00	0.04 ± 0.00
		particulate fractions			
FOM	15.6 ± 0.0	27.9 ± 1.0	0.01 ± 0.00	0.02 ± 0.00	0.02 ± 0.00
$CHBr_3$–MeOH floating	17.0 ± 0.2	26.3 ± 4.4	0.02 ± 0.00	0.07 ± 0.00	0.09 ± 0.00
humin (alkali insoluble residue)	ND	ND	0.02	ND	0.26

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

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

Analyses by ^{13}C NMR Spectrometry. The ^{13}C NMR spectra of the whole compost and its different fractions are shown in **Figure 1**. The spectral region with a maximum at ca. 135 ppm may be attributed to C_1 and C_4 in S lignin and to C_1 in G units (*16*). In this region, also consider that the major lignin signals at ca. 153 and 145 ppm may be overlapped with those of tannins (*17*). The alkyl region (50−0 ppm) showed a maximum at ca. 21 ppm, which is frequently attributed to acetate groups in hemicellulose (*18*), but the most typical signal in this region, with a maximum at ca. 30 ppm, corresponds to a large extent to protein-like structures and polymethylene carbons in lipids and lipid macromolecules. The signal ca. 172 ppm, in the soil humic substances frequently attributed to carboxyl groups, showed the highest intensity in the most oxidized soil fractions (FAs and to a lesser extent HAs). However, the area of this peak in the composts under study (**Table 3**) is similar in colloidal and particulate fractions and has an even greater intensity in the case of HAs than of FAs. These facts could be interpreted that the intensity in the carbonyl region was in part due to aliphatic esters, which could be the case with hemicellulose esters (*18*). In addition, one may also take into account that the amides also show a typical signal in this region: in fact, protein-like compounds in compost produce diagnostic

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 ppm	190–160 ppm	160–140 ppm	140–110 ppm	110–100 ppm	100–90 ppm	90–60 ppm	60–50 ppm	50–0 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
humin (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.

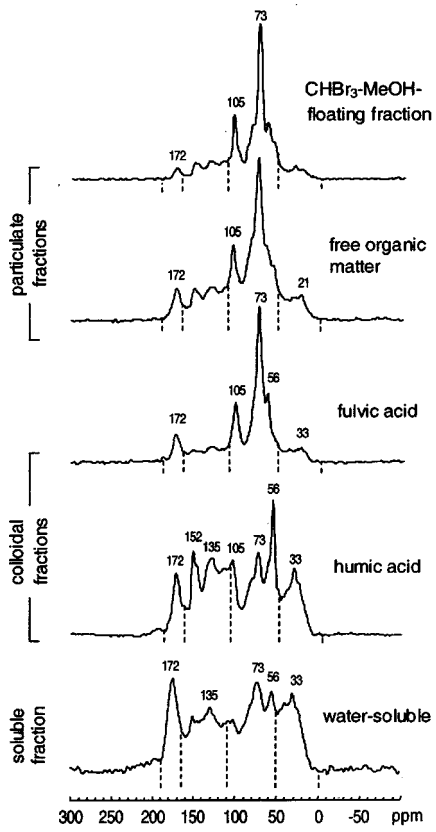

Figure 1. ^{13}C CPMAS spectra of soluble, colloidal, and particulate organic fractions from mineral soil treated with ^{15}N-labeled compost soil after 80 days of incubation.

NMR signals in the 50−45 ppm range (Cα 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 C_2, C_3, and C_5 of pyranoside rings in sugar constituents of celluloses and hemicelluloses). In addition, some ill-resolved resonances for the sugar ring carbon at the C_6 position (shoulder at ca. 63 ppm), for C_4 in amorphous cellulose (shoulder at 84 ppm), and for C_1 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 C_3 and C_5 in etherified syringil (S) structures and also to C_3 and C_4 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*).

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	12.7	1.2		69.0	6.9	5.6
water soluble	6.9	3.3	6.9	67.6	8.4	6.9
HA	0.2	2.3	5.0	77.8	6.8	7.9
FA	1.4	2.4	4.8	79.0	4.8	7.6
humin (alkali insoluble residue)	0.6	3.4	5.2	80.0	6.5	4.3
compost-treated soil						
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.

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

The FA showed a ^{13}C NMR profile strikingly different from the other fractions. The spectrum almost completely consisted of the main peaks of sugars with no signal intensity in the aromatic/unsaturated region. The very low intensity of the 56 ppm peak, the lack of a substantial signal intensity in the 0−50 ppm region, and the presence of a clear but low intensity 174 ppm peak can be interpreted as colloidal hemicellulose or a polyuronid moiety conforming to most of the FA fractions under study. Concerning the $CHBr_3$−MeOH floating humin fraction, the ^{13}C NMR spectra also point to a composition comparable to that of the whole labeled compost but with a higher relative intensity of the peaks in the *O*-alkyl region.

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

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

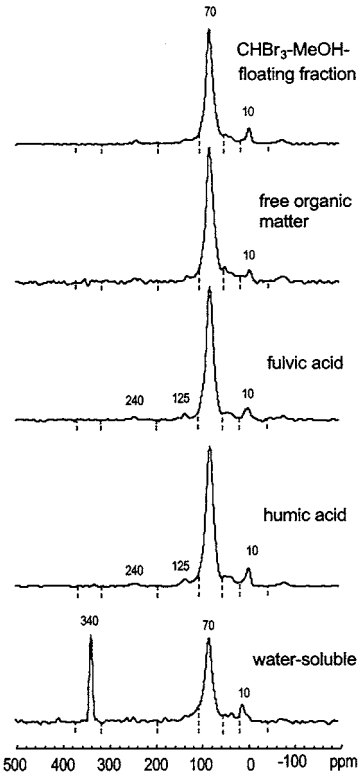

Figure 2. ^{15}N CPMAS spectra of soluble, colloidal, and particulate organic fractions from mineral soil treated with ^{15}N-labeled compost soil after 80 days of incubation.

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

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

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

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