Characterization of Organic Matter in Soils by Thermochemolysis Using Tetramethylammonium Hydroxide (TMAH)

Benny Chefetz,* Yona Chen, C. Edward Clapp, and Patrick G. Hatcher

ABSTRACT

Tetramethylammonium hydroxide (TMAH) thermochemolysis–gas chromatography/mass spectrometry (GC/MS) was employed to study the chemical structure of soil organic matter sampled from a soil plot in which corn (*Zea mays* L.) was farmed continuously for 15 yr. The chromatograms exhibited peaks related to compounds derived from lignin, fatty acid methyl esters (FAMEs), non-lignin aromatic structures, and heterocyclic N compounds. The dominant lignin-derived peaks in the TMAH thermochemolysis–GC/MS chromatograms were mainly derivatives of *p*-hydroxyphenyl and guaiacyl structures, suggesting a non-woody (grass) lignin type. With depth, the ratio of syringyl to guaiacyl compounds (S/G) decreased, suggesting a preferential degradation of the syringyl units by microorganisms. Fatty acid methyl esters of varying C-chain length (C_7 to C_{27}) were identified in the soil chromatograms. Both TMAH–GC/MS and ^{13}C-NMR (nuclear magnetic resonance) data suggested a relative increase of long-chain fatty acids with soil depth (or degree of humification), suggesting a refractory nature for these compounds. The heterocyclic N compounds yielded from the TMAH thermochemolysis were mainly pyrroles, pyridines, and pyrazoles. In addition, low levels of methylated amino acids (phenylalanine, leucine, and valine) were detected. The presence of the amino acids in the bottom layer of the soil suggests a preservation mechanism. The changes identified in the chemical components provide clues as to the nature of the humification processes in the soil profile and also yield information on the nature of the sources of soil organic matter.

Thermochemolysis in the presence of tetramethylammonium hydroxide (TMAH; off-line thermochemolysis) is a novel technique that provides new detailed information on the structure and composition of soil organic matter (SOM). This technique and pyrolysis in the presence of TMAH (in situ methylation) have been introduced as effective techniques for analyzing a large variety of natural macromolecules. These techniques were used to characterize lignin (Hatcher et al., 1995; Clifford et al., 1995; Hatcher and Minard, 1996), humic substances (Hatcher and Clifford, 1994; Martin et al., 1994, 1995; del Rio and Hatcher, 1996), coalified woods (McKinney and Hatcher, 1996), carbohydrates (Fabbri and Helleur, 1999), lipids (Challinor, 1996; Ishida et al., 1999), and cutan (McKinney et al., 1996). It has been demonstrated that the TMAH technique is a chemolytic procedure that hydrolyzes and methylates esters and ether linkages, assisting depolymerization and methylation. Methylation makes the polar products of the reaction volatile enough for gas chromatographic analysis. The TMAH thermochemolysis was found to be effective in cleaving the major linkage in native lignin, the β-O-4 ester bond (Hatcher and Minard, 1996). A detailed study of the TMAH reaction mechanism using ^{13}C-labeled TMAH suggests that the cleavage of the β-O-4 ester bond in lignin proceeds through a base-catalyzed intermolecular epoxidation, with the α or γ hydroxyl acting as a nucleophile, resulting in a loss of the phenoxide group (Filley et al., 1999).

Soil organic matter–extracted fractions such as humic acid (HA), fulvic acid (FA), and humin have been analyzed by TMAH thermochemolysis. Hatcher and Clifford (1994) and del Rio et al. (1994) have shown that the TMAH thermochemolysis of HA yields monomethoxy, dimethoxy, and trimethoxy benzecarboxylic acid methyl esters and fatty acid methyl esters (FAMEs) originating from structural components of the HA.

Soil organic matter in agricultural soils consists of a mixture of plant, microbial, and insect residues; dissolved organic matter; and humic substances (HS). Humic substances are formed during microbial decomposition of plant litter (Stevenson, 1994). With soil depth, SOM is mineralized and humified, resulting in a mixture of structurally identifiable materials as plant residue macromolecules and HS. Therefore, knowing the chemical structure of plant tissues and their transformation in soils is essential to understanding the humification processes that occur in soils. In this study, we applied the off-line TMAH thermochemolysis–GC/MS together with solid-state ^{13}C-NMR, using the cross polarization magic angle spinning (CPMAS) with the ramp-CP technique, for the analysis of bulk (untreated) agricultural soil profile. Our goal is to use these chemical approaches on untreated samples to develop a better understanding of how humification occurs in a well-defined profile from a cultivated soil.

MATERIALS AND METHODS

Soil Profile Samples

The soil samples used in this study were provided from a field experiment at Waseca, MN. In short, soil samples (Webster clay loam) from four depths, 0 to 7.5, 7.5 to 15, 15 to 30, and 30 to 45 cm, were collected from a 9.9- by 9.1-m plot in which only corn was grown continuously for 15 yr. The samples were air-dried, sieved through a 2-mm sieve, and oven-dried

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Abbreviations: C/G, cinnamyl phenol to guaiacyl ratio; CPMAS, cross polarization magic angle spinning; FA, fulvic acid; FAMEs, fatty acid methyl esters; G, lignin derived guaiacyl compounds (methylated); GC, gas chromatograph; HA, humic acid; HS, humic substances; MS, mass spectrometry; NMR, nuclear magnetic resonance; P, lignin-derived *p*-hydroxyphenyl compounds (methylated); S, lignin-derived syringyl compounds (methylated); S/G, syringyl to guaiacyl ratio; SOM, soil organic matter; TMAH, tetramethylammonium hydroxide.

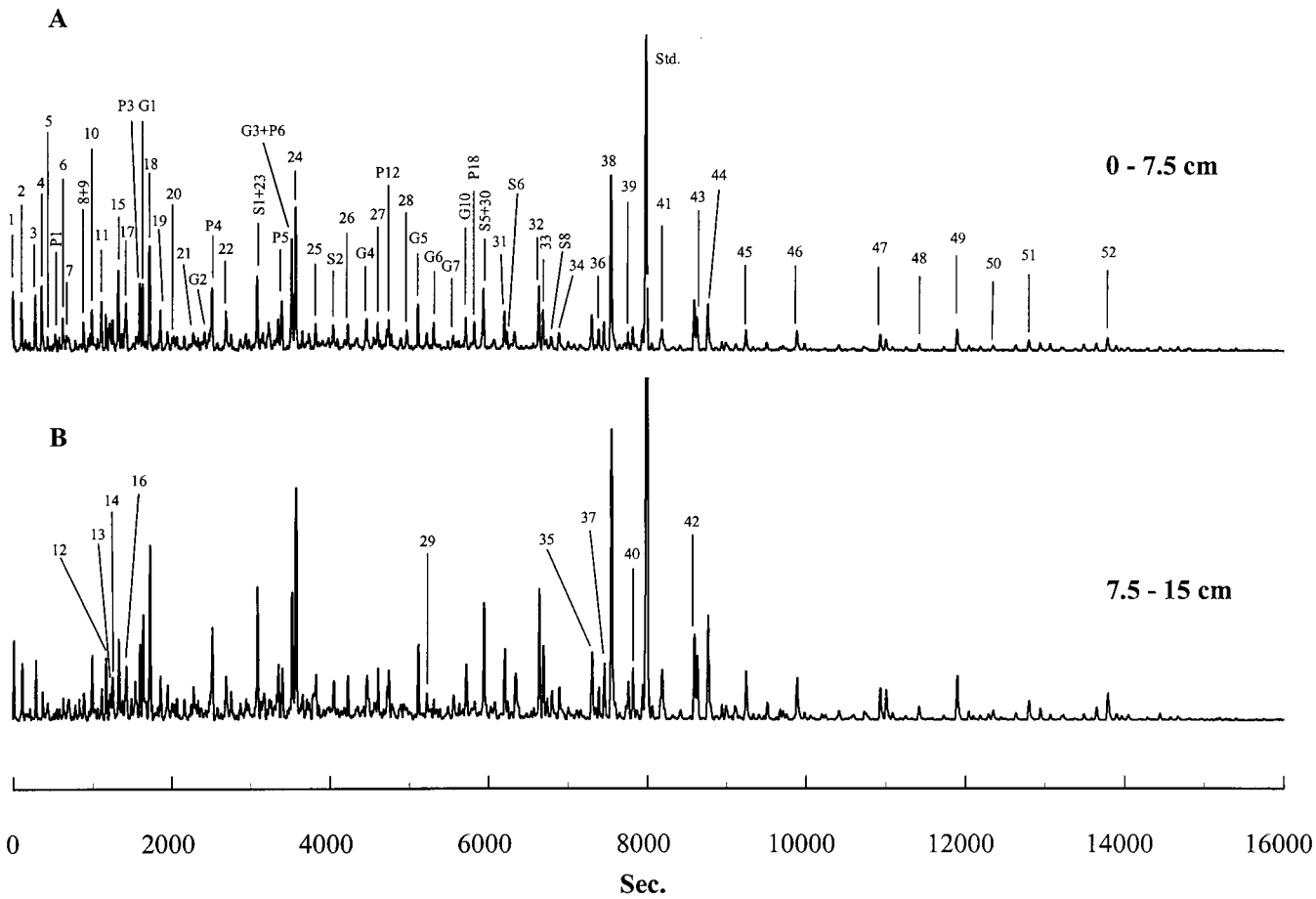

Fig. 1. (A) Tetramethylammonium hydroxide (TMAH) thermochemolysis–gas chromatography/mass spectrometry chromatogram of 0- to 7.5-cm soil layer, and (B) 7.5–15 cm soil layer. For peak identification refer to Table 1.

(65°C) before analysis. Total SOM content was measured by loss of weight on ignition at 550°C for 8 h.

Tetramethylammonium Hydroxide Thermochemolysis–Gas Chromatography/Mass Spectrometry

For the TMAH analysis, 1 to 2 mg of soil organic matter was used. Therefore, 50 mg of oven-dried soil samples from the 0- to 7.5-cm and 7.5- to 15-cm layers (3–4.5% organic matter), and 100 mg of samples from the 15- to 30-cm and 30- to 45-cm layers (1.5–2% organic matter) were weighed and placed in glass tubes (three replicates). Two hundred milliliters of TMAH (25% in methanol; Aldrich, Milwaukee, WI) was added to the tubes containing the soil samples and gently mixed before the methanol was evaporated under a stream of N_2. Then, the tubes were sealed under vacuum and subsequently placed in an oven at 250°C for 30 min. After cooling, the tubes were cracked open, internal standard (1951 ng of *n*-eicosane) was added, and the inside surfaces were extracted (three times) using ethyl acetate. The combined extracts were reduced to ≈50 mL under a stream of N_2. Gas chromatographic analyses were performed using a Hewlett-Packard 6890 gas chromatograph (Hewlett-Packard, Palo Alto, CA) equipped with a 15-m fused silica capillary column coated with chemically bound DB-5 (0.25-mm i.d., film thickness of 0.1 mm; Supelco, Bellefonte, PA). Samples (1 mL) were injected using an autoinjector (Hewlett-Packard 7683 series), with a split ratio of 5 and a front inlet temperature of 310°C. Helium was used as a carrier gas with a flow rate of 1 mL min^{-1}; electronic flow control was set for constant flow. The GC oven temperature was programmed from 40 to 300°C at a rate of 8°C min^{-1}. The GC was directly coupled to a Pegasus II (Leco, St. Joseph, MI) time-of-flight mass spectrometer by a deactivated fused silica transfer line heated to 300°C. Mass spectra, from 33 to 700 m/z, accumulated at a rate of 9 scans s^{-1}. Most peaks were identified by comparison with the NIST (version 1.6) library.

For quantitative purposes, a single response factor was used for all syringyl lignin–derived compounds and another for all guaiacyl lignin–derived compounds. The response factors were calculated by taking the average of the response factors of the commercial standards dimethoxybenzaldehyde and trimethoxybenzaldehyde (G4 and S4, respectively) and dimethoxybenzoic and trimethoxybenzoic acid methyl ester (G6 and S6, respectively) relative to the standard (*n*-eicosane). The syringyl to guaiacyl ratios (S/G) were calculated by summing the responses for amounts of the compounds containing a syringyl structure divided by the sum of responses for compounds containing a guaiacyl structure.

Carbon-13 Nuclear Magnetic Resonance Spectroscopy

Solid-state CPMAS ^{13}C-NMR spectra were obtained using a Bruker DPX 300-MHz NMR-spectrometer (Bruker Analytic GmbH, Germany). The spectrometer operates at a ^{1}H frequency of 75 MHz and a ^{13}C frequency of 300 MHz. The experimental parameters were the following: contact time of 3 ms; recycle delay time of 1 s; sweep width of 27 kHz

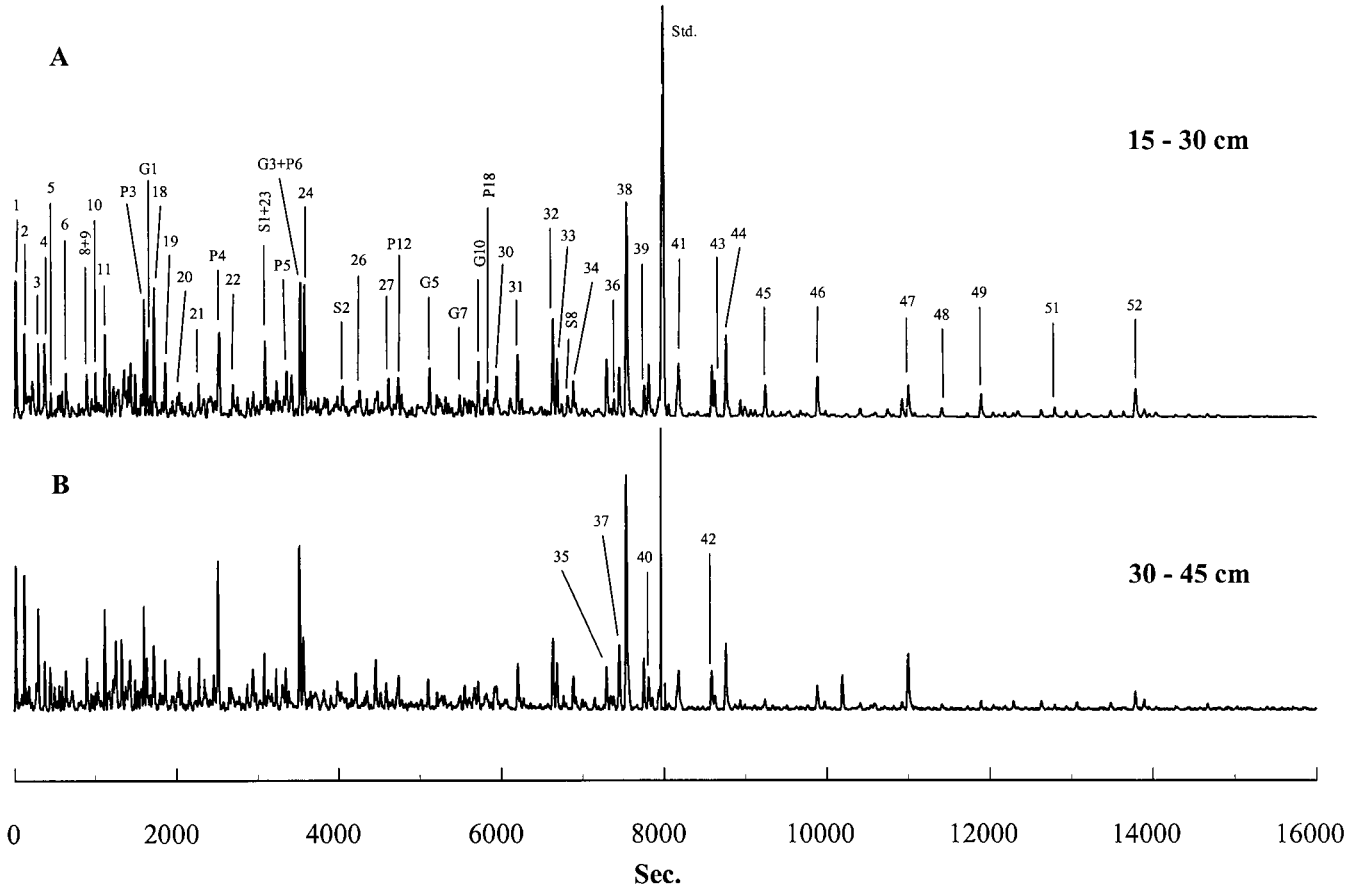

Fig. 2. (A) Tetramethylammonium hydroxide (TMAH) thermochemolysis–gas chromatography/mass spectrometry chromatogram of 15–30 cm soil layer, and (B) 30–45 cm soil layer. For peak identification refer to Table 1.

(368 ppm), and line broadening of 100 Hz. Freeze-dried samples were placed into a 4-mm rotor and spun at a frequency of 13 kHz at the magic angle (54.7° to the magnetic field). The CP method described by Cook and Langford (1998) was used to obtain quantitative CPMAS spectra at this field. The two-pulse phase-modulation procedure was also used.

RESULTS AND DISCUSSION

The TMAH thermochemolysis is highly selective for specific polar groups such as esters, phenols, and acids. It provides information concerning the molecular fragments comprising the SOM. Therefore, to elaborate the overall structural changes in the SOM and the associated humification process in soils, this technique was complemented with ^{13}C-NMR, which provides a more global perspective on structural information from bulk SOM. The TMAH thermochemolysis of the four soil layers (Fig. 1 and 2) yielded methylated *p*-hydroxyphenyl, guaiacyl and syringyl compounds (lignin-derived structures), aromatic (non-lignin–derived) structures, heterocyclic N (protein-derived), and FAMEs. Peak identifications are presented in Table 1.

Lignin is an important component in the structure of the surface-layer SOM and most HS. Derivatives from lignin can become incorporated into the HS macromolecular structure. Thus, it is important to follow the chemical transformation of lignin by-products since they are subject to humification. The dominant peaks derived from lignin are (4-methoxyphenyl)-ethene (P3), 4-methoxybenzaldehyde (P4), 4-methoxyacetophenone (P5), 4-methoxybenzoic acid methyl ester (P6), 1,2-dimethoxybenzene (G1), 3,4-dimethoxystyrene (G3), 1,2,3-trimethoxybenzene (S1), and 3,4,5-trimethoxyacetophenone (S5). Less intense peaks were methoxybenzene (P1), 3-(4-methoxyphenyl)-2-propanoic acid methyl ester (P12), trans-3-(4-methoxyphenyl)-2-propenoic acid methyl ester (P18), 3,4-drimethoxytoluene (G2), 3,4-dimethoxybenzaldehyde (G4), 3,4-dimethoxyacetophenone (G5), 3,4-dimethoxybenzoic acid, methyl ester (G6), cis 1-(3,4-dimethoxyphenyl)-methoxypropene (G10), 3,4,5-trimethoxytoluene (S2), 3,4,5-trimethoxybenzoic acid methyl ester (S6), and trans 2-(3,4,5-trimethoxyphenyl)-1-methoxyethylene (S8). Typical woody plants yield high portions of syringyl and guaiacyl structures consistent with the results from the TMAH thermochemolysis (Challinor, 1995; Clifford et al., 1995). But the dominant peaks in the TMAH thermochemolysis–GC/MS chromatograms of the studied soil samples were mainly derivatives of *p*-hydroxyphenyl and guaiacyl structures (P and G compounds, respectively). The presence of methylated cinnamyl lignin derivatives (P18) and the dominance of the P3 and G3 peaks are typical for a non-woody (grass)-type lignin (Clifford et al., 1995). Therefore, the abundance of grass-type lignin

Table 1. Peak identification of tetramethylammonium hydroxide (TMAH) thermochemolysis products.

	Compounds derived from p-hydroxyphenyl structures
P1	Methoxybenzene
P3	(4-methoxyphenyl)-ethene
P4	4-methoxybenzaldehyde
P5	4-methoxyacetophenone
P6	4-methoxybenzoic acid methyl ester
P12	3-(4-methoxyphenyl)-2-propanoic acid methyl ester
P18	3-(4-methoxyphenyl)-2-propenoic acid methyl ester
	Compounds derived from guaiacyl structures
G1	1,2-dimethoxybenzene
G2	3,4-dimethoxytoluene
G3	3,4-dimethoxystyrene
G4	3,4-dimethoxybenzaldehyde
G5	3,4-dimethoxyacetophenone
G6	3,4-dimethoxybenzoic acid methyl ester
G10	cis 1-(3,4-dimethoxyphenyl)-methoxypropene
	Compounds derived from syringyl structures
S1	1,2,3-trimethoxybenzene
S2	3,4,5-trimethoxytoluene
S5	3,4,5-trimethoxyacetophenone
S6	3,4,5-trimethoxybenzoic acid methyl ester
S8	trans 2-(3,4,5-trimethoxyphenyl)-1-methoxyethylene
	Other compounds
1	Butanoic acid, 4-methoxy, methyl ester
2	Benzaldehyde
3	Benzene (methoxymethyl)
4	Pentanoic acid 4-oxo, methyl ester
5	1H-Pyrrole-2-carboxaldehyde, 1-methyl
6	Heptanoic acid, methyl ester
7	2-Cyclopenten-1-one,2,3-dimethyl
8	Acetophenone
9	Benzaldehyde, 3-methyl
10	Ethanone, 1-cyclohexyl
11	Benzoic acid, methyl ester
12	3H-Pyrazol-3-one,2,4-dihydro-2,4,4,5-tetramethyl
13	2,5-Pyrrolidinedione, 1-methyl
14	3H-Pyrazol-3-one,2,4-dihydro-2,4-dimethyl
15	Unidentified N compound
16	Octanoic acid, methyl ester
17	2-Pyrrolidinone, 4,4-dimethyl-5-methylidene
18	1,4-deimethoxybenzene (isomer of G1)
19	Benzoic acid, methyl ester
20	Benzaldehyde 3-methoxy
21	Nonanoic acid, methyl ester
22	Benzene propanoic acid, methyl ester
23	Decanoic acid, methyl ester
24	1,2,4-Trimethoxybenzene (isomer of S1)
25	1,3,5-Trimethoxybenzene (isomer of S1)
26	2,4 (1H, 3H)-Pyrimidinedione, 1,3,5-trimethyl
27	Dodecanoic acid, methyl ester
28	Nonanedioic acid, methyl ester
29	Tridecanoic acid, methyl ester
30	Methyl tetradecanoate
31	iso Methyl tetradecanoate
32	iso Pentadecanoic acid, methyl ester
33	Pentadecanoic acid, methyl ester
34	Tetradecanoic acid 12 methyl, methyl ester
35	Pentadecanoic acid 14 methyl, methyl ester
36	7-Hexadecenoic acid, methyl ester
37	9-Hexadecenoic acid, methyl ester
38	Hexadecenoic acid, methyl ester
39	Hexadecenoic acid 15 methyl, methyl ester
40	Heptadecanoic acid, methyl ester
41	Heptadecanoic acid 10 methyl, methyl ester
42	9-Octadecanoic acid, methyl ester (Z)
43	9-Octadecenoic acid, methyl ester (E)
44	Octadecenoic acid, methyl ester
45	Nonedecanoic acid, methyl ester
46	Eicosanoic acid, methyl ester
47	Docosanoic acid, methyl ester
48	Docosanoic acid, ethyl ester
49	Tetracosanoic acid, methyl ester
50	Pentacosanoic acid, methyl ester
51	Hexacosanoic acid, methyl ester
52	Heptacosanoic acid, methyl ester

peaks in the top layer of the soil (0–7.5 cm depth) suggested that the main pool of fresh organic matter originated from grass-type plants. This evidence is in accordance with the 15 yr of continuous corn farming in the studied soil and prove the characteristic pattern of the surface soil layer to plant residue.

With depth, a few changes in relative intensity of the grass lignin–derived peaks were observed (Fig. 1 and 2). The relative intensity of the all lignin–derived peaks was reduced, but the S compounds (S1 and S5) exhibited the sharpest decrease. In addition, the peak of G3 decreased, compared with a relative increase of the peaks for P4, P5, and P6. The intensity of S5, which was one of the major peaks in the chromatogram of the top soil layer, decreased significantly with depth and could not be observed in the deepest layer (30–45 cm). This phenomenon indicates a decomposition and transformation mechanism of the lignin building blocks as humification processes with increasing depth. Lignin decomposition and its structural changes can be monitored by the S/G (syringyl to guaiacyl) and C/G (cinnamyl phenol to guaiacyl) ratios. The use of the S/G ratio as a parameter for lignin degradation is based on the assumption that condensed lignin G-type units are more resistant to biological degradation than lignin with a larger proportion of methoxyl groups (S-type; Kogel-Knabner, 1997). With increasing depth (Fig. 3), the S/G ratio decreased, suggesting a preferential degradation of the syringyl units by microorganisms. The decrease of the S/G ratio resulted mainly from the sharp decrease of the S1 and S5 peaks. However, the C/G ratio exhibited no changes with soil depth and was stable at a level of 0.2. In sedimentary systems, the C/G ratio increases with increasing contributions of non-woody plants, while increases of the S/G ratio suggest input of woody plant (Hedges and Mann, 1979). In our study, we can assume that there were no changes to the vegetation over the course of humification; thus, the structural changes of lignin recorded by the S/G ratio are solely related to biodegradation and humification processes.

In addition to the major lignin-derived peaks, the compounds 1,4-dimethoxybenzene and 1,2,4-trimethoxybenzene were present as major peaks in the TMAH thermochemolysis chromatograms of the soil layers (Fig. 1 and 2). These two compounds are not derived from the lignin structure. Recently, Fabbri and Helleur (1999) reported that TMAH thermochemolysis of cellulose and starch yield 1,2,4-trimethoxybenzene, which was also identified in TMAH thermochemolysis of beech (*Fagus sylvatica* L.) leaf litter (Hermosin and Saiz-Jimenez, 1999). In our study, it is difficult to relate the 1,2,4-trimethoxybenzene peak to cellulose. Cellulose is considered to be a readily degraded biopolymer, which is rapidly used by the soil fauna. Thus, the intensity of this peak is expected to decrease with depth (i.e., humification). But, the 1,2,4-trimethoxybenzene peak was still one of the major peaks present in the chromatograms of the bottom soil layers.

In support of this view, the carbohydrate peaks in the ^{13}C-NMR spectra (72 and 105 ppm; Fig. 4) exhibited a sharp decrease with depth, suggesting decomposition of

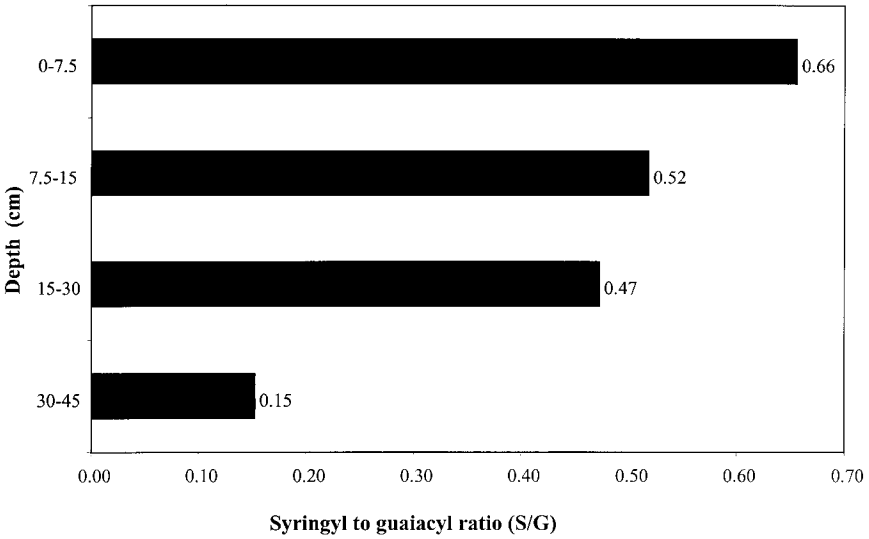

Fig. 3. Syringyl to guaiacyl ratio (S/G) as calculated from the tetramethylammonium hydroxide (TMAH) thermochemolysis–gas chromatography/mass spectrometry chromatograms vs. soil depth.

this carbohydrate component of SOM. The polysaccharide level, calculated from the ^{13}C-NMR spectra (60–112 ppm), decreased 21% (from 38–30% of the total C) with depth. A similar trend was reported by Zech et al. (1997) and Kögel-Knabner (1997) for the cellulose content in a forest soil profile. These studies have reported a decrease in the carbohydrate content with humification. The lack of carbohydrate-derived peaks in the TMAH chromatogram is due to poor sensitivity of this technique to carbohydrates (Clifford et al., 1995). Thus, we conclude on the basis of the above findings that the origin of the 1,2,4-trimethoxybenzene peak in the soil chromatogram was not cellulose but potentially refractory organic-matter compounds of soil HS.

The relative intensity of other non-lignin aromatic compounds (such as benzaldehyde, benzene-methoxymethyl, acetophenone, and benzoic acid methyl ester) increased with depth, suggesting that their presence is tied to humification, whereas compounds from plants are oxidized and transformed to aromatic humic-like structures. This hypothesis is supported by the ^{13}C-NMR CPMAS spectra (Fig. 4). The phenolic C peak (150 ppm), which is related to lignin-type compounds, decreased with depth, while a reversed trend was exhibited by C-substituted aromatic structures (130 ppm). These findings indicate a relative enrichment of non-lignin aromatic structures with depth (i.e., progressive decomposition and humification). The relative increase of the carboxyl C peak (175 ppm) in the NMR spectra with depth may also be related to oxidation of lignin side chains during microbial degradation of lignin (Stevenson, 1994). But, this hypothesis cannot be evaluated with TMAH thermochemolysis because of poor resolution of the acid-lignin (G6 and S6) and aldehyde-lignin (G4 and S4) derivative peaks in the soil chromatograms. Thus, it is more likely that in the soil studied, the increase of the carboxyl-C peak is mainly due to accumulation of refractory FAMEs as correlated to the 32-ppm peak in the NMR spectra.

Under TMAH thermochemolysis conditions, the soil sample throughout the profile yielded saturated, unsaturated, and branched FAMEs of varying C-chain length (from C_7 to C_{27}). The presence of long-chain fatty acids in the soil may be primarily due to the input of aboveground plant aliphatic biopolymers such as cutin (McKinney et al., 1996), plant root aliphatic biopolymers such as suberin, and microbial activity byproducts. The presence of FAMEs originating from aliphatic biopolymers in soil is supported by the characteristic 32-ppm peak in the solid-state ^{13}C-NMR (Fig. 4). Several studies have shown that aliphatic biopolymers (e.g., cutan) are non-hydrolyzable and can be preserved in sediments with minor alteration (Nip et al., 1986; Hatcher et al., 1983). Moreover, FAMEs were produced by TMAH from low-rank coal and soil HAs, and the fatty acids were suggested to be linked to the HA macromolecule network via ester bonds (del Rio et al., 1994; Hatcher and Clifford, 1994). A similar series of FAMEs was observed from milled beech leaf litter (Hermosin and Saiz-Jimenez, 1999) and a soil humin fraction (Fabbri et al., 1996). The humin and beech leaf yielded long-chain saturated and unsaturated FAMEs that probably originated from cutin and cutan. The product 1,3,5-trimethoxybenzene, which was found to be a major part of cutan's structure (McKinney et al., 1996), was observed only in the upper 15-cm layer and was not present in the chromatographs from the 30- to 45-cm layer. This suggests that cutan might not be the only source for aliphatic biopolymers in the soil that might yield long-chain FAMEs.

In agricultural soils, where most of the aboveground biomass is removed, the contribution of the underground biomass (roots) to the SOM increases. Thus, the origin of the fatty acids (FAMEs, produced by the TMAH thermochemolysis) could be the corn root system. Nierop (1998) reported that pyrolysis of roots revealed alkane and alkene patterns, which can be attributed to suberin present in the roots. In addition, the unsaturated and branched fatty acids could originate

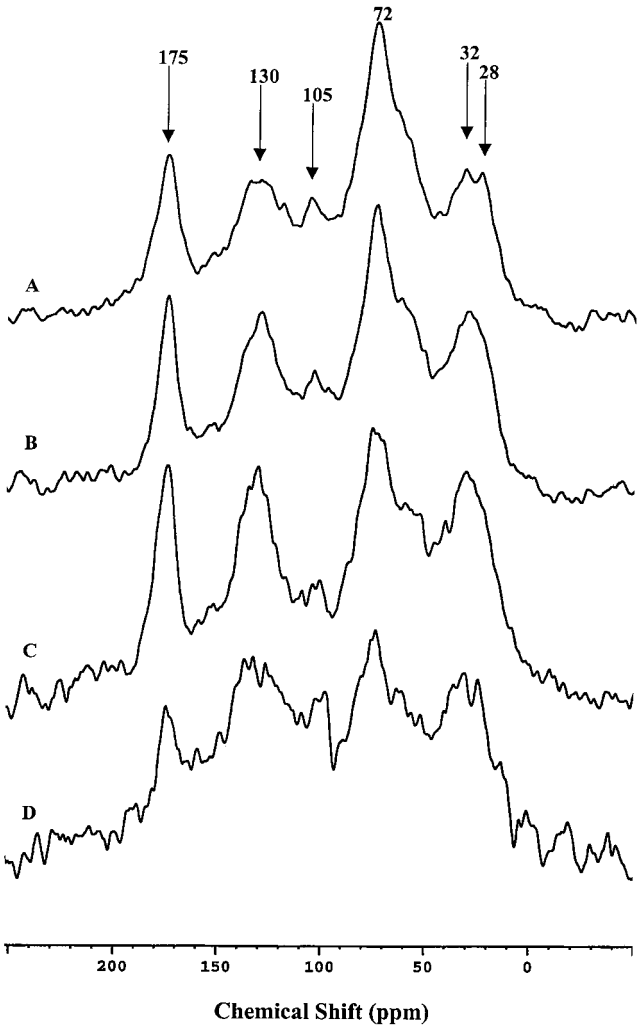

Fig. 4. Cross polarization magic angle spinning (CPMAS) ^{13}C-nuclear magnetic resonance (NMR) spectra of the soil layers at (A) 0–7.5 cm, (B) 7.5–15 cm, (C) 15–30 cm, and (D) 30–45 cm.

from microbial activity. The solid-state NMR spectra exhibited a relative increase of the paraffinic-C region (0–50 ppm) with depth (from 21–25% of the total C). The increasing level of aliphatic C recorded from the NMR spectra and the unchanged levels of fatty acids with depth, suggest that the structures yielding these aliphatic compounds in the soil are highly refractory. Thus, their relative concentration increased with humification. A similar trend for the aliphatic C structures was reported for forest soil horizons using NMR analyses (Zech et al., 1997).

Tetramethylammonium hydroxide thermochemolysis of the studied soil samples yielded heterocyclic N products as pyrroles, pyridines, and pyrazoles (Table 1). These peaks were present in all four soil layers, suggesting low bioavailability of these N forms. A series of N-heterocyclic structures were identified in pyrolysis GC/MS chromatogram of several SOM samples (Leinweber and Schulten, 1999). These authors suggested that these structures are original N structures present in the soil. But ^{15}N-NMR spectroscopic investigation of soils revealed that the major part of organic N is bound in amide-N fanctional groups, while heterocyclic-N compounds were not detected (Kögel-Knabner, 1997). Moreover, it was observed that during pyrolysis, dipeptides have been formed to cyclic dipeptides and amino acids were transformed by intermolecular condensation (Chiavari et al., 1992; Hendricker and Voorhees, 1998). Thus, the heterocyclic N compounds identified in the TMAH thermochemolysis chromatograms originate from peptides and proteins in refractory forms of the SOM (Knicker and Hatcher, 1997). The TMAH thermochemolysis analysis of the soil profile samples studied yielded, in addition to the N-heterocyclic structures, methylated amino acids (phenylalanine, leucine, and valine). These amino acids were present in relatively high levels in the higher top layer (0–7.5 cm) than in the deep layers. The dominant source for amino acids in soils is cell walls of microorganisms and plant roots exudates (Stevenson, 1994). Therefore, the presence of amino acids in the deep soil layers suggests a preservation mechanism, which protects the labile proteins from biodegradation. Encapsulation of organic N (mainly proteins) in refractory SOM has been suggested as a possible mechanism (Knicker and Hatcher, 1997).

CONCLUSION

In this research, we studied chemical structures in SOM using TMAH thermochemolysis: a novel, advanced technique that allows analysis without extraction of the SOM. The research aim was to elaborate SOM structural changes with soil depth. The TMAH thermochemolysis was shown to be a useful technique for characterizing non-extracted SOM. The main lignin-derived products were monomethoxyphenyl (P) and dimethoxyphenyl (G), suggesting that the main source of organic-matter inputs to the soil was grass-type lignin litter. With depth, the S/G ratio decreased, suggesting preferential degradation processes of the syringyl units by microorganisms. The decrease of the S/G ratio and the relative increase of the aromatic and carboxylic C–containing groups exhibited in the ^{13}C-NMR spectra, support the theory that side-chain oxidation of lignin structures is one of the major humification processes occurring in soils.

In addition to lignin-derived compounds, the TMAH thermochemolysis indicated the presence of a relatively large fraction of long-chain fatty acids. The long-chain fatty acids, which were also found in TMAH analyses of HAs, seem to be highly resistant to biological degradation. This hypothesis was also supported by the ^{13}C-NMR spectra, which exhibited an increase of the paraffinic C with depth.

Refractory and highly aged organic matter dominates the soil C storage. However, surface soil layers seem to contain mostly organic materials exhibiting pattern characteristics of plant litter. With humification and/or soil depth, the resemblance of both the ^{13}C-NMR spectra and TMAH thermochemolysis chromatograms to those

of plant components decreases as a result of mineralization, decomposition, and repolymerization processes.

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REFERENCES

Challinor, J.M. 1995. Characterisation of wood by pyrolysis derivatisation-gas chromatography/mass spectrometry. J. Anal. Appl. Pyrolysis 35:93–107.

Challinor, J.M. 1996. A rapid simple pyrolysis derivatization gas chromatography-mass spectrometry method for profiling of fatty acids in trace quantities of lipids. J. Anal. Appl. Pyrolysis 37:185–197.

Chiavari, G., and G.C. Galletti. 1992. Pyrolysis–gas chromatography/mass spectrometry of amino acids. J. Anal. Appl. Pyrolysis 24: 123–137.

Clifford, D.J., D.M. Carson, D.E. McKinney, J.M. Bortiatynski, and P.G. Hatcher. 1995. A new rapid technique for the characterization of lignin in vascular plants: Thermochemolysis with tetramethylammonium hydroxide (TMAH). Org. Geochem. 23:169–175.

Cook, R., and C.H. Langford. 1998. Structural characterization of fulvic acid and humic acid using solid-state ramp-CP-MAS 13C nuclear magnetic resonance. Environ. Sci. Technol. 32:719–725.

del Rio, J.C., and P.G. Hatcher. 1996. Structural characterization of humic substances using thermochemolysis with tetramethylammonium hydroxide. p. 77–95. *In* J.S. Gaffney et al. (ed.) Humic and fulvic acids: Isolation, structure, and environmental role. ACS Symposium Series 651, Am. Chem. Soc., Washington, DC.

del Rio, J.C., F.J. Gonzalez-Vila, F. Martin, and T. Verdejo. 1994. Characterization of humic acids from low-rank coals by 13C-NMR and pyrolysis-methylation. Formation of benzenecarboxylic acid moieties during the coalification process. Org. Geochem. 22: 885–891.

Fabbri, D., and R. Helleur. 1999. Characterization of the tetramethylammonium hydroxide thermochemolysis products of carbohydrates. J. Anal. Appl. Pyrolysis 49:277–293.

Fabbri, D., G. Chiavari, and G.C. Galletti. 1996. Characterization of soil humin by pyrolysis (/methylation) – gas chromatography/ mass spectrometry/; structural relationships with humic acids. J. Anal. Appl. Pyrolysis 37:161–172.

Filley, T.R., R.D. Minard, and P.G. Hatcher. 1999. Tetramethylammonium hydroxide (TMAH) thermochemolysis: Proposed mechanisms based upon the application of ^{13}C-labeled TMAH to synthetic model lignin dimer. Org. Geochem. 30:607–621.

Hatcher, P.G., E.C. Spiker, N.M. Szeverenyi, and G.E. Maciel. 1983. Selective preservation and origin of petroleum-forming aquatic kerogen. Nature (London) 305:498–501.

Hatcher, P.G., and D.J. Clifford. 1994. Flash pyrolysis and in situ methylation of humic acids from soil. Org. Geochem. 21:1081–1092.

Hatcher, P.G., M.A. Nanny, R.D. Minard, S.C. Dible, and D.M. Carson. 1995. Comparison of two thermochemolytic methods for the analysis of lignin in decomposing wood: The CuO oxidation method and the method of thermochemolysis with TMAH. Org. Geochem. 23:881–888.

Hatcher, P.G., and R.D. Minard. 1996. Comparison of dehydrogenase polymer (DHP) lignin with native lignin from gymnosperm wood by thermochemolysis using tetramethylammonium hydroxide (TMAH). Org. Geochem. 24:593–600.

Hedges, J.I., and D.C. Mann. 1979. The lignin geochemistry of marine sediments from the southern Washington coast. Geochim. Cosmochim. Acta. 43:1809–1818.

Hendricker, A.D., and K.J. Voorhees. 1998. Amino acid and oligopeptide analysis using Curie-point pyrolysis mass spectrometry with in-situ thermal hydrolysis and methylation: Mechanistic considerations. J. Anal. Appl. Pyrolysis 48:17–33.

Hermosin, B., and C. Saiz-Jimenez. 1999. Thermally assisted hydrolysis and methylation of milled beech leaf litter. J. Anal. Appl. Pyrolysis 49:417–424.

Ishida, Y., S. Wakamatsu, H. Yokoi, H. Ohtani, and S. Tsuge. 1999. Compositional analysis of polyunsaturated fatty acid oil by one-step thermally assisted hydrolysis and methylation in the presence of trimethylsulfonium hydroxide. J. Anal. Appl. Pyrolysis 49: 267–276.

Knicker, H., and P.G. Hatcher. 1997. Survival of protein in an organic-rich sediment. Possible protection by encapsulation in organic matter. Naturwissenschaften 84:231–234.

Kögel-Knabner, I. 1997. ^{13}C and ^{15}N NMR spectroscopy as a tool in soil organic matter studies. Geoderma 80:243–270.

Leinweber, P., and H.-R. Schulten. 1999. Advances in analytical pyrolysis of soil organic matter. J. Anal. Appl. Pyrolysis 49:359–383.

Martin, F., F.J. Gonzalez-Vila, J.C. del Rio, and T. Verdejo. 1994. Pyrolysis derivatization of humic substances: I. Pyrolysis of fulvic acids in the presence of tetramethylammonium hydroxide. J. Anal. Appl. Pyrolysis 28:71–80.

Martin, F., J.C. del Rio, F.J. Gonzalez-Vila, and T. Verdejo. 1995. Pyrolysis derivatization of humic substances: II. Pyrolysis of soil humic acids in the presence of tetramethylammonium hydroxide. J. Anal. Appl. Pyrolysis 31:75–83.

McKinney, D.E., and P.G. Hatcher. 1996. Characterization of peatified and coalified wood by tetramethylammonium hydroxide (TMAH) thermochemolysis. Int. J. Coal Geol. 32:217–228.

McKinney, D.E., J.M. Bortiatynski, D.M. Carson, D.J. Clifford, J.W. De Leeuw, and P.G. Hatcher. 1996. Tetramethylammonium hydroxide (TMAH) thermochemolysis of the aliphatic biopolymer cutan: Insights into the chemical structure. Org. Geochem. 24: 641–650.

Nierop, K.G.J. 1998. Origin of aliphatic compounds in a forest soil. Org. Geochem. 29:1009–1016.

Nip, M., E.W. Tegelaar, H. Brinkhuis, J.W. De Leeuw, P.A. Schenck, and P.J. Holloway. 1986. Analysis of modern and fossil plant cuticles by Curie point pyrolysis gas-chromatography and Curie point pyrolysis-gas chromatography-mass spectrometry: Recognition of a new, highly aliphatic and resistant biopolymer. Org. Geochem. 10:769–778.

Stevenson, F.J. 1994. Humus chemistry: Genesis, composition, reactions. 2nd ed. John Wiley & Sons, New York.

Zech, W., N. Senesi, G. Guggenberger, K. Kaiser, J. Lehmann, T.M. Miano, A. Miltner, and G. Schroth. 1997. Factors controlling humification and mineralization of soil organic matter in the Tropics. Geoderma 79:117–161.