Chapter 17

Characterization of Soil Organic Nitrogen after Addition of Biogenic Waste Composts by Means of NMR and GC–MS

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Application of composts to soils leads to increases in soil organic carbon and nitrogen. The effect of biowaste compost on the chemical composition of the organic N fraction was examined in different soils. Sandy and loamy soils were mixed with biowaste composts, both fresh and mature, and incubated for eighteen months. NMR spectroscopic characterization of the bulk samples and their residues after hydrolysis with 6 N HCl revealed no major changes in the organic nitrogen functionality as a result of the compost application. These spectra show that more than 80% of the total organic nitrogen of all samples was derived from peptide-like material. This conclusion was supported by the results obtained from tetramethylammonium hydroxide (TMAH) thermochemolysis.

Biogenic waste composts are added to agricultural and reclaimed soils to reduce landfill volume for municipal waste. The application of biowaste composts is expected to increase soil organic carbon and nitrogen and therefore to improve the nutrient supply of soils. This treatment also leads to improvement of the physical structure of soils (*1-5*). The efficiency of such a procedure for recultivation is strongly influenced by the properties and the amount of newly formed humic material, but it also depends upon the availability of added nitrogen after recultivation.

The processes involved in the decomposition of organic biowaste appear to be comparable to those involved in the degradation of organic matter. The decomposition of organic wastes follows an exothermic process by biological oxidation. During degradation, materials are chemically and physically transformed into stable humified products (*6-9*). Biddlestone et al. (*6*) described the composting of

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In Nitrogen-Containing Macromolecules in the Bio- and Geosphere; Stankiewicz, B. Artur, et al.;
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organic waste as a"building up" and "breaking down" process. Senesi (*3*) divided the degradation of organic materials into three stages. First, readily decomposable organic compounds, such as sugars, starch, hemicellulose, amino acids and some cellulose, are converted to CO_2 and other volatile compounds such as N_2O and ammonia. In the second stage, organic metabolites, biomass, and the remnants of cellulose, as well as parts of lignin, are incorporated into new biomass and metabolized to CO_2. The final stage is characterized by a gradual degradation of the more resistant compounds such as lignin, which are converted into humic substances.

However, while the soil organic carbon has been the subject of many investigations (*10*, *11*), little is known about the fate of soil organic nitrogen (*12*). Characterization of the organic nitrogen pool is still incomplete and knowledge about the chemical structure and the linking of nitrogen compounds to humic substances is lacking (*13*).

Approximately 90% of the total nitrogen in soils is incorporated into the organic fraction. Generally, this organic nitrogen is analyzed by hot acid hydrolysis with 6 N HCl for 12 to 24 h. Applying this technique, 20 to 35% and 5 to 10% of the organic nitrogen can be found in amino acids and amino sugars, respectively. Twenty to 35% of the organic nitrogen is present as ammonium, while 10 to 20% of the organic nitrogen are hydrolyzed compounds not yet identified (hydrolyzable unknown nitrogen = HUN). Twenty to 35% of the organic nitrogen remains as acid insoluble nitrogen, which may be incorporated into refractory organic materials and protected against biological degradation (*12-16*). This resistance might be explained by the incorporation of nitrogen into heterocyclic compounds such as pyridines, indoles and pyrroles (*16, 17*). Recent evidence, on the other hand, indicated that most of the soil organic nitrogen is in amide functional groups, most probably of biogenic origin (*18-20*).

The intention of this study was to investigate the forms of soil organic nitrogen, found in soils after addition of fresh and mature biowaste composts by means of solid-state ^{15}N NMR and thermochemolysis with tetramethylammonium hydroxide (TMAH) (*21-25*). To learn more about the acid insoluble organic-N forms, these techniques were also applied to the residues obtained after acid hydrolysis of the composts and the soil/compost mixtures (*26*).

Materials and Methods

For incubation experiments soil substrate of a Haplic Luvisol under agricultural use near Witzenhausen, Germany (sand: 6.7 %, silt: 73.8 %, clay: 19.6 %) and a coal mine spoil of the Lusatian lignite mining district of Germany (sand: 93%, silt: 4.6%, clay: 2.4%) were each mixed with 70 t ha^{-1} of a fresh biowaste compost. In a second study, the substrates of the same soil and mine spoil were treated with 65 t ha^{-1} of a mature biowaste compost. The maturity of the composts was determined by the self-heating test (*27*). Under controlled conditions, at a temperature of 14°C and 50% maximum water holding capacity (WHC), the samples were incubated in a microcosm system, as described by Siebert et al. (*28*), for 18 months. Soil substrates without addition of compost were incubated as controls. Immediately after application of the composts and after 18 months of incubation, samples were taken, freeze-dried

and ground. The nitrogen and carbon contents were determined for all samples using an CHN-1000 elemental analyzer (LECO).

Acid Hydrolysis. For the determination of the a-amino acid content, 2 g of each sample were hydrolyzed with 10 ml 6 N HCl for 12 h at 105°C (*29*, *30*). The hydrolyzates were filtered (blue band filter No. 589) and washed with distilled water. The filtrates were lyophilized and freeze-dried. After addition of a sodium citrate solution and a solution containing a ninhydrin reagent, the supernatants were boiled for 20 minutes in a water bath. During this boiling time ninhydrin was allowed to react with α-amino acids to form purple-colored complexes. Their concentration was determined by measuring the optical density of the reacted solution at 570 nm. The amount of a-amino acid N was calculated by a calibration curve with a standard solution of 28 μg leucine ml^{-1}. The non-hydrolyzable nitrogen was obtained by measuring the nitrogen content of the hydrolytic residues.

^{13}C and ^{15}N NMR Spectroscopy. To improve the sensitivity of the NMR experiment, the samples and the hydrolytic residues were treated with 10 % hydrofluoric acid (HF), as described by Schmidt et al. (*31*). HF-treatment of soil samples was shown to increase the concentration of soil organic matter by removal of the mineral matter without any major alteration of the soil organic matter composition. Solid-state cross polarization magic angle (CPMAS) ^{13}C NMR spectra were taken on a Bruker MSL 100. A contact time of 1.0 ms and pulse delays between 100 and 600 ms were used. The magic angle spinning speed was 4.3 kHz. The ^{13}C chemical shifts are referred to external tetramethylsilane (= 0 ppm). Solid-state CPMAS ^{15}N NMR spectra were obtained on a Bruker MSL 300 (7.05T) with a contact time of 1 ms, a pulse delay of 100 ms, and a magic angle spinning speed of 4.3 kHz. The chemical shift assignment is referenced to the nitromethane scale (= 0 ppm) (*32*). A more detailed description of the applied parameters can be found in Knicker and Lüdemann (*20*).

Thermochemolysis with Tetramethylammonium Hydroxide (TMAH). Samples were subjected to thermochemolysis with TMAH in sealed tubes. Previously this technique was shown to cleave peptide-bonds in albumin and to lead to specific methylated derivatives of amino acids (*26*). For their identification, soil and compost samples and their residues (1 mg - 10 mg of sample) were heated with 100 μl TMAH (25% in methanol) for 30 minutes at 250°C. The thermochemolysis products were extracted with methylene chloride and concentrated to 50 μl. An aliquot of 1 μl was injected onto a J&W DB5 MS capillary column (30 m x 0.25 mm) of a combined gas chromatograph mass spectrometer (GC/MS) system (Fison, MD 800). The GC was programmed with an initial temperature of 40°C, a heating rate of 15°C min^{-1} to 100°C. The heating rate was then minimized to 6°C min^{-1} to a final temperature of 300°C. The methylated compounds were identified by their retention times and mass spectra.

Results

Characterization of SOM after Additon of Biogenic Composts to Soils. The biowaste composts are characterized by a low C to N ratio (C/N=13 fresh compost; C/N=12 mature compost). After addition of these composts to the loamy substrate of the Luvisol and to the sandy substrate of the mine spoil, the carbon and nitrogen content of the soil substrates increased. The carbon content increased from 13 mg C g^{-1} soil to 34 mg C g^{-1} soil in the Luvisol after compost addition. As well as the carbon content, the amount of nitrogen increased from 1.7 mg N g^{-1} soil to 3.7 mg N g^{-1} soil. The addition of biowaste composts to the mine spoil resulted in an enrichment of the C and N content from 0 to 17 mg C g^{-1} soil and to 1.8 mg N g^{-1} soil (Table I).

Table I: Chemical Characterization of Biowaste Composts and Soils

	C_t	N_t	C/N	N_{org}	N_{inorg}
	---- mg g^{-1} ----			% of total N	
Compost					
fresh compost	232.7 ± 3.6	18.5 ± 0.3	12.6 ± 0.3	99.9 ± 0.0	0.1 ± 0.0
mature compost	186.1 ± 1.9	15.9 ± 0.3	11.7 ± 0.3	99.5 ± 0.0	0.5 ± 0..0
Mine spoil (0 months)					
soil	n.d.	n.d.	-	-	-
soil + fresh compost	19.1 ± 0.0	2.1 ± 0.0	9.1 ± 0.0	99.6 ± 0.0	0.4 ± 0.0
soil + mature compost	15.2 ± 0.0	1.5 ± 0.0	10.1 ± 0.0	99.0 ± 0.0	1.0 ± 0.0
Mine spoil (18 months)					
soil	n.d.	n.d.	-	-	-
soil + fresh compost	14.7 ± 0.0	1.6 ± 0.1	9.2 ± 0.4	91.2 ± 0.7	8.8 ± 0.7
soil + mature compost	11.9 ± 0.7	1.2 ± 0.1	9.9 ± 0.1	90.2 ± 1.0	9.8 ± 1.0
Luvisol (0 months)					
soil	13.0 ± 0.0	1.7 ± 0.0	7.7 ± 0.0	98.5 ± 0.0	1.5 ± 0.0
soil + fresh compost	33.7 ± 0.0	3.5 ± 0.0	9.6 ± 0.0	99.3 ± 0.0	0.7 ± 0.0
soil + mature compost	35.0 ± 0.0	3.8 ± 0.0	9.2 ± 0.0	99.0 ± 0.0	1.0 ± 0.0
Luvisol (18 months)					
soil	12.7 ± 0.0	1.7 ± 0.0	7.5 ± 0.2	94.6 ± 0.4	5.4 ± 0.4
soil + fresh compost	26.5 ± 0.1	3.3 ± 0.0	8.0 ± 0.1	88.6 ± 0.1	11.4 ± 0.1
soil + mature compost	27.0 ± 1.3	3.4 ± 0.1	8.0 ± 0.2	90.5 ± 0.0	9.5 ± 0.0

n.d = not detectable; Standard error (compost: n = 6; soil, soil + compost: n = 2)

After an incubation time of 529, days the total C content of the Luvisol treated with fresh compost decreased from 33.7 mg C g^{-1} soil to 26.5 mg C g^{-1} soil. A comparable decrease from 35 mg C g^{-1} soil to 27 mg C g^{-1} soil was determined for the Luvisol treated with mature compost. During the same time period, the nitrogen content of the Luvisol treated with fresh compost decreased only from 3.5 mg N g^{-1} soil to 3.3 mg N g^{-1} soil and in the Luvisol incubate mixed with mature compost from

3.8 mg N g^{-1} soil to 3.4 mg N g^{-1} soil. Consequently, the C/N ratios of the soil compost mixtures declined from approximately 9 and 10 at the beginning of the experiment to 8 after 18 months of incubation. In contrast to the Luvisol, the mine spoil, as a sandy sterile substrate, contains almost no organic material (Table I). Therefore, changes in organic matter composition of the mine spoil/compost mixture occurring during incubation are only related to the decomposition of the mature and fresh compost. During incubation, a decrease in the total C and N contents of approximately 23% and 24%, respectively, was observed in the mine spoil sample mixed with fresh compost. In the mine spoil substrate mixed with mature compost, the total C declined to 22% and the total N to 20% as result of 18 months incubation. This indicates that the changes in elemental composition occurring during incubation of the mine spoil/compost mixtures, but also in the Luvisol/compost mixtures, are mainly induced by the degradation of the compost materials. However, in both the incubates of the Luvisol and the mine spoil mixed with composts, the total nitrogen and carbon content is still higher than in the control samples (Table I). From these results it can be assumed that additon of biowaste compost to arable and reclaimed soils leads to an increase in the soil organic matter level.

For all samples, with the exception of the mine spoil substrate, the relative concentration of organic and inorganic nitrogen in relation to the total nitrogen of the samples was determined (Table I). Less than 2% of the total nitrogen was assigned to inorganic nitrogen in the pure composts and soil substrates. At the beginning of the incubation experiment, less than 1% of the total nitrogen originates from inorganic nitrogen. After 529 days of incubation, the inorganic N fraction increased in the soil and compost mixtures to 10% of total nitrogen, indicating that at least some mineralization of organic N occurred. The N mineralization in the soil without composts yielded only 4% of total nitrogen. However, at this time approximately 90% of the total nitrogen in all incubates were still found in the organic fraction, revealing that organic nitrogen of the composts was incorporated into the stable organic fraction of the soil.

Characterization of SON by Acid Hydrolysis. The characterization of SON by wet chemical analysis with 6 N HCl shows that 17 to 31% of the organic N is present as a-amino acids in the composts, Luvisol and in the soils-compost mixtures (Table II). The maturity of biowaste composts did not affect the yield in acid hydrolyzable amino acid content. After hydrolysis, a high N amount (>60% of organic N) remained in the hydrolytic residues of the composts, showing that only a small portion of the nitrogen pool is liberated by acid hydrolysis of compost materials. This agrees with the results of Bremner (*33*), who found that 20 to 60% of the nitrogen in humic acids is not solubilyzed by hydrolysis. Addition of biowaste composts to soils leads to a relative decrease of the N content in the hydrolytic residues. However, 28 to 43% of the organic N still remained in the refractory N fraction after compost addition to soil. Without compost addition the soils yielded the lowest amounts of acid insoluble N (<25% of organic N), while the a-amino N content in the soil was more or less the same as in the soil and compost mixture. After addition of composts to the soil, the hydrolyzable unknown N fraction yielded 36 to 47% of organic N. This fraction

consisting of amino sugars and ammonia from the partial destruction of amino acids, such as serine, threonine and tryphtophan during hydrolysis (*13*). Schnitzer (*16*) and others have pointed out that some of the unknown nitrogen may be present in heterocyclic structures. However, with the exception of a small amount of purines and pyrimidines, such heterocyclic compounds could not be identified in soils in relevant amounts (*16*).

Table II: Organic Nitrogen Fractions after Acid Hydrolysis

	a-amino N	HUN	Hydrolytic residue
		% of organic nitrogen	
Compost			
fresh compost	30.9 ± 0.7	5.2 ± 1.7	63.9 ± 1.1
mature compost	24.0 ± 0.3	0.0 ± 1.6	76.0 ± 1.4
Mine spoil (0 months)			
soil	n.d.	n.d.	n.d.
soil + fresh compost	21.8 ± 0.6	44.7 ± 1.8	33.5 ± 2.4
soil + mature compost	16.6 ± 0.0	43.1 ± 3.3	40.3 ± 3.3
Mine spoil (18 months)			
soil	n.d.	n.d.	n.d.
soil + fresh compost	34.8 ± 0.5	37.8 ± 0.6	27.4 ± 0.0
soil + mature compost	26.9 ± 0.2	36.1 ± 0.7	37.0 ± 0.5
Luvisol (0 months)			
soil	18.1 ± 3.3	58.1 ± 3.3	23.8 ± 0.0
soil + fresh compost	16.0 ± 0.7	40.8 ± 0.8	43.2 ± 1.4
soil + mature compost	19.8 ± 0.5	40.3 ± 1.8	39.9 ± 1.3
Luvisol (18 months)			
soil	21.8 ± 0.9	53.3 ± 1.3	24.9 ± 0.9
soil + fresh compost	19.5 ± 1.3	46.4 ± 1.2	34.1 ± 0.2
soil + mature compost	21.0 ± 0.3	46.5 ± 1.6	32.5 ± 1.5

HUN = hydrolyzable unknown nitrogen; n.d. = not detectable;
Standard error (compost: n = 6 ; 0 months: n = 2 ; 18 months: n = 4)

The results listed above in Table II indicate that the addition of composts to the soils leads to an increase in the nitrogen content of the hydrolytic residues. By this it can be concluded that, already in the compost material, some of the nitrogen is incorporated into the organic fraction. It has been further shown that detailed information about the linking of the organic nitrogen in the soils and composts cannot be obtained by acid hydrolysis alone.

Characterization of Soil Organic Matter by ^{13}C NMR. The ^{13}C NMR spectra of the composts and their hydrolytic residues are presented in Figures 1a and 1b. As revealed in Table III, the fresh and mature compost do not show major differences in the bulk chemical composition. Both ^{13}C NMR spectra are characterized by intense signals in the chemical shift region of 0-alkyl carbons (110 to 45 ppm), most tentatively assigned to carbohydrates. However, carbon bound in ether or alcoholic groups may also contribute to the signal intensity in this chemical shift region. It is also worthwhile to mention, that besides methoxyl carbon, amine-substituted carbon may add to the signal in the chemical shift region between 60 and 45 ppm. The peaks in the chemical shift region between 160 and 110 ppm are assigned to aromatic carbons. The signals between 220 and 160 ppm are attributed to carboxylic C and/or amide C, while those between 45 and 0 ppm originate from alkyl carbons, i.e. in aliphatic chains. The similarity between the ^{13}C NMR spectra of the fresh and mature composts suggests that most of the chemical alterations during degradation occurred during an earlier stage of the decomposition process. In general, such alterations are characterized by a loss of carbohydrates and a relative enrichment of aromatic, carboxylic and aliphatic carbons (*11*, *20*, *34*, *35*). With compost maturity the degradation process slows down. Changes in the chemical composition of the degrading material become less detectable by ^{13}C NMR spectroscopy.

Table III: Relative Carbon Distribution of the Composts, the Luvisol, the Luvisol/Compost Mixtures after 18 Months of Incubation and their Hydrolytic Residues determined by Solid-State ^{13}C NMR

	Carboxyl C 220-160	Aromatic C 160-110	O-alkyl C 110-45	Aliphatic C 45 - -10
			ppm	
		% of total signal intensity		
Untreated materials				
Fresh compost	11	19	49	22
Mature Compost	9	22	54	15
Luvisol	10	23	49	18
Luvisol/fresh compost	10	25	45	20
Luvisol/mature compost	10	25	45	20
HCl - residues				
Fresh compost	6	30	40	23
Mature compost	7	32	42	19
Luvisol	7	34	36	23
Luvisol/fresh compost	7	35	36	22
Luvisol/mature compost	7	36	35	22

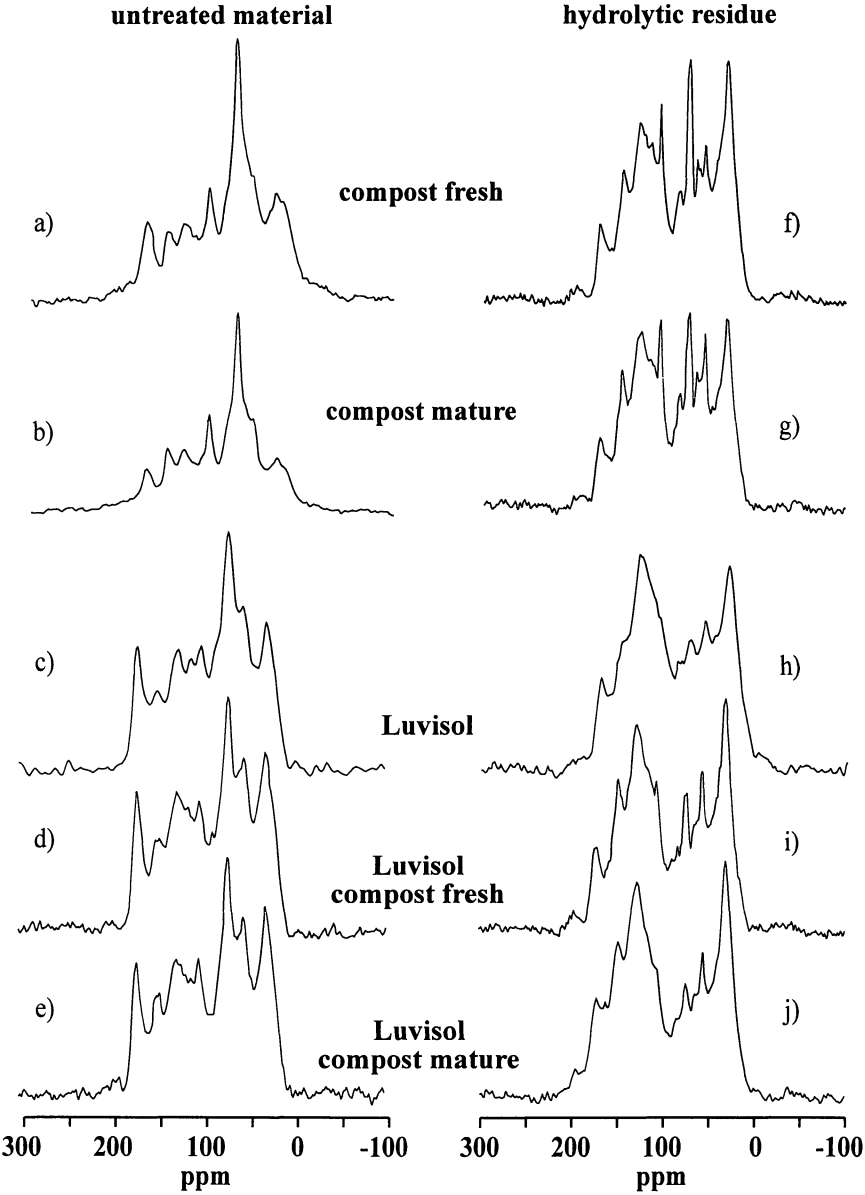

Figure 1. Solid-state ^{13}C NMR spectra of the composts, the Luvisol, the Luvisol/compost mixtures and their hydrolytic residues.

The ^{13}C NMR spectrum of the untreated Luvisol (Figure 1c) still shows its major signal intensity in the chemical shift region assigned to carbohydrates (110 to 45 ppm). However, compared to the ^{13}C NMR spectra of the composts, the relative intensity in this region is diminished in favor of the relative intensity in the region assigned to aromatic carbons. The ^{13}C NMR spectra of the Luvisol, obtained 18 months after addition of the composts (Figure 1d, e) reveal a similar pattern to that of the natural soil. From this, it can be assumed that the composition of the organic material in the soil/compost mixture is still determined by the soil substrate. The application of biowaste composts to the soil, therefore, did not lead to major alterations in the soil organic matter of the Luvisol.

Acid hydrolysis with 6 N HCl of the composts and the soil substrate/compost mixtures resulted in a relative enrichment of aromatic compounds. This is revealed by comparison of the intensity distribution in the ^{13}C NMR spectra of the untreated samples and the hydrolytic residues (Table III; Figure 1a-j). The decrease of signal intensity in the chemical shift region between 110 and 45 ppm (O-alkyl carbon) and 220 to 160 ppm (carbonyl carbon, carboxyl- and amide carbons) as a result of the hydrolysis indicates the loss of labile compounds such as carbohydrates and amino acids. Compared to the ^{13}C NMR spectrum of the hydrolytic residue of the Luvisol (Figure 1h), a more intense signal at 72 ppm in the hydrolytic residues of the compost and soil/compost mixtures is observed. This can be explained by the presence of higher amounts of acid insoluble cellulosic material in the composts than in the more humified organic material of the soil.

Characterization of Soil Organic Nitrogen by ^{15}N NMR. The ^{15}N NMR spectra of the composts and their hydrolytic residues are presented in Figure 2a, b, f, g. The untreated composts and the hydrolytic residues of the composts show similar spectra. A broad signal is found in the chemical shift region between -220 and -285 ppm, most probably assigned to amide/peptide functional groups (*20*). No signals, distinguishable from the noise, are observed in the chemical shift region of pyridinic N (-40 to -145 ppm), indicating that such compounds were not formed in higher amounts during decomposition. However, nitrogen in acetylated amino sugars, lactams, unsubstituted pyrroles, indoles and carbazoles may contribute to the intensity of the main peak. The heterocyclic N in histidine, nucleic acid derivatives and substituted pyrroles is expected to contribute to the chemical shift region between -145 and -220 ppm, and may be hidden by the broad main signal at -260 ppm. Had such compounds been major contributors to the total nitrogen, the main peak at -260 ppm would be shifted towards lower field.

The ^{15}N NMR spectra of the Luvisol and the soil substrate/compost mixtures in Figure 2 c-e show a comparable pattern to that of the ^{15}N NMR spectra of the untreated composts. They are also dominated by a peak at -260 ppm, assigned to amide functional groups. The similarity of the ^{15}N NMR spectra of the composts, soil and soil substrate compost mixtures indicates that, in all of these samples, the nitrogen is bound in similar functional groups. Even after 18 months of incubation, no major alterations in N functionality are detected by ^{15}N NMR spectroscopy. In these spectra, no major signal intensity can be observed in the chemical shift region of

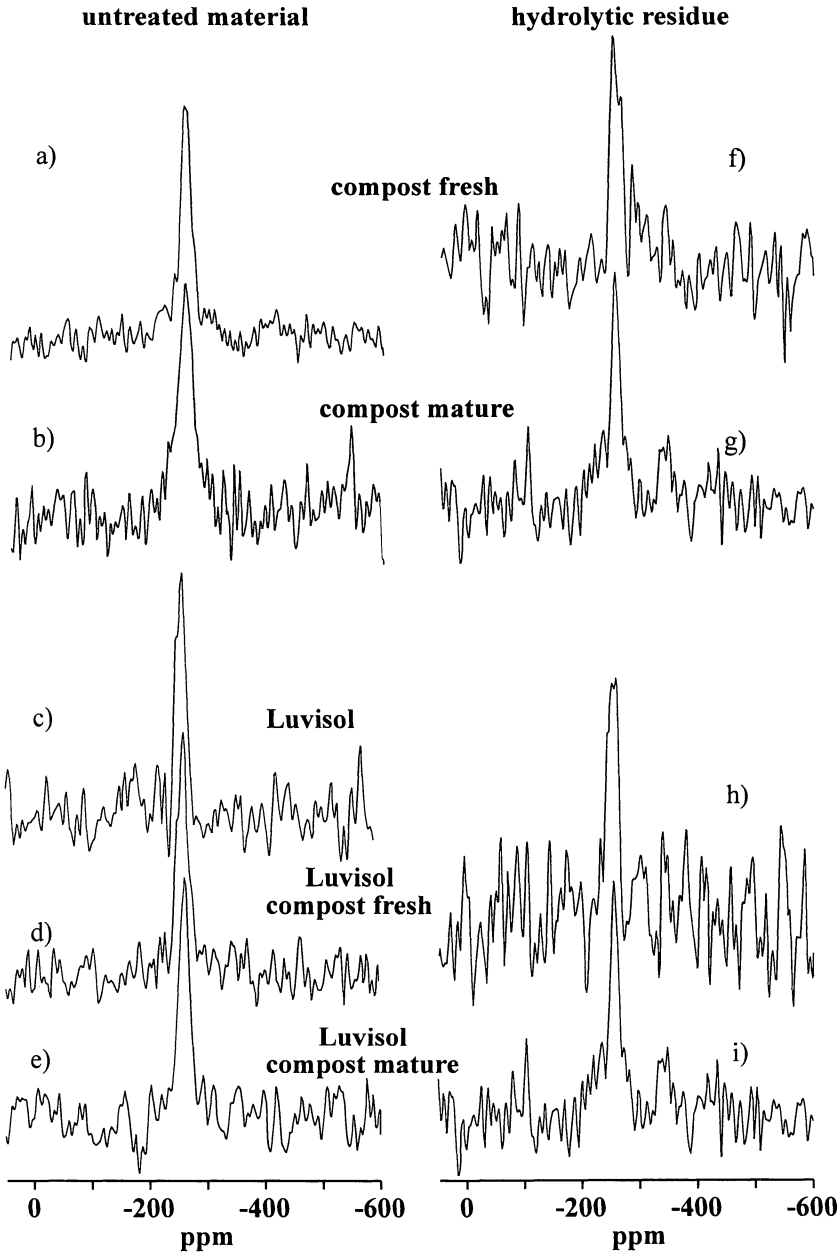

Figure 2. Solid-state ^{15}N NMR spectra of the composts, the Luvisol, the Luvisol/compost mixtures and their hydrolytic residues.

pyridinic N or pyrrolic N. From these results, it can be concluded that heterocyclic nitrogen is not a major form of organic nitrogen in these samples. The ^{15}N NMR spectra shown here rather support the assumption that some amide functional groups, presumably of biogenic origin, are more resistant to microbial degradation than previously thought (*18-20*, *26*, *36*, *37*).

It is generally believed that most of the proteinaceous material is hydrolyzed by acid hydroylsis. In our experiments, on the other hand, amino acid N, liberated with acid hydrolysis, can only explain less than 30% of the total signal intensity observable in ^{15}N NMR spectra of the composts, Luvisol and soil substrate/compost mixtures. From this observation it can be concluded that some amides, identified with ^{15}N NMR spectroscopy, are not hydrolyzed by the commonly used acid hydrolysis.

To verify this conclusion, the acid insoluble residues obtained after HCl-hydrolysis of the composts and the soil substrate/compost mixtures were also subjected to ^{15}N NMR spectroscopy. In order to increase the sensitivity of the ^{15}N NMR experiment, the residues, depleted in organic material, were treated with HF. As revealed from Table IV, applying this technique increased the relative amount of organic nitrogen of the samples to a factor which allowed us to obtain ^{15}N NMR spectra with acceptable S/N ratios.

Table IV: Enrichment of C and N in the Hydrolytic Residue due to Treatment with 10% (v/v) HF

	Before treatment	After treatment*	Enrich-ment[1]	Before treatment	After treatment*	Enrich -ment[1]
	C mg g^{-1}			N mg g^{-1}		
Compost mature	189.4 ± 0.3	552.2	2.9	12.0 ± 0.1	33.8	2.8
Compost fresh	251.4 ± 0.6	571.2	2.3	11.8 ± 0.0	26.0	2.2
Luvisol/ compost mature	16.0 ± 0.1	467.4	29.0	1.2 ± 0.0	22.3	25.7
Luvisol/ compost fresh	17.0 ± 0.3	472.7	27.3	1.0 ± 0.0	25.7	22.3

[1] Enrichment = [content of C or N after HF treatment]/[content of C or N before HF treatment]. Standard error (n = 2); * - single analyzed.

The non-hydrolyzable residues (Figure 2 f-i) show the main intensity in the chemical shift region assigned to amide functional groups. Thus, hydrolysis with 6 N HCl failed to significantly alter the spectral signature of this refractory amide-like N. Similar to the ^{15}N NMR spectra of the bulk samples, no signals indicative of pyrrolic N or pyridinic N, can be observed in the ^{15}N NMR spectra of the residues. This result strongly contradicts common models, in which acid insoluble nitrogen in soils is explained by the formation of nitrogen heterocyclic aromatics during the humification process. The ^{15}N NMR spectra of the hydrolytic residues presented here rather

support the above mentioned assumption that most of the N in the non-hydrolyzable residues of our samples is present in form of amides, which are protected from the intense chemical hydrolysis.

Characterization of SON by Thermochemolysis with Tetramethylammonium Hydroxide (TMAH). The procedure of thermochemolysis with tetramethylammonium hydroxide (TMAH) has been demonstrated to cleave ester and ether bonds (*38*) and only recently was shown to efficiently cleave peptide bonds (*26*). Also, Schulten and Sorge (*39*) determined nitrogen compounds in the form of N,N-dimethylamides in soils applying a comparable technique, the pyrolysis methylation reaction with TMAH. If some of the amide nitrogen observed in the ^{15}N NMR spectra of the hydrolytic residues originate from proteinaceous or peptide-like material, such compounds should be cleaved during thermochemolysis with TMAH. Their methylated products were then identified with gas chromatography/mass spectrometry. Figure 3 represents the chromatogram of the TMAH/thermochemolysis products obtained from the hydrolytic residue of the mature compost. The numbered peaks (A-7 to A-17) indicate products, which were also identified after TMAH/thermochemolysis of bovine serum albumin (*26*). These products and their possible origin are listed in Table V. We did not find any peaks indicating higher amounts of TMAH/thermochemolysis products of pyridinic N in the hydrolytic residues of our samples.

Table V: Peak Assignments for Proteinaceous Material of Hydrolyzed Mature Compost by Thermochemolysis with TMAH

Peak	Compound	Possible origin
A-7	Dimethylalanine, methyl ester	Alanine
A-10	Benzaldehyde	Phenylalanine
A-11	Dimethylvaline, methyl ester	Valine
A-12	Benzene, (methoxymethyl)-	Thyrosine
A-14	Butanediocic acid, dimethyl ester	
A-15	N-methyl-, L-proline, methyl ester	Proline
A-17	N,N dimethyl, leucine (or isoleucine), methyl ester	Leucine or Isoleucine

Similar results were obtained for the soil substrate/compost mixtures. The identification of proteinaceous materials in the hydrolytic residues of our samples confirms the results of Knicker and Hatcher (*26*) that some peptide-like compounds in decaying organic material are protected against acid hydrolysis. Due to the low mineral matter content of the sediment, the resistance of proteinaceous material in the sapropel of Mangrove Lake was explained by the encapsulation of peptides into a refractory network of algal material. In soil with considerable mineral content, this mineral matrix may be involved in the stabilization of otherwise labile compounds. Peptide-like material can be adsorbed onto clay minerals and organic matter (*13*, *40*).

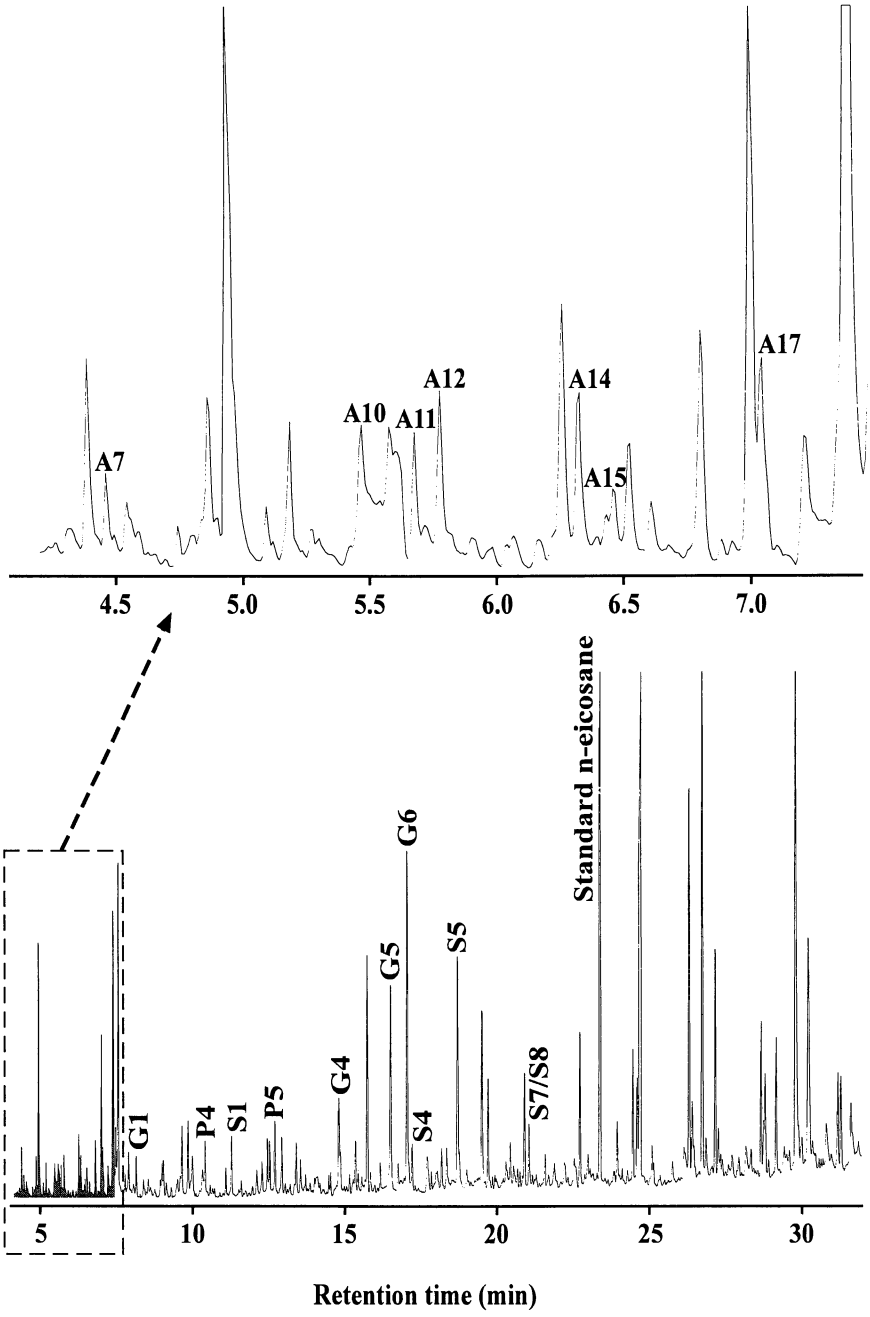

Figure 3. Gas chromatogram of the TMAH/thermochemolysis products of the hydrolytic residue of the mature compost.

Proteinaceous material protected in such a fashion may resist hydrolysis with 6 N HCl.

Summary and Conclusions

The objective of this study was to characterize the soil organic nitrogen pool after the amendment of biowaste compost to an agricultural soil and to a reclaimed sandy substrate of a mine spoil. For the characterization of SON, the composts, the soils and the soil/compost mixtures were analyzed by acid hydrolysis and by means of ^{13}C and ^{15}N NMR spectroscopy. Detailed information about the refractory nitrogen fraction was obtained by the examination of the acid hydrolysis residues from the different materials by ^{15}N NMR spectroscopy and by thermochemolysis with TMAH.

The addition of biogenic waste composts to soils led to an increase in soil organic carbon and nitrogen. After acid hydrolysis 17 to 31% of the organic N were identified as a-amino acids, 36 to 47% of the organic N was lost by hydrolysis as unknown nitrogen. 28 to 43% of organic N remained in the hydrolytic residue. ^{13}C NMR spectroscopic characterization of a Luvisol before and 18 months after application of biowaste composts indicated no major alterations of the soil organic matter composition. Acid hydrolysis resulted in a loss in intensity in the chemical shift region of O-alkyl carbons and carboxyl carbons of the ^{13}C NMR spectra of the composts, the substrate of a Luvisol, and mixtures of a Luvisol with biowaste composts. Increase in relative intensity was observed in the chemical shift region of aliphatic and aromatic carbons.

Characterization of the soil organic nitrogen pool was done by solid-state ^{15}N NMR spectroscopy. The organic nitrogen of the soils and composts is characterized by a broad signal in the chemical shift region between -220 and -285 ppm, which is tentatively assigned to amide/peptide functional groups. More than 80% of the total organic nitrogen is bound in peptide-like structures. To characterize the nature of the acid insoluble nitrogen, solid-state ^{15}N NMR spectroscopy was applied to the hydrolytic residues of the biowaste composts and the Luvisol/compost mixtures. Generally, it is assumed that peptide-like material in soils is quickly hydrolyzed with 6 N HCl. Only nitrogen bound in chemically- or physically-protected compounds should remain in the acid insoluble residue. In common models, it is assumed that this refractory nitrogen is incorporated into N-heterocyclic structures. However, in the studies presented here, such heterocyclic structures could not be identified by ^{15}N NMR spectroscopy. For detailed characterization we subjected the hydrolytic residues to a new technique of thermochemolysis with tetramethylammonium hydroxide (TMAH) and analyzed the products with gas chromatography/mass spectrometry. Several methylated products of amino acid derivatives were identified, but methylated compounds related to heterocyclic nitrogen compounds could not.

Our results confirm that refractory nitrogen in soil organic matter is probably composed of peptide-like material (*19*, *20*). They further support the assumption of previous studies (*27*) that acid hydrolysis with 6 N HCl fails to attack all proteinaceous compounds in organic material of soils and sediments. The mechanisms, responsible for the resistance of such compounds towards acid

hydrolysis may be similar to those involved in their protection against microbial degradation. Such mechanisms could be of physical or chemical nature involving the adsorption to clay minerals (*15*) or humic material incorporation into mineral sheets (*41*) but also as part or in connection with refractory biopolymers (*26*, *36*). However, regardless of the mechanism involved in the protection of these generally thought labile compounds in soil, their presence in soils could partly explain the nature of the commonly-termed unidentified nitrogen.

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