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The influence of topography on the nature of humic substances in soil organic matter at a site in the Atlantic Coastal Plain of South Carolina

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Abstract. The effect of topography on the nature of humic substances, isolated as water soluble organic carbon (WSOC), fulvic acid (FA), and humic acid (HA) was evaluated by comparing relative proportion and chemical characteristics of these fractions in upland and bottomland Coastal Plain soils in South Carolina. The fractions were characterized by elemental analysis and ^{13}C cross-polarization magic angle spinning nuclear magnetic resonance (CPMAS NMR) spectroscopy. The majority of humic substances occur as humic acids, with bottomland soils having higher HA/FA ratios when compared to upland soils. We found no significant differences between upland and bottomland humic substances with respect to yields of WSOC and fulvic acids, and in the C and N content of humic and fulvic acids. Carbon-13 CPMAS NMR spectroscopy revealed that the WSOC and fulvic acid fractions were composed largely of O-alkyl-C structures with bottomland soils having higher amounts of these groups. Humic acid C distribution was similar between upland and bottomland soils and was largely composed of aromatic groups. Our results demonstrate that topography influences the formation of humic acid and the structural and chemical properties of the various humic fractions.

Introduction

Soils along a hillslope usually exhibit markedly differing morphologies that may be explained by differences in moisture status, microclimate and vegetation (Birkeland 1974). Accumulations and losses of soil organic matter (SOM) have been attributed to the movement of water over or

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through a soil. For example, low amounts of SOM typically accumulate on steeply sloping, well-aerated soils, because of erosion or rapid oxidation (Kleiss 1970; Malo et al. 1974; & Schimel et al. 1985). Large organic matter accumulations are reported for soils in swamps and marshes (Reiners 1973; Schlesinger 1977), and these accumulations usually are attributed to slow decomposition in waterlogged conditions (Williams & Gray 1974) and to poor substrate quality (Day 1982).

Finney et al. (1962) measured higher SOM in some Ohio soils on NE vs SE-facing slopes and attributed this condition to higher moisture levels and more vegetation cover. Woods and Schuman (1988) measured SOM along a hillslope transect in Colorado, where erosion caused an accumulation of organic-rich sediment in downslope positions.

In these studies, differences in the structural composition of humic substances in SOM was not evaluated. Our study was undertaken to provide basic information on the chemical characteristics of humic substances in SOM from a toposequence (upland vs bottomland) of soils. We hypothesize that topography regulates the formation of humic substances and influences their distribution in the organic pool.

Materials and methods

Study site

The study was conducted in the Upper Three Runs Creek (UTRC) watershed (Fig. 1) on the US Department of Energy's Savannah River Site (SRS) within the Aiken plateau of the Upper Atlantic Coastal Plain physiographic region of South Carolina (Siple 1967). The UTRC watershed has broad to steeply-sloping upland ridges and wide floodplains (Rogers 1990). The surface of the study area is covered by marine sediments of late Eocene age (Barnwell group) and is underlain in places by middle age Eocene sediments (McBean material) (Dennehy et al. 1989). The Barnwell formation typically has strata of quartz gravel, sands to loamy sands, and kaolinitic clays, whereas the McBean formation has strata of sands and smectitic clays (Rogers 1990). Most of the original surficial sediments have been eroded and transported downslope producing deposits of colluvium and colluvium grading into alluvium (Dennehy et al. 1989). Examination of soil morphology confirmed that most of the soils in the study have formed in colluvial sands from Barnwell material, whereas a few soils adjacent to UTRC have formed in a mixture of colluvium and alluvium from Barnwell and McBean material.

We established three transects 130 to 160 m in length (Fig. 1) extend-

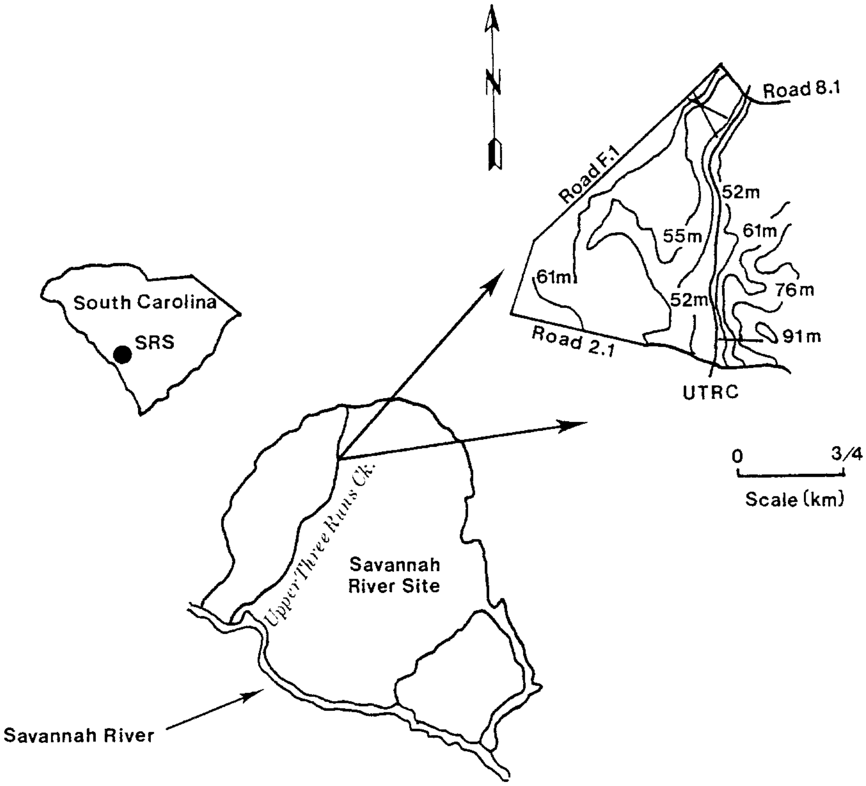

Fig. 1. Location of Savannah River Site (SRS), Upper Three Runs Creek (UTRC), and study transects.

ing from the summit (2—5% slope) of the floodplain areas, along the side (5—35%) slope and into the floodplain (1—2% slope), terminating near UTRC. The transects were divided into upland and bottomland regions based upon their drainage class and OC content. Selected positions in each region were determined at random and a total of 13 upland and 10 bottomland sites were sampled. A detailed soil description was performed at each sampling site to identify the soil series (Table 1). The upland soils are well to somewhat excessively drained, are subject to drought, and have a lower OC content relative to the bottomland soils. The bottomland soils are poor to very poorly drained and have water standing on or below the surface during the fall to early spring of most years.

Vegetation in the bottomland area is composed of plants tolerant to wet conditions such as *Acer rubrum, Liquidambar styraciflura, Nyssa aquatica, Pinus taeda, Quercus nigra, Q. alba, Ilex opaca* and ferns. In

Table 1. Taxonomic, chemical and physical properties of soils used in study.

Series	Sample No.	Taxonomy (Subgroup)	A horizon Depth (cm)	pH (2:1)	OC content g kg^{-1}	Sand %	Silt %	Clay %
Upland								
Bassfield	1	*Typic Hapludults*	0—8	5.6	18.1	88.6	8.1	3.3
Lakeland	2	Typic Quartzipsamments	0—13	5.0	16.5	92.5	5.1	2.5
Lakeland	3	Typic Quartzipsamments	0—8	4.8	15.4	91.1	5.8	3.1
Lakeland	4	Typic Quartzipsamments	0—14	5.0	10.8	91.1	6.6	2.3
Lakeland	5	Typic Quartzipsamments	0—20	5.2	23.2	94.0	3.5	2.5
Lucy	6	Arenic Paleudults	0—10	4.9	16.6	91.9	5.0	3.1
Lucy	7	Arenic Paleudults	0—15	5.7	15.5	84.7	10.8	4.5
Springhill	8	Typic Kanhapludults	0—6	5.0	56.5	85.3	8.7	6.0
Troup	9	Grossarenic Paleudults	0—11	4.6	16.0	91.0	5.7	3.3
Troup	10	Grossarenic Paleudults	0—15	4.6	14.7	91.9	4.9	3.2
Troup	11	Grossarenic Paleudults	0—15	4.8	9.8	91.4	5.0	3.6
Troup	12	Grossarenic Paleudults	0—10	4.8	23.2	93.3	3.7	3.0
Troup	13	Grossarenic Paleudults	0—13	5.4	35.5	88.5	8.0	3.5
Bottomland								
Osier	14	*Typic Psammaquents*	0—15	4.2	86.0	96.8	2.4	0.8
Osier	15	Typic Psammaquents	0—24	4.8	35.5	98.5	1.3	0.2
Pickney	16	Cumulic Humaquepts	0—18	4.4	194.6	88.1	10.5	1.4
Pickney	17	Cumulic Humaquepts	0—19	3.3	340.3	90.7	4.7	4.6
Pickney	18	Cumulic Humaquepts	0—16	5.3	54.2	77.7	14.6	7.7
Pickney	19	Cumulic Humaquepts	0—13	5.3	32.9	90.9	5.8	3.3
Pickney	20	Cumulic Humaquepts	0—13	4.4	53.0	85.9	9.9	4.2
Pickney	21	Cumulic Humaquepts	0—13	4.8	30.5	91.6	5.5	2.9
Pickney	22	Cumulic Humaquepts	0—13	4.7	251.0	76.7	17.2	6.1
Williman	23	Arenic Ochraquults	0—16	4.8	38.8	91.3	6.5	2.2

contrast, mainly *Quercus laurifolia, Q. marilandica, Q. laevis, Q. stella*, with some *Carya* sp., *Cornus florida, Magnolia virginiana*, and *Pinus taeda* grow on the dry upland ridges. Aerial photos revealed that the trees have not been harvested for more than 40 years. Climatically the region is humid subtropical, with total annual rainfall of approximately 122 cm (Rogers 1990). In winter and summer the average daily temperatures are 9 and 26 °C, respectively (Rogers 1990).

Collection and analyses of soils

The fresh litter layer was removed and composite surface horizon soil samples were collected within a 1-m radius of each sampling site. These samples were air-dried, ground to pass a 2-mm sieve, and stored in the dark at 4 °C. The OC content of each soil sample was measured using a N and C analyzer (Carlo Erba/model NA 1500, Milan, Italy). Soil pH was measured using a pH meter (Radiometer Copenhagen Corp., Ion 85 analyzer, Cleveland, OH) with a combination electrode and Millipore Milli-Q H_2O at a solution to soil ratio of 2:1. Particle-size analysis was performed using the micro-pipet method of Miller and Miller (1988). Prior to particle-size analysis, all soils with >20 g OC kg^{-1} soil were pretreated with H_2O_2 to remove OC.

Water soluble organic carbon extraction

Water soluble organic carbon was isolated by placing 150 g of air-dried 2-mm sieved soil into 250 mL centrifuge tubes and then adding 150 mL of Millipore Milli-Q H_2O. The tubes were shaken at 1.6 Hz for 45 min at 25 ± 2 °C, then centrifuged at 3400 X g for 45 min. The supernatant was prefiltered through a Milli-Q H_2O pre-rinsed Whatman No. 42 filter paper then immediately filtered through a pre-rinsed 0.4-um polycarbonate (Nuclepore Corp., Pleasanton, CA) filter.

Results from preliminary experiments employing 8 sequential extractions on 2 soils, indicated that approximately 90% of the total WSOC extracted was recovered after 3 extractions. We defined the quantity of WSOC extracted after 3 extractions as the WSOC fraction. All WSOC extracts were stored in the dark at 4 °C. The WSOC extracts were not treated to remove inorganic material. Samples of WSOC (#7, 11, 14 and 19) were freeze-dried for ^{13}C NMR analysis, and the ash contents were determined by dry combustion at 550 °C for 24 h. The WSOC fraction had a median ash content of 100 mg ash g^{-1} WSOC.

Alkali-extraction and purification

Following removal of WSOC from the soils, humic and fulvic acids were extracted and purified from the soils using a modified method outlined in Schnitzer (1982). The modifications involved conducting the extraction under a Ar atmosphere, dialyzing the humic acids against Millipore Milli-Q H_2O (Spectra/Por dialysis tubing, MWCO-1000, Fisher Scientific Co., Atlanta, GA) and passing the fulvic acids through Bio-Rad AG 50W-X8 cation exchange resin (Bio-Rad Labs, Richmond, CA). The humic acids were then freeze-dried, while the fulvic acids were evaporated to dryness at 40 °C. The ash contents were determined by dry combusion at 550 °C for 24 h. The median fulvic and humic acid ash content was 91 and 48 mg ash g^{-1}, respectively.

Humic substance characterization

The OC content of the humic substances in solution was determined using a OC analyzer (OI Corp., model 24, College Station, TX) with dextrose used as a C standard. The C and N contents of humic and fulvic acids were determined using the N and C analyzer with acetanilide, benzylisothiourea phosphate, sulphanilamide, and phenthrene as C and N standards. Because of limited quantities of WSOC extracted, the C and N content was not determined.

Solid-state ^{13}C CPMAS NMR spectroscopy was performed on select WSOC, humic and fulvic acid fractions from upland and bottomland soils. Spectra were obtained on a custom built spectrometer (Nicolet 1180B data system, and 293B pulse programer, General Electric, Los Angeles, CA) at the Colorado State University Nuclear Magnetic Resonance Center operating at 25.3 MHz. Hexamethylbenzene was used as an external chemical shift reference compound for spectrometer fine-tuning and optimization. Spectra based on 50,000 scans were obtained using a contact time of 1 ms, and a recycle time of 1 s. Each spectrum was divided into the following regions (Novak and Smeck 1991): 0 to 50 ppm (aliphatic-C), 50 to 110 (O-alkyl-C), 110 to 160 (aromatic-C), 160 to 190 (COOH-C) and 190 and 240 (carbonyl-C).

Recently, Schnitzer (1990) has indicated that the carbohydrate content cannot be quantitatively estimated from ^{13}C NMR spectra because a significant portion of the signal intensity between 50 to 110 ppm is due to non-carbohydrate structures (i.e., aliphatic C's bonded to OH groups, ether O's or occurring in saturated five- or six-membered rings bonded to O's and C's of certain amino acids) as well as carbohydrates. Thus, we chose to assign the 50 to 110 ppm region to O-alkyl-C structures, which contain a wide range of C-O and C-N moieties (Malcolm 1989). Peak areas were measured with an integrator and reported as a percentage of the total OC content. Malcolm (1989) and Wilson (1989) have cautioned that data obtained using ^{13}C NMR spectroscopy are semiquantitative because of differences in C relaxation times and interferences of paramagnetic species that cause line broadening. We minimized these differences by analyzing all samples using a similar cross-polarization and recycle delay time to ensure that a relative comparison of spectral data could be made.

Statistical analyses

Means and standard deviations were used to summarize the data, and statistical significance was determined by using a combination of the Mann-Whitney test and the Wilcoxon paired-sample test (Minitab, Inc., State College, PA, 1986). Rejection or acceptance of significant data was made at the 0.05 level of rejection.

Results

Proportion and elemental composition of humic substances

Despite bottomland soils having a higher SOM content, similar amounts of WSOC ($P = 0.182$) were extracted from upland and bottomland soils (Table 2). The mean yields of fulvic acid extracted from upland and bottomland soils were 74.7 and 58.5 g kg^{-1} OC, respectively ($P = 0.180$). Bottomland soils had higher yields of humic acid ($P = 0.017$) and, thus, higher HA/FA ratios ($P = 0.001$) compared to upland soils. The humic and fulvic acid fractions obtained from the upland and bottomland soils were similar ($P > 0.251$) in C and N contents and C/N ratio (Table 3).

Carbon-13 nuclear magnetic resonance spectra

Water soluble organic carbon

The ^{13}C NMR spectra for upland (Fig. 2, spectra A and B) and bottomland (Fig. 2, spectra C and D) WSOC show signals between 16 to 24 ppm in the aliphatic-C region due to methylene groups in alkyl chains and branched structures (Malcolm 1989). Upland soils have a higher proportion of aliphatic structures than bottomland soils (Table 4). There are sharp signals for O-alkyl compounds between 71 to 73, and 97 and 103 ppm in all spectra, due to structures containing C-O and C-N moieties

Table 2. Yield of humic substances extracted from upland and bottomland soils.

Series	Sample No.	Yield of humic substances (g kg^{-1} OC)				HA/FA Ratio
		WSOC	FA	HA	Humin	
Upland						
Bassfield	1	7.6	76.0	149.3	767.1	2.0
Lakeland	2	4.9	61.1	107.1	826.9	1.8
Lakeland	3	7.8	90.2	297.7	604.3	3.3
Lakeland	4	5.9	43.1	188.7	762.3	4.4
Lakeland	5	6.7	72.9	175.2	744.3	2.4
Lucy	6	10.9	80.2	206.2	702.7	2.6
Lucy	7	7.4	60.2	153.5	778.9	2.6
Springhill	8	3.8	78.7	109.6	807.9	1.4
Troup	9	9.1	99.1	187.9	703.9	1.9
Troup	10	13.4	86.0	210.2	690.4	2.4
Troup	11	16.5	100.9	312.9	569.7	3.1
Troup	12	8.2	48.1	43.9	899.8	0.9
Troup	13	3.7	75.0	132.5	788.8	1.8
	Mean$^+$	8.2	74.7	175.0	742.1	2.4
	SD	(3.5)	(17.1)	(71.1)	(85.6)	(0.9)
Bottomland						
Osier	14	14.2	65.1	223.4	697.3	3.4
Osier	15	9.3	44.3	475.6	470.8	10.7
Pickney	16	10.5	21.3	149.8	818.4	7.0
Pickney	17	3.5	11.9	254.0	730.6	21.4
Pickney	18	2.3	71.1	220.7	705.9	3.1
Pickney	19	7.7	114.5	296.4	581.4	2.6
Pickney	20	2.5	76.5	449.1	479.1	5.9
Pickney	21	3.4	50.8	226.5	719.3	4.5
Pickney	22	3.2	5.4	164.8	826.8	30.5
Williman	23	6.0	124.2	299.1	570.7	2.4
	Mean$^+$	6.3	58.5	275.2*	660.0	9.2**
	SD	(3.8)	(38.2)	(102.6)	(121.5)	(9.4)

* Differences between means of upland and bottomland humic substances, significant at 0.05*, and 0.01** level of confidence

(Malcolm 1989), with a higher proportion occurring in bottomland soils. Spectra of WSOC are devoid of signals between 150 to 163 ppm, which are attributed to ROH groups (Schnitzer and Preston 1987). Resonance signals between 173 to 178 and 190 to 240 ppm are due to COOH, amides, esters and carbonyl compounds, respectively, and the quantities of these groups were similar in the upland and bottomland soils (Table 4).

Table 3. Mean C and N contents (dry, ash-free basis) of fulvic and humic acids extracted from upland and bottomland soils.

Soils	Fulvic acids C g kg^{-1}	Fulvic acids N	Fulvic acids C/N	Humic acids C g kg^{-1}	Humic acids N	Humic acids C/N
Upland ($n = 13$)	410 (30)	19 (5)	21	519 (36)	26 (5)	20
Bottomland ($n = 10$)	422 (37)	17 (4)	24	515 (44)	24 (5)	21

Standard deviations are in parentheses

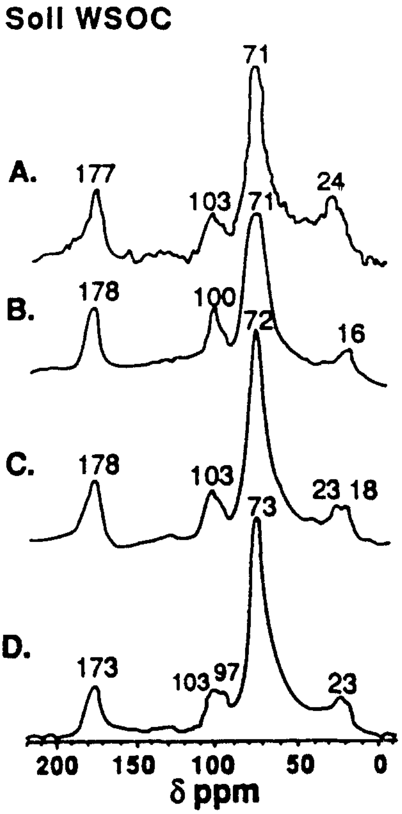

Fig. 2. Carbon-13 CPMAS NMR spectra of the WSOC fraction from upland (A-Lucy # 7 and B-Troup # 11) and bottomland (C-Osier # 14 and D-Pickney # 19) soils.

Table 4. Distribution of carbon in water soluble organic carbon (WSOC), fulvic and humic acid fractions extracted from upland and bottomland soils as determined by ^{13}C CPMAS NMR.

Humic material	Sample No.	Landscape position	Distribution of carbon (% of the total OC content)				
			Aliphatic-C	O-Alkyl-C	Aromatic-C	COOH-C	Carbonyl-C
WSOC	7	Upland	24	54	5	12	5
	11	Upland	27	51	6	10	6
	14	Bottomland	15	64	4	12	5
	19	Bottomland	15	65	7	11	2
Fulvic acid	7	Upland	21	40	15	18	6
	13	Upland	23	36	15	21	5
	19	Bottomland	21	50	5	19	5
	21	Bottomland	18	46	13	18	5
Humic acid	7	Upland	31	23	29	12	5
	13	Upland	23	28	32	14	3
	19	Bottomland	24	22	33	16	5
	21	Bottomland	23	23	35	15	4

Fulvic acids

The NMR spectra of fulvic acids from the upland (Fig. 3, spectra A and B) and bottomland (Fig. 3, spectra C and D) samples showed that aliphatic structures were present (Malcolm 1989) and that the amounts of aliphatic groups were similar between soil types (Table 4). In all spectra, strong signals in the O-alkyl-C region suggest structures containing C-O and C-N moieties were present (Malcolm 1989). Bottomland soils had higher amounts of these groups when compared to upland soils (Table 4). Weak aromatic signals at 120 to 131, and 149 to 163 ppm were present in all spectra, with upland soils having a slightly higher distribution of aromatic

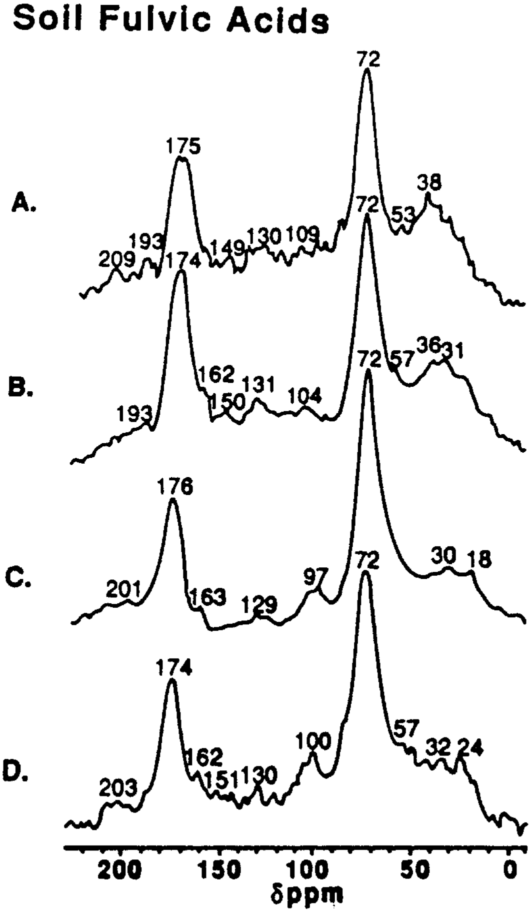

Fig. 3. Carbon-13 CPMAS NMR spectra of the fulvic acid fraction from upland (A-Lucy # 7 and B-Troup # 13) and bottomland (C-Pickney # 19 and D-Pickney # 21) soils.

groups compared to bottomland soils (Table 4). Fulvic acids from bottomland and upland soils have very weak signals indicative of ROH groups (150 to 163 ppm). All spectra exhibited peaks between 174 to 176 and 190 to 240 ppm, but there were no differences in distribution of these groups between soil types (Table 4).

Humic acids

The NMR spectra of humic acid from the upland (Fig. 4, spectra A and B) and bottomland (Fig. 4, spectra C and D) samples show that aliphatic, O-alkyl, aromatic, COOH, amide and esters and carbonyl groups are present, and the distribution of these groups is similar between soil regimes (Table 4). All spectra exhibit signals between 54 to 57 ppm due

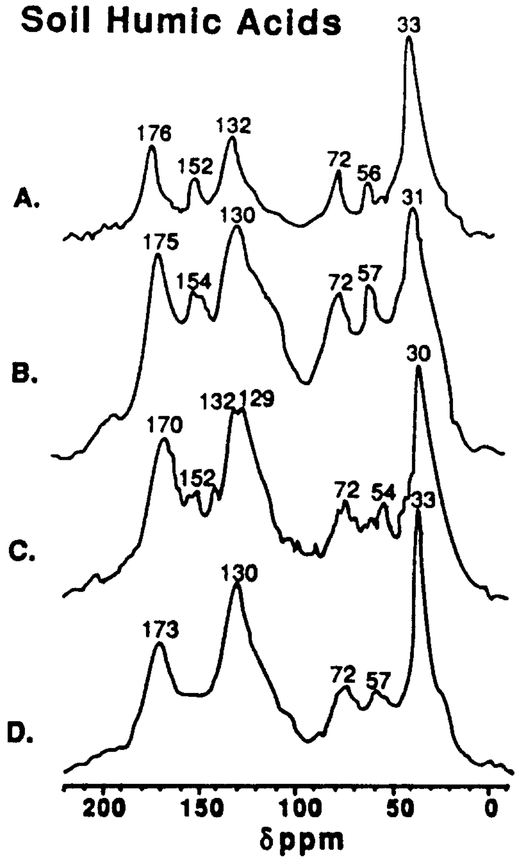

Fig. 4. Carbon-13 CPMAS NMR spectra of the humic acid fraction from upland (A-Lucy # 7 and B-Troup # 13) and bottomland (C-Pickney # 19 and D-Pickney # 21) soils.

to methoxy structures, and most have a signal between 152 to 154 ppm due to ROH groups. The occurrence of these two resonance peaks suggests the presence of lignin and lignin-like components (Hatcher et al. 1983). The peaks due to ROH groups are more distinct in spectra from upland than bottomland soils, which suggest a relatively higher proportion of lignin and lignin-like structures in the humic acid fraction of upland soils.

Discussion

In order to evaluate the influence of topography on the nature of humic substances in forested Coastal Plain soils, we fractionated the SOM into 3 humic fractions: WSOC, fulvic and humic acids. We found that relatively low and nearly equal quantities of WSOC were extracted from upland and bottomland soils. Small amounts of WSOC are usually extracted from soils because water does not dissolve humic compounds and cannot readily disrupt the chemical or physical bonds with organic components (Hayes et al. 1975). The yield of WSOC in this study were similar to those reported by Hayes et al. (1975).

In general, bottomland soils had higher OC contents compared to upland soils (Table 1). The accumulation of OC in bottomland soils may be due to the influence of topography modifying rates of organic matter decomposition. Poor drainage and frequent flooding create anoxic conditions which limits organic matter decomposition (Kononova 1966; Stevenson 1982).

The C and N contents of humic and fulvic acids (Table 1) are within the range reported by Stevenson (1982). The C/N ratios in the fulvic acids are similar to a typical soil fulvic acid C/N ratio (20:1), however, the C/N ratios in the humic acids are higher than a typical soil humic acid C/N ratio (10:1) (Thurman 1985).

There was a high proportion of O-alkyl compounds (structures containing C-O and C-N moieties) in the ^{13}C NMR spectra of all WSOC and fulvic acid fractions, with bottomland soils having higher amounts of these compounds. These structures constitute the majority of organic components in the WSOC fraction (Table 4) of both soil regimes which is consistent with the results of Candler et al. (1988) who used ^{13}C NMR spectroscopy to characterize the WSOC leachate collected from an Inceptisol formed under a mixed-forest vegetation in Germany. The high quantity of O-alkyl structures which occur in the surface horizons of both soil regimes is also consistent with reports by Zech et al. (1989) who characterized humic fractions in several European soil profiles. The fulvic acid spectra from the upland and bottomland soils differed from spectra of fulvic acids from two Georgia Ultisols as reported by Lobartini et al. (1989), which had a higher aromatic and aliphatic C content compared to fulvic acids in this report. The humic acid spectra from upland and bottomland soils were similar to spectra of humic acids from Georgia Ultisols, as reported by Lobartini and Tan (1988).

A number of hypotheses can be proposed to explain the trends in the OC contents and the chemical nature of humic substances in the OC pool of forested Coastal Plain soils of South Carolina. Topography may modify the formation and nature of humic substances in SOM by influencing the drainage, vegetation and litter quality. The low OC contents in the upland soils may be due to the export of soluble DOC leachate and transport of potential humic acid precursor compounds to bottomland soils. Sand and loamy sand textures coupled with high infiltration rates in the upland soils could augment the flushing effect. Rapid C efflux from the upland soils would also reduce the residence time of humic acid precursors and thus, minimize potential polymerization reactions resulting in a low quantity of humic acid and hence low HA/FA ratios (Table 2).

Selective transport of certain classes of humic acid precursor compounds in the DOC leached from upland soils may also favor the formation of relatively high amounts of humic acids and HA/FA ratios in bottomland soils. Hydrophilic organic acids would readily move through the upland sandy Entisols and Ultisols and contribute humic acid precursors to the bottomland soils.

Litter quality may also influence the qualitative nature of humic substances in SOM. Lignin and lignin-like structures in the ^{13}C NMR spectra of humic acids in the upland soils may be due to the presence of *Quercus* and *Pinus* spp. which are known to contain high amounts of lignin (Day 1982). On the other hand, vegetation in the bottomland areas contains species that can lose up to 17% of their initial dry weight within 48 h after contact with water (Kuenzler et al. 1980). Brinson (1977) reported that $>25\%$ of the soluble leachate from fresh *Nyssa aquatica* leaves consisted of phenols that were rapidly released (6—29 h) upon contact with water. We found more O-alkyl structures in the WSOC and fulvic acid fractions of bottomland SOM when compared to upland SOM. The leaching of WSOC structures from litter and transport of hydrophilic humic acid precursors from upland areas could contribute to higher amounts of humic acid and higher HA/FA ratios in bottomland SOM when compared to upland SOM.

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