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Analysis of ^{13}C and ^{15}N CPMAS NMR-spectra of soil organic matter and composts

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^{15}N and ^{13}C CPMAS spectra of composted plants are presented. The plants (*L. rigidium* and *Zea mays*) were grown in ^{15}N enriched medium and fermented for several months until an approx. 80% of the dry matter was lost. In all ^{15}N spectra the secondary amide/peptide peaks at 87 ppm contributes more than 80% of the total intensity. No new ^{15}N peaks are formed during the fermentation process. Older attempts to assign a significant fraction of humic acid nitrogen to heteroaromatic structures formed in the fermentation process are thus most probably wrong.

^{15}N-NMR; ^{13}C-NMR; CPMAS; Compost; Humic acid

1. INTRODUCTION

The ^{13}C NMR spectroscopy has contributed significantly to our knowledge of the chemical structure of soil organic matter. The application of the solid-state-high-resolution-CPMAS-technique even permits the study of complete soils and native humic material without any prior chemical treatment, as for instance the extraction by aqueous sodium hydroxide [1,2].

^{15}N NMR at natural abundance, however, encounters even greater sensitivity problems, caused by the low natural abundance of this isotope and its small and negative gyromagnetic ratio [3]. Consequently no serious attempts have been made to determine the chemical structure of natural soil organic matter nitrogen by the CPMAS-technique, although in spite of the central biological importance of this element the chemical structure of nitrogen in humic and fulvic material is still a matter of controversy [4,5]. In the following first attempts to learn about the practical limits of the ^{15}N CPMAS technique as applied to biodegraded plants grown on ^{15}N enriched fertilizer are presented. The spectral changes observed are compared to ^{13}C CPMAS spectra of the same composts. A previous similar ^{15}N CPMAS study dealt with the structure of melanoidins and similar compounds [6].

2. MATERIALS AND METHODS

The soils and composts were freeze-dried and thoroughly milled. *Lolium rigidum* was grown on Hoaglands solutions containing potassium nitrate (90% ^{15}N enriched) as the sole nitrogen source. The neat harvested plants were composted under aerobic conditions at 30°C in the dark. Samples were collected after 14, 28, 46, 52 and 70 days. The complete samples were freeze-dried and milled. In addition, the freeze-dried humic acid fraction obtained from a composted mixture of 10 g corn straw *(Zea mays)* 0.8% N with 25% ^{15}N above natural abundance with 50 g quartz sand each saturated with water after 150 days of fermentation was studied. This humic acid sample contained 8% N. The ^{13}C CPMAS spectra were taken at 2.3 Tesla (25 MHz) in a Bruker MSL 100 spectrometer equipped with a commercial 7 mm CPMAS probe at a rotation fequency of 4 kHz. The ^{15}N CPMAS spectra were studied in a Bruker MSL 300 spectrometer at 7 Tesla (30.4 MHz), in an identical CPMAS probe at the same rotation frequency.

The proton spin lattice relaxation time T_{1H} was determined indirectly by detecting the ^{13}C or ^{15}N magnetisation [7]. By variation of the contact time in a series of CPMAS spectra, the time relevant for the polarisation transfer T_{NH} and the proton spin lattice relaxation in the rotating coordinate system $T_{1\rho H}$ were determined. The ^{13}C chemical shifts are given in the TMS (=0 ppm) scale. ^{15}N spectra are referenced to glycine (=0 ppm).

3. RESULTS AND DISCUSSION

In Fig. 1 ^{15}N and ^{13}C CPMAS spectra from the *L. rigidium* compost are shown. The bottom spectrum shows the unfermented starting material, while the spectra of the complete fermentation products sampled after 2–10 weeks are presented above. In the ^{15}N spectra of the starting material signals are found at 344, 139, 114 (weak shoulder), 87, 52, 40 and 1 ppm. The signals at 355, 221 and −48 ppm are spinning side bands (first and second order) of the strong signal at 87 ppm. In the figure they are marked by asterisks respectively arrows. Their distance from the peak is the rotation frequency ν_R (4000 Hz) for the first order and twice ν_R (8000 Hz) for the second order. The signal centered at 87 ppm represents 82–87% of the total intensity and is assigned to the peptide nitrogens of proteins. In the range between 95 and 120 ppm also the ^{15}N signals of quinonimines should occur. The strong peptide signals, however, render them

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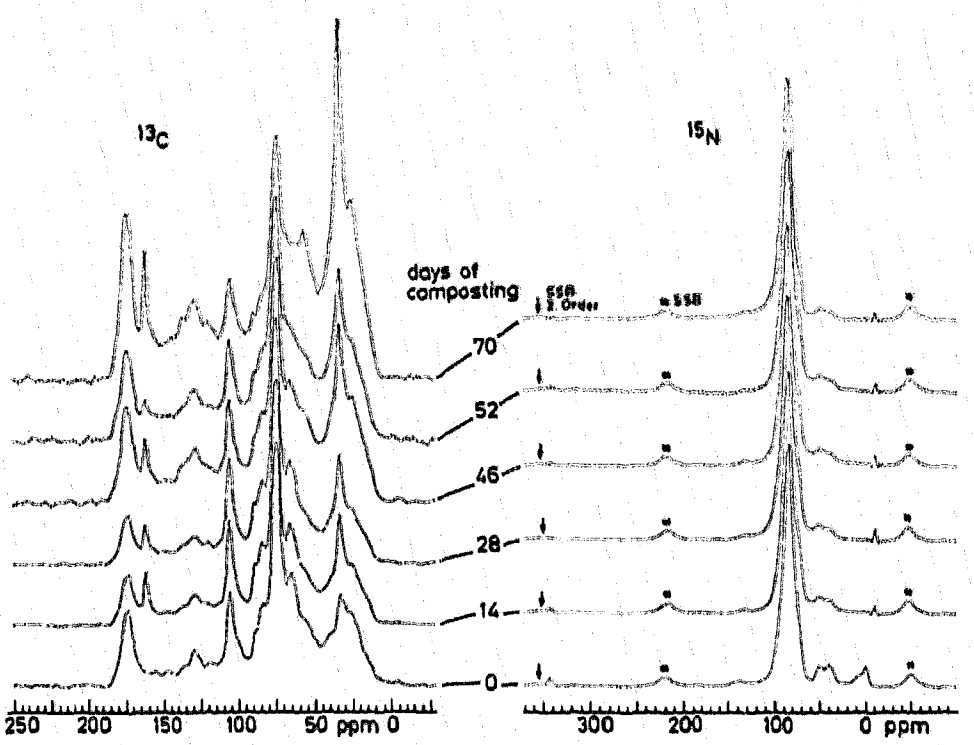

Fig. 1. ^{13}C and ^{15}N CPMAS spectra of the complete freeze-dried composts of *L. rigidum* plant material. (*) SSB = spinning side bands 1st order; (↓) SSB = spinning side bands 2nd order.

unobservable. The most plausible assignments of the resonances are shown in Table I [3].

During composting the signals from the free amino groups and the nucleic acids are diminished most rapidly. Simultaneously NH_4^+ (−9 ppm) is formed. No new signals appear in this series of spectra. It is thus improbable, that the composting process leads to the formation of new heteroaromatic nitrogen containing ring structures to Schiff-bases, as has been claimed previously (see reviews [4,5]). The ^{15}N signals expected for such structures are indicated as missing signals at the bottom of Table I.

The parts of the composts soluble in aqueous sodium hydroxide were also measured in solution by the inverse gated decoupling technique. No additional signals were found, thus indicating, that no ^{15}N signals were suppressed in the cross-polarization experiments.

Compared to the ^{13}C CPMAS spectra the changes observed appear to be less dramatic and of smaller magnitude. Furthermore most of the changes in the ^{15}N-

Table I

Possible assignments of the ^{15}N-NMR spectra of the *L. rigidum* composts given in Fig. 1

Signal	Rel. intensity (%)		Assignment
	I[a]	F[a]	
344 ppm	2	>1	residual nitrate ions
114–140 ppm	4	5	nitrogen at position 9 in purine base of nucleic acids
87 ppm	82	87	peptide nitrogen of proteins
40–55 ppm	8	5	$-NH_2$ groups in bases of nucleic acids
0 ppm	4	>1	free amino groups of peptides, amino acids and amino sugars
−10 ppm	0	4	NH_4^+
Missing signals:			
95–120 ppm			quinonimines[b]
160–180 ppm			pyrrols
280–320 ppm			Schiff-bases
~290 ppm			phenazine derivatives

[a] Intensities: I = iniatial, F: final

[b] Possibly hidden under the strong peptide signal

Table II

Composition of the composted material as determined by integration of the ^{13}C-CPMAS spectra

Plausible assignment of the ranges (see [1]):
220–160: carboxyl/carbonyl
160–110: most aromatic structures
111– 60: carbohydrate derived structures, C-α of peptides
60– 45: methoxylgroups
45– 0: aliphatic structures

Days of composting	Integration range (ppm)				
	220–160 (%)	160–110 (%)	110–60 (%)	60–45 (%)	45–0 (%)
0	9.7	10.4	48.4	9.7	21.8
14	9.0	11.7	50.2	8.3	20.9
28	8.8	11.5	51.3	8.2	20.3
42	10.9	13.5	40.0	9.8	25.6
56	10.6	14.0	40.1	9.8	26.3
70	13.4	15.6	30.8	10.4	30.0

Table III

Relaxation times for ^{15}N in a CPMAS experiment

Sample	Signal (ppm)	T_{NH} (ms)	$T_{1\rho}$ (ms)	T_{1H} (ms)
Starting material (0 days)	86	0.23	5.3	220
	40	0.09	5.5	
	0	0.30	6.9	
Complete compost after 70 days	86	0.09	6.2	10[a]
	40	0.06	6.4	
	0	n.d.	n.d.	

[a] Determined in the ^{13}C spectra

spectra seem to occur in the first 14 days of fermentation, while those for the carbon spectra occur more steadily during the whole fermentation period. At the end of the composting the *Lolium* biomass had lost approximately 80% of the carbon but most of the nitrogen was still present. Table II compiles the relative intensies of the ^{13}C spectra given in Fig. 1. It becomes obvious from the integrals, that in the composting process carbohydrate-derived structures are most rapidly consumed and thus the spectral range between 110 and 60 ppm is reduced in intensity compared to the carboxyl, the aromatic and the aliphatic region. Decomposition of the carbohydrate structures is not likely to influence the nitrogen-containing moieties of the plant material.

In order to learn the quantitative limits of the ^{15}N CPMAS spectra the relevant relaxation times for two probes the starting material and the compost after 70 days are shown in Table III. Contrary to the relaxation times determined for ^{13}C [2], where al T_{CH} values were

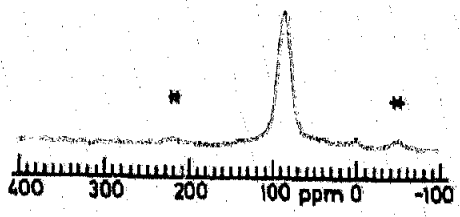

Fig. 2. ^{15}N CPMAS spectrum of the humic acid fraction of a *Zea mays* compost after 150 days of fermentation.

fairly similar, significant differences in the individual T_{NH} are seen at least for the unmodified starting material. The T_{NH} appear to be significantly reduced by the composting procedure. The T_{1H} time, relevant for the repetition of the CPMAS pulse sequence and thus for the signal-to-noise ratio is reduced by more than an order of magnitude in the composts probably through the formation of free organic radicals.

In Fig. 2 the ^{15}N spectrum of the humic acid fraction from a corn straw/sand mixture after 150 days of fermentation is given. The spectrum is very similar to those from the complete humified *L. rigidum* biomass. Again the signal at 87 ppm contains more than 80% of the total intensity and the small well-defined signal at 0 ppm can be ascribed to free amino groups. From the data presented here it appears probable that most of the nitrogen in recent soil organic matter remains in at most slightly modified protein-like secondary amide structures. This conclusion is in contradiction to the results obtained from classical chemical analysis, where approximately 50% of the total nitrogen remains unidentified [4,5].

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