

TRANSFORMATIONS IN SOIL OF THE ORGANIC FRACTIONS FROM ¹⁵N LABELLED COMPOST AS REVEALED BY ¹³C AND ¹⁵N NMR SPECTROSCOPIES AND ANALYTICAL PYROLYSIS

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1. ABSTRACT

A molecular assessment of different C and N forms from a compost-treated soil has been performed by NMR spectroscopy and pyrolysis-gas chromatography-mass spectrometry. A ¹⁵N-enriched mixture of urban compost (30%), wheat straw (64%) and K¹⁵NO₃ (6%) incubated for 80 days was used to isolate different fractions: water-soluble (WS), humic acid-like (HA), fulvic acid-like (FA), and alkali-insoluble fractions. This compost (21.8% N as ¹⁵N) was added to a mineral soil and incubated for 80 days. These fractions and two particulate ones were isolated from the compost-treated soil. The stable isotope ratios (¹⁵N/¹⁴N) of each fraction were compared to monitor the N speciation patterns in soil. Most of the newly-formed N-compounds concentrate in WS (96% ¹⁵N) and in the insoluble residue (>25% ¹⁵N) but about 28% consists of amide N in the HA. According to spectroscopic and pyrolytic data the HA resembles to a lignoprotein, whereas the FA consists of a C=O-containing carbohydrate material. Alkyl compounds suggesting the presence of lipid macromolecules are released mainly by particulate fractions. The WS (1.7% of total N) showed heterogeneous composition yielding methoxyphenols, furans, fatty acids and N-compounds (mainly pyrroles). Our results suggest that non-selective biodegradation of the different C and N forms dominates over the formation of humic substances.

2. INTRODUCTION

The sustainable improvement of soil properties based in periodic organic inputs (Parr et al., 1989) largely depends on the extent to which such external inputs lead to the formation of active colloidal material referred to as humic substances. Apart from biological and toxicological criteria, the concept of compost maturity implies the presence of stabilized organic matter suitable to long-term enhancement of soil physical and physico-chemical properties. Nevertheless, no extensive information exists on the fate in soil of the different organic fractions of compost. There is some controversy on whether the compost is heavily transformed in soil, leading to stable humic-like substances or, on the contrary, it is degraded by soil microorganisms while only a minor portion is stabilized by humification or association with mineral components (Terry et al., 1979; Almendros et al., 1990). The assessment of the C and N forms in soil is of special interest in the case of composts, where the presence of humic substances (viz., HA, FA and humin) should not be defined by operative laboratory methods used in soil organic matter studies, but requires molecular characterization techniques (González-Vila et al., 1999). Laboratory incubation using ¹⁵N allows quantitative monitoring of exogenous N-forms, and also permits ¹⁵N NMR analyses in reasonable acquisition times (K-gel-Knabner, 1997). Assuming the above considerations, the present experimental approach included: i) preparation of a ¹⁵N-enriched compost by incubating 80 days a mixture of wheat straw, urban waste and K¹⁵NO₃, ii) further 80-day incubation of the soil-compost mixture, iii) laboratory isolation of soluble, colloidal and particulate fractions from soil and compost, and iv) pyrolysis (Py) combined with gas chromatography and mass spectrometry (GC-MS) and ¹³C and ¹⁵N NMR. Such an experimental design is expected to shed some light about the fate and the speciation patterns of the compost C and N forms after their transformation in the soil.

3. MATERIAL AND METHODS

3.1. Incubation experiments

The ¹⁵N-labelled compost was prepared by incubating a mixture of urban compost, from the Valdemingümez landfill, Madrid (29.55% by weight) wheat straw (64%) and K¹⁵NO₃ (6.05%) for 80 days. These proportions were calculated to obtain a starting C/N ratio= 20, where 50% of the N was as ¹⁵N (25% ¹⁴N from straw, and 25% from urban waste). A standard dose of CaSO₄ (0.25% by weight) was added to prevent N losses by volatilization. The starting materials were homogenized to 2 mm. After measuring the water holding capacity of the mixture, the moisture was adjusted to 54.44 dry weight percentage. After 80 days at 27°C (36% wt loss by mineralization), a portion of the compost was added to a mineral soil in order to study its further transformation, and the remainder was liophylized for chemical characterization. The soil was taken from the upper 20 cm of a Calcic Luvisol in the experimental farm 'La Higuera' (CSIC, Toledo, Spain), with a pH= 7.4, organic C= 5 g kg⁻¹, CO₃²⁺= 10 g kg⁻¹, cation exchange (pH= 7)= 125 mmol_ckg⁻¹, sand= 780 g kg⁻¹, silt= 80 g kg⁻¹, clay= 140 g kg⁻¹. The amount of organic matter was reduced to ca. 0.02% with 10% by wt H₂O₂ at 60°C for 2 h (× 3 times); the floating straw particles were removed with a spoon. Finally, the soil was

washed, centrifuged, air-dried, and homogenized to 2 mm. 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 at 27°C for 80 days. Two replications of the experiments (incubations, chemical analyses, Py-GC-MS and ^{13}C NMR) were carried out, but using K^{14}NO_3 .

3.2. Fractionation protocols

The major organic fractions in compost and compost-treated soil were isolated by physical and chemical procedures. An extraction with water at room temperature was carried out after 2-h shaking, repeated twice. The suspension was centrifuged and the supernatant solution was collected and lyophilized (WS fraction). From the previous residue, further separation by flotation in 2 M H_3PO_4 (Dabin, 1971) was performed in order to isolate the particulate organic matter not yet incorporated to the soil organo-mineral matrix (free organic matter, FOM). The yellowish supernatant solution was left to aggregate to the humic extract described below. The soil residue was washed and centrifuged, then extracted with 0.1 M $\text{Na}_4\text{P}_2\text{O}_7$ followed by repeated extractions with 0.1 M NaOH. The extract was used to separate the HA (precipitation with HCl) from the FA (soluble) fraction. These colloidal fractions (including the whole FA extract) were dialyzed to remove the salts introduced during the extraction process, and lyophilized. After these extractions, some amounts of organic matter remained with the soil residue. In order to remove this particulate organic fraction, the residue was washed with 0.01 M HCl and centrifuged, dried and homogenized to <0.25 mm, then a humin (insoluble) fraction was recovered by flotation in a CHBr_3 -EtOH mixture of $r = 1.8 \text{ g mL}^{-1}$. This floating fraction is quite analogous to the "inherited humin" of French literature (Chouliaras et al., 1975) consisting of organic particles originally encapsulated in stable soil aggregates.

3.3. Analysis of stable isotope ratios in compost and soil

^{15}N abundances were determined with a Finnigan MAT delta S spectrometer (Finnigan, Bremen, Germany) connected to a CN elemental analyser (SCA CNRS, Vernaison, France)

3.4. Curie-point pyrolysis-gas chromatography-mass spectrometry

The samples on ferromagnetic wires were heated for 5 s to a Curie temperature of 510°C in a Horizon Instruments pyrolyser connected to a Varian Saturn 2000 GC-MS. The GC oven was set from 50 to 100°C at $32^\circ\text{C min}^{-1}$ and then up to 320°C at a rate of 6°C min^{-1} . The injection port, with a liquid CO_2 cryogenic unit, was adjusted from -30°C (1 min) to 300°C at $20^\circ\text{C min}^{-1}$, while the GC interface was at 300°C . A CPSil-5 fused-silica column (25 m \times 0.32 mm, film thickness 0.4 μm), with He as carrier gas was used.

3.5. Solid-state ^{13}C and ^{15}N NMR spectroscopy

The solid-state ^{13}C NMR spectra were acquired at 25.1 MHz in a Bruker MSL 100 (2.3 Tesla) using cross-polarization/magic angle spinning (CPMAS). A total of 1000 free induction decays were accumulated for each spectrum. The contact time was 1 ms and the pulse repetition rate was set to 5 s. The sweep width was 37.5 kHz and the acquisition time was 0.016 s. The MAS was performed at 4 kHz. The chemical shift range was referred to tetramethylsilane (= 0 ppm). The ^{15}N CPMAS spectra were obtained on a Bruker MSL 300 spectrometer at 7.05 Tesla (^{15}N resonance frequency 30.4 MHz). The rotation frequency was 4-4.5 kHz, the contact time 0.7 ms and the pulse delay 4 s. The chemical shift range was referred to ammonium chloride (= 0 ppm).

4. RESULTS AND DISCUSSION

4.1. Organic fractions isolated from soil and compost

Table 1 shows the different fractions of the compost, and of the compost-treated soil. The compost has a substantial concentration of WS, amounting to ca. 5% of its total C. The colloidal fractions (HA+FA) amounted to ca. one third of the total C. After incubation of the soil-compost mixture, there was a decrease in amount (mineralization and/or insolubilization) of both soluble and colloidal fractions, and a relative accumulation of particulate organic matter. Of the latter, only about 5.6% of the C added to soil remained as non-decomposed particles (FOM), whereas about 18% corresponded to the particulate fraction separated by flotation in CHBr_3 -EtOH.

	¹⁵ N-labelled compost		Soil treated with ¹⁵ N compost	
	g C kg compost ⁻¹	g C 100 g C ⁻¹	g C kg soil ⁻¹	g C 100 g C ⁻¹
Total sample	340	1000	10.0	1000
Water soluble	18 ±1	53	0.6 ±0.0	19
Humic acid	70 ±8	206	1.5 ±0.0	150
Fulvic acid	39±3	115	0.6>±0.0	55
Free organic matter			0.6 ±0.4	56
CHBr ₃ -EtOH-floating			1.9 ±0.2	182
Non-extractable humin	213 ± 6	627	5.5 ±0.0	539

Table 1. Organic fractions in compost and in compost-amended soil

4.2. Stable isotope analysis

Table 2 shows the N concentration and ¹⁵N richness of the fractions in the compost and the compost-amended soil. The values calculated as percentages of the total N showed a considerable ¹⁵N enrichment in the colloidal fractions mainly HA. In fact, in lignocellulosic composts this fraction frequently consists of lignoproteins (Jenkinson and Tinsley, 1959).

	Total N content		Isotopic abundance				Total N	
	g kg ⁻¹	¹⁵ N richness	¹⁵ N		¹⁴ N		N g kg sample ⁻¹	
Total compost:	17.7±0.1	21.8 ±4.2	3.85	(255)	13.8	(749)	17.65	(1000)
Water soluble	3.4±0.4	95.7 ±5.0	0.53	(30)	0.02	(1)	0.55	(31)
Humic acid	42.0±0.0	14.5 ±2.9	0.73	(41)	4.29	(243)	5.02	(284)
Fulvic acid	19.2±0.0	12.7 ±1.1	0.27	(15)	1.87	(106)	2.14	(121)
Non-extractable humin	16.5±0.2	29.7 ±4.4	2.98	(169)	7.03	(399)	10.01	(567)
Compost-treated soil:								
Water soluble	2.6±0.5	96.4 ±2.8	0.01	(17)	0.00	(1)	0.01	(17)
Humic acid	35.5±0.1	8.6 ±1.0	0.01	(17)	0.10	(185)	0.11	(203)
Fulvic acid	21.5±1.8	8.8 ±1.4	0.00	(7)	0.04	(69)	0.04	(76)
Free organic matter	15.6±0.0	27.9 ±1.0	0.01	(12)	0.02	(31)	0.02	(43)
CHBr ₃ -EtOH-	17.0±0.2	26.3 ±4.4	0.02	(44)	0.07	(124)	0.09	(168)

	Total N content		Isotopic abundance			Total N	
floating							
Non-extractable humin			0.02		0.26		(494)

Table 2. Isotopic abundances and partition of the total N (^{14}N = original, ^{15}N = added) in the different organic fractions from compost and from compost-amended soil In brackets: proportion of the total nitrogen (=1000) in g kg^{-1} .

In both soil and compost fractions the highest ^{15}N richness corresponded to the WS but, when expressed as a percentage of the total N, about 40% of the total compost ^{15}N is in particulate fractions, suggesting a microbial biomass containing altered protein and chitin. The percentages of the total N pool showed that, in both compost and soil, more than half of the total N is in the particulate fractions, but about a third part is in the colloidal fractions (HA+FA). The stable isotope distribution indicates that the N pool in the WS was lower in soil than in compost, pointing to a rapid mineralization or incorporation of N forms to the insoluble fractions. In fact, the percentages of ^{15}N as regards total N indicate that the tendency in soil was to insolubilization, i.e., the highest stability of soil N occurs in the insoluble fractions, whereas the N in FA is more readily metabolized, then its total amount and the newly-incorporated N drastically decreased after the transformation of the compost in the soil.

4.3. Analytical pyrolysis

Figures 1 and 2 show the pyrograms at 510°C of the compost and soil fractions. The major pyrolytic compounds, and their possible precursors are listed in Table 3. The compounds arising from carbohydrate and protein dominate in the more labile fractions (WS and FA), whereas the pyrograms of the HA included large peaks of aromatic compounds. A series of diagnostic methoxyphenols traditionally ascribed to lignin Py fragments were among the major products from the whole compost and the different fractions. Such compounds correspond to typical guaiacyl (G) and syringyl (S) structures with characteristic methyl-, ethyl-, vinyl-, propenyl- and keto-substitutions, the most abundant being vinylguaiacol and dimethoxyphenol.

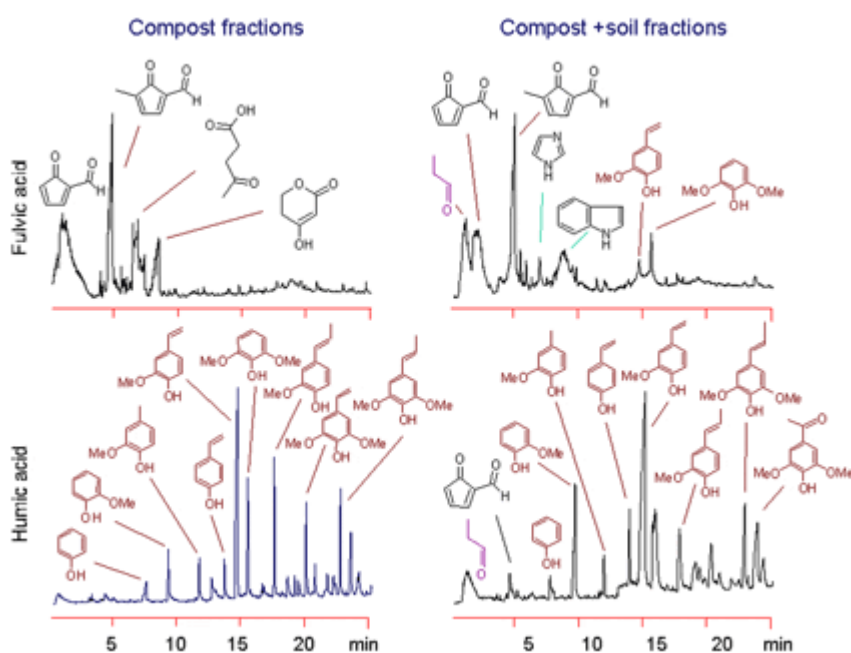


Fig. 1. Pyrolytic products from colloidal fractions from compost and compost-treated soil.

In the FA the Py molecular assemblages consisted mainly of furanes accompanied by other characteristic carbohydrate dehydration products such as 4-oxo-pentanoic acid. Most of the N-bearing products occur in the WS and FA, the most representative being pyrroles and pyrrolines arising from aminoacids and the tetrapyrrole moiety of chlorophylls, indoles (probably derived from aminoacids), pyridin derivatives (also pointing to polypeptides and chitin) and nitriles (Van der Kaaden et al., 1984). No specific patterns were observed regarding these N forms, which were similar in the compost and in the soil fractions, i.e., the transformation of the compost in the soil does not lead to the identification of substantial amounts of newly formed organic N forms, others than those present in the original compost. Alkyl compounds, consisting of alkanes and fatty acids, were found mainly in particulate fractions. Both light and heavy

fractions physically isolated from the soil (FOM and CHBr_3 -EtOH-floating) yielded a substantial amount of compounds arising from lipid material. Our results point to an accumulation of aliphatic microbial metabolites in soil particulate fractions, whereas lignin-derived structures accumulate in soluble and colloidal fractions.

MW	Compound [origin]
58	Propanal [C]
60	Ethanoic (Acetic) acid [C]
67	Pyrrole [P]
68	Imidazole [P]
68	Pentadiene
79	Pyridine [P]
81	Methylpyrrole [P]
82	2-Methylfuran [C]
84	2H-Pyran, 3,4-dihydro- [C]
94	Phenol [L,P]
96	Dimethylfuran [C]
96	2-Furancarboxaldehyde [C]
96	Heptadiene
98	2-Furanmethanol [C]
98	2(3H)-Furanone, 5-methyl- [C]
98	1-Hexene, 3-methyl-
110	2-Furancarboxaldehyde, 5-methyl [C]
114	4-hydroxy-5,6-dihydro-(2H)-pyran-2-one [C]
116	4-oxo-pentanoic (Levulinic) acid [C]
117	Indole [P]
124	Phenol, 2-methoxy (Guaiacol) [L]
126	2-Furancarboxaldehyde, 5-hydroxymethyl- [C]
131	Methylindole [P]
138	Phenol, 2-methoxy, 4-methyl (Methylguaiacol) [L]
138	Phenol, methoxymethyl [L]
150	Vinylphenol [L]
154	Phenol, 2,6-dimethoxy (Syringol) [L]
164	Phenol, 2-methoxy, 4-propenyl (Propenylguaiacol) [L]
164	Phenol, 2-methoxy, 4-(1-propenyl) (<i>Cis</i> -isoeugenol) [L]
164	Phenol, 2-methoxy, 4-(1-propenyl) (<i>Trans</i> -isoeugenol) [L]
166	Ethanone, 1-(4-hydroxy, 4-methoxyphenyl) (Acetovanillone) [L]
168	Phenol, 2,6-dimethoxy, 4-methyl (Methysyringol) [L]
168	Phenol, dimethoxymethyl [L]
180	Phenol, 2-methoxy, 4-vinyl (Vinylguaiacol) [L]
180	Phenol, 2,6-dimethoxy, 4-vinyl (Vinylsyringol) [L]
194	Phenol, 2,6-dimethoxy, 4-propenyl (Propenylsyringol) [L]
194	Phenol, dimethoxypropenyl [L]

Table 3. Tentative assignation and possible origin of the major pyrolysis compounds.

Square brackets: C= carbohydrate-derived, L= lignin-derived, P= protein-derived.

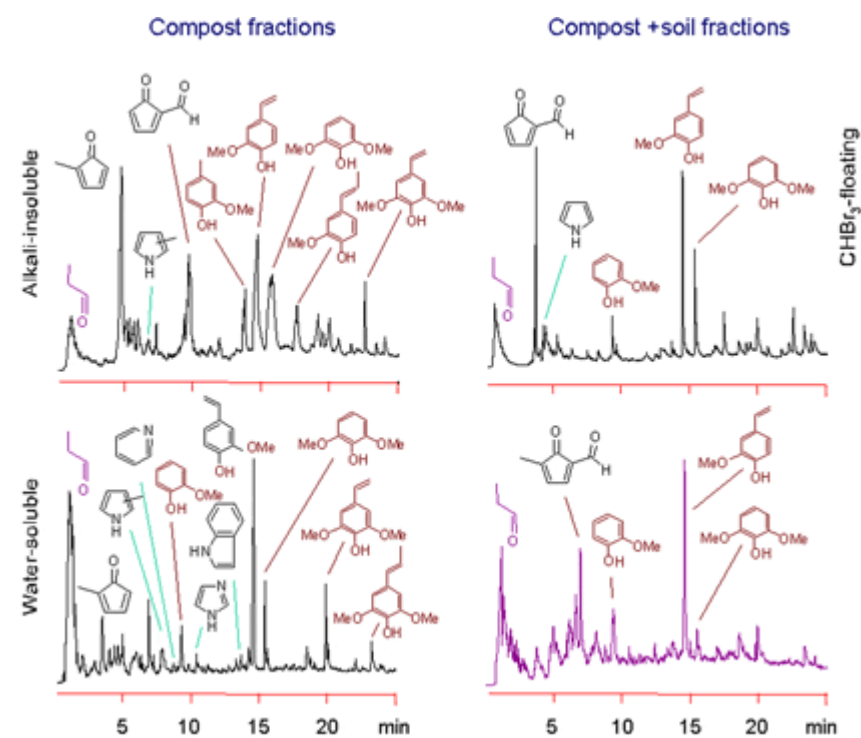


Fig. 2. Pyrograms from soluble and particulate compost and compost-treated soil fractions.

4.5. Analyses by ^{13}C and ^{15}N NMR

Figure 3 shows both the ^{13}C and ^{15}N NMR spectra of the whole compost and the isolated soil and compost fractions, and Table 4 includes the area measurements in the most diagnostic ^{13}C chemical shift ranges (Wilson, 1987). The ^{13}C spectrum of the whole compost showed a profile similar to that of a lignocellulosic material. The prominent 73 ppm signal is traditionally assigned to C₂, C₃ and C₅ of pyranoside rings. Additional ill-resolved resonances coincided with those produced by the C₆ sugar carbon (shoulder at ca. 63 ppm), by C₄ in amorphous cellulose (shoulder at 84 ppm) and by C₁ in carbohydrate (anomeric C at 103 ppm) (Kolodziejcki et al., 1982). It has been reported that the intensity of the signal at ca. 103 ppm could to some extent be increased by non-protonated carbons from tannins (Preston et al., 1997). The range for aromatic carbons (160-110 ppm) could be divided into two regions (at between 160-140 ppm, mainly produced by aromatic carbons linked to O or N, and that at between 140-110 ppm for H-substituted and C-substituted carbons). According to the structures found in the pyrolytic analysis, the signal at ca. 153 ppm could be assigned to C₃ and C₅ in etherified S structures, as well as to C₃ and C₄ in G units (L, demann and Nimz, 1973).

C-type	Carbonyl carbons	Aromatic carbons		O-alkyl carbons				Alkyl carbons
		220–160 ppm	160–140 ppm	140–110 ppm	110–100 ppm	100–90 ppm	90–60 ppm	
<i>Compost:</i>	7.3	6.8	13.3	8.3	6.1	39.5	7.1	11.6
WS	17.4	9.0	15.8	4.7	3.7	20.5	8.3	20.5
HA	7.8	11.4	21.2	8.5	4.0	22.7	10.6	13.8
FA	5.2	3.2	5.7	10.9	6.3	58.9	3.9	5.9
NEH	4.4	5.4	12.1	9.5	6.2	48.1	6.7	7.5
<i>Compost-treated soil:</i>								
WS	18.3	9.0	15.5	4.7	4.1	19.8	7.1	21.4
HA	10.1	11.5	21.6	7.2	3.5	18.7	10.0	17.3
FA	9.4	4.7	9.0	8.8	6.2	47.4	5.4	9.1
FOM	7.6	5.4	11.3	11.1	5.1	40.9	6.2	12.2
Humins	3.2	5.2	11.8	10.0	5.3	49.2	5.3	7.9

Table 4. Signal area measurements in the ^{13}C NMR spectra of different organic fractions from compost and from compost-amended soil

WS= water soluble fraction, HA= humic acid, FA= fulvic acid, FOM= free organic matter, humin= CHBr_3 -EtOH-floating, NEH= non-extractable humin.

The spectral region with maximum at ca. 135 ppm could be assigned to C_1 and C_4 in S lignin and to C_1 in G units (Haw et al., 1984), but the major lignin signals at ca. 153 and 145 ppm may be overlapped with those of tannins (Preston et al., 1997). The alkyl region (50-0 ppm) showed a peak at ca. 30 ppm, often ascribed to polymethylene carbons in lipids and lipid macromolecules, but its intensity can also be enhanced by proteins. The signal at ca. 21 ppm can be ascribed to acetate groups in hemicellulose (Kolodziejewski et al., 1982). The carbonyl region (220-160 ppm) was dominated by a carboxyl signal ca. 172 ppm. Nevertheless, it must be taken into account that the intensity of this peak (Table 4) is similar in colloidal and particulate fractions, and has even greater intensity in HA than in FA. These facts point out that this signal is to some extent due to aliphatic esters, such as hemicellulose esters (Kolodziejewski et al., 1982), or amides. Out of the different fractions, the ^{13}C NMR spectra of both compost and soil WS showed the most heterogeneous composition, with a large variety of aromatic extractives, carbohydrate, alkyl structures and carboxyl groups. Considering the shape of the ^{15}N NMR spectrum (Fig. 3) it is probable that aminoacids and/or peptides are also responsible for the intensity enhancement in most of the above spectral regions such as the 160-220 and the 0-50 ppm ranges, as well as in the most specific 56 ppm signal, overlapped with that of methoxyl groups. The ^{13}C NMR spectra of the HA suggested a lignoprotein, with relatively sharp peaks in the methoxyl/a-amino region, intense signals at 73 and 105 ppm and minor peaks or shoulders at ca. 64 and 84 ppm. The signal intensity at 105 ppm (which is higher than expected when compared with the other carbohydrate bands) points to the presence of tannins or cinnamic acids, typical of lignin-hemicellulose complexes from grass lignins.

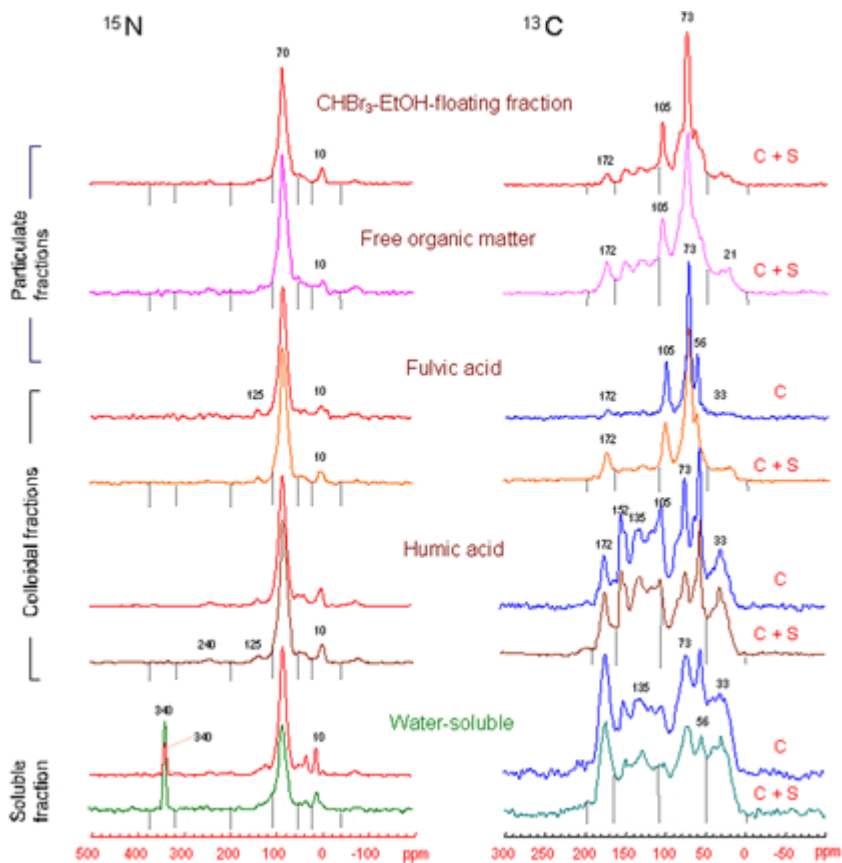


Fig. 3. ^{15}N and ^{13}C and CPMAS NMR spectra of organic fractions from ^{15}N -labelled compost (C) and compost-treated mineral soil (C+S). Dotted lines indicate the major chemical shift ranges. For ^{13}C : 0-50 ppm= alkyl (13= methyl, 21= acetate, 30= polymethylene), 50-110 ppm= O-alkyl (56= methoxyl/a-amino, 73= major carbohydrate signal, 103= anomeric C in carbohydrate, 105= quaternary aromatic carbons in tannins); 110-160 ppm= aromatic/unsaturated; 160-220 ppm= carbonyl (172= carboxyl/amide, 198= ketone/aldehyde). For ^{15}N : 370-320 ppm= nitro groups, 320-200 ppm= nitrile, oximes, 200-110= indole, imidazole, pyrrole, 110-50= amide, pyrrole, lactame, 50-20 ppm= $-\text{NH}_2$, $-\text{NR}_2$, and 20-(-29) ppm: terminal amino groups in aliphatics, NH_4 .

The ^{13}C NMR profile of the FA strongly differed from the other fractions and is also quite different from those of typical soil FAs (Fr,nd et al., 1989). The spectrum shows prominent sugar peaks with no aromatic/unsaturated compounds. The negligible 56 ppm peak, the weak signal intensity in the 0-50 ppm range, and the 174 ppm peak point to hemicelluloses or polyuronid materials. The ^{13}C NMR spectra of the fractions from the compost-amended soils were quite similar to those of the original compost, which suggests that the incorporation of the organic matter to the soil is mainly physical, and non-selective biodegradation of the different C and N forms prevails on the formation of humic substances.

As a whole, the results of the ^{13}C NMR indicate that, in spite of the appreciable losses of weight during the two incubation experiments, the different C types are degraded to a similar extent, whereas selective concentration of e.g., aromatic or alkyl structures, a characteristic of the formation processes of humic substances in soil, comparatively requires a longer residence time with continuous inputs of organic matter (Almendros et al., 1990).

Concerning the ^{15}N NMR spectra (Fig. 3), the prominent peak ca. 70 ppm is assigned to amide structures (Almendros et

al., 1991). Since it has been reported that this peak remains even in samples subjected to acid or enzymatic degradation (K-gel-Knabner et al., 1997) it has been inferred that most of the recalcitrant "unknown" N-forms in soils and composts did not consist of heterocyclic rings (200-110 ¹⁵N range) postulated in classical literature but to amine and amide structures stable against chemical or biological degradation. The diagnostic peak ca. 340 ppm enables the quantitation of the residual nitrate, indicating that, in the whole compost, almost all the ¹⁵N used for the labelling has turned into microbial N metabolites. In the WS from the compost-treated soil, the comparatively greater intensity (¥ 3) of this ¹⁵NO₃⁻ signal (Figure 3) suggests that this peak has an origin in the mineralization of organic N forms, rather than in the presence of residual nitrate not yet incorporated to the microbial biomass.

5. CONCLUSIONS

1. Either extended composting or further transformation of the compost in soil did not lead to organic matter structurally comparable to typical soil humic substances.
2. A substantial amount of the ¹⁵N added turned into amide forms and remained in the insoluble, particulate fractions (on average 27% ¹⁵N richness) instead of in the colloidal fractions. This could indicate that the structural macromolecules in microbial biomass are the major pool of newly-formed N compounds.
3. The FA almost completely consisted of readily biodegradable carbohydrate material, whereas the HA included a significant aromatic domain.
4. The WS showed heterogeneous composition and large amounts of aromatic, O-alkyl and alkyl structures and a carbonyl content higher than in FA. It represents about 8% of the total soil N, and a 96% ¹⁵N enrichment, but the ¹⁵N NMR spectrum shows that only 20% of its N consists of nitrate, the remainder consisting of amide and amine compounds.
5. The structural similarity between the organic fractions from the original compost and the compost-amended soil indicates that the physical incorporation and the non-selective biodegradation of the different C and N forms prevail on the selective preservation of recalcitrant macromolecules, and the latter prevails on the formation of humic substances.

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