Nature of Clay-Humic Complexes in an Agricultural Soil: I. Chemical, Biochemical, and Spectroscopic Analyses

D. A. Laird,* D. A. Martens, and W. L. Kingery

ABSTRACT

Soil management systems that encourage the formation and stabilization of recalcitrant clay–humic complexes will have the greatest long-term impact on C sequestration and soil quality. The objective of this study was to determine the relationship between the chemical, biochemical, and spectroscopic characteristics of humic substances and clay mineralogy. Clay–humic complexes were separated from the Ap horizon of a Webster soil (fine-loamy, mixed, superactive Typic Endoaquoll) by an invasive sonication–centrifugation technique. The samples were analyzed for mineralogy by XRD (x-ray diffraction), chemical composition by inductively coupled plasma-atomic emission spectorscopy (ICP-AES), C and N by thermal combustion, C chemistry by solid state ^{13}C magic angle spinning-nuclear magnetic resonance spectroscopy (MAS-NMR) and both gas chromatography (GC) and high performance liquid chromatography (HPLC) analyses of extractable organic compounds. The coarse, medium, and fine clay fractions are dominated by quartz, a low-charged interstratified phase, and smectite, respectively. Extractable organic compounds, 30 to 52% of the total C, are dominated by basic amino acids and polyunsaturated fatty acids. Less than 3% of the extractable C is monosaccharides and amino sugars and only trace levels of phenolic acids were found. The C/N ratios of humic substances associated with the coarse, medium, and fine clay fractions are 17, 10, and 10, respectively. The coarse clay fraction has stronger carboxyl and O-alkyl ^{13}C-NMR peaks and lower levels of extractable amino acids, fatty acids, monosaccharides, and amino sugars than humics associated with the fine clay fraction. The results indicate that the biochemistry of the clay–humic complexes differs substantially from that of whole soils and that soil clay mineralogy strongly influences humification.

Agricultural soils have the potential to make a significant contribution towards reducing net emissions of greenhouse gases (CO_2 and CH_4) to the atmosphere through increased C sequestration in soil organic matter (OM). In a recent analysis, Bruce et al. (1999) estimated that with prudent management about 1.1×10^{15} g of C could be sequestered in USA and Canadian soils over the next two decades, and that C sequestration in agricultural soils could contribute 15% of the net reductions in C emissions needed to bring the USA and Canada in compliance with the Kyoto Protocols (United Nations, 1997). Increasing soil OM has the added benefit of improving soil quality and thereby enhancing the long-term sustainability of agriculture. However, development of management systems that optimize C sequestration in agricultural soils will require increased understanding of the biogeochemical processes that influence the complex cycling of organic C (OC) in soils.

D.A. Laird, USDA-ARS, National Soil Tilth Lab., 2150 Pammel Drive, Ames, IA 50011. D.A. Martens, USDA-ARS, Southwest Watershed Research Center, 2000 E. Allen Road, Tucson, AZ 85719-1596. W.L. Kingery, Dep. of Plant and Soil Sciences, Mississippi State Univ., P.O. Box 9555, Mississippi State, MS 39762. *Corresponding author (laird@nstl.gov).

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The biogeochemical cycling of OC in soils is divided into two general processes, (i) microbially mediated decomposition of biological materials to either volatile gases (CO_2 and CH_4) or humic monomers; and (ii) abiotic polymerization of humic monomers to form humic substances (Stevenson, 1994) (Note, in this paper the term *humic substances* refers to the products of secondary synthesis and is distinguished from the terms *humic acid* and *fulvic acid* which refer to materials obtained by a particular extraction procedure). Soil clay minerals are believed to serve three critical functions in the formation and stabilization of humic substances: First, humic monomers are adsorbed on clay surfaces where they accumulate over time. Second, clay minerals are believed to catalyze the abiotic polymerization of the adsorbed humic monomers. And third, adsorbed humic substances may be physically sequestered by clay minerals and hence unavailable to soil microorganisms.

The ability of clay minerals to adsorb and catalyze polymerization of synthetic humic monomers has been demonstrated in numerous laboratory studies. For example, nontronite, montmorillonite, illite, and kaolinite have all been shown to adsorb and significantly enhance the polymerization of polyphenols and the copolymerization of amino acids and polyphenols (Wang and Huang, 1989; Wang, 1991; Bosetto et al., 1997). Clay mineralogy and the type of inorganic cations adsorbed on the clay significantly effect both the rate and the products of the polymerization reactions.

Considerable evidence indicates that OM associated with fine soil clay particle-size fractions is more aliphatic, has relatively lower C/N ratios, younger radiocarbon ages, and greater lability during laboratory incubations compared with OM associated with coarse clay particle-size fractions (Arshad and Lowe, 1966; Ladd et al., 1977; Anderson et al., 1981; Tiessen and Stewart, 1983; Catroux and Schnitzer, 1987). However, the cause of these differences is poorly understood and evidence relating the observed differences to clay mineralogy is often equivocal. Common to many studies are fractionation schemes that seek to minimize disruption energy, in an attempt to isolate ecologically significant fractions. Such schemes, however, typically fail to isolate clay-organic complexes of mineralogical significance. For example, Catroux and Schnitzer (1987) used a mild ultrasonic technique to isolate particle-size fractions from a surface horizon sample of an Ontario Aquoll. Mechanical analysis revealed that the sample contained 22%

Abbreviations: CEC, cation-exchange capacity; FAME, methylated fatty acids; GC, gas chromatography; HPLC, high performance liquid chromatography; ICP-AES, inductively coupled plasma-atomic emission spectroscopy; MAS-NMR, magic angle spinning-nuclear magnetic resonance spectroscopy; OC, organic C; OM, organic matter; XRD, x-ray diffraction.

clay, 45% silt, and 35% sand, while recoveries following the mild ultrasonic dispersion were 15% clay, 55% silt, and 30% sand. Furthermore, only 1% of the mass was recovered as fine clay (<0.2 μm), whereas from a mineralogical perspective such soils are dominated by fine clays. As further example, Arshad and Lowe (1966) characterized OM associated with coarse-, medium-, fine-, and very fine-size fractions of a Bnt horizon from a soil dominated by smectitic clays. They found more OM associated with the coarse clay fraction which was predominantly smectitic but also contained kaolinite. From a mineralogical perspective, smectites are a component of the fine clay fraction and only present in coarse clays as a contaminant due to incomplete dispersion.

The goal of our research is to develop an understanding of the influence of clay mineralogy on the formation and stabilization of humic substances in soils. The specific objective of this study was to determine the relationship between mineralogy and the chemical, biochemical, and spectroscopic characteristics of humic substances associated with mineralogically significant clay fractions separated from a typical agricultural soil.

MATERIALS AND METHODS

The soil sample used in this study was collected from an agricultural field located on the University of Minnesota Southern Agricultural Experiment Station, near Waseca, MN. The sample was collected from the Ap horizon (0–15 cm) of a Webster pedon. The soil was mechanically dispersed in distilled water without chemical pretreatments and a bulk sample of the soil clay (<2 μm particle-size fraction) was separated by sedimentation and air dried as described previously (Laird et al., 1991a). The soil clay sample has been used in several previous investigations, and detailed analyses of the clay mineralogy (Laird et al., 1991a; Laird and Nater, 1993) and reactions between atrazine and various soil clay fractions (Laird et al., 1994) have been reported.

A Na-saturated clay suspension was prepared by shaking 60 g of soil clay with 2 L of 2 *M* NaCl. After the clay settled (24 h), the supernatant was discarded and then the sample was dispersed by shaking in 2 L of deionized water. Approximately 25% of the clay suspension was retained as the whole clay (<2.0 μm) sample. The remaining 75% of the suspension was fractionated using a sonication-centrifugation-decantation technique to separate the coarse (0.2–2.0 μm), medium (0.02–0.2 μm), and fine (<0.02 μm) particle-size fractions. To do so, 33 mL of the suspension (1 g clay) were transferred to 50 mL centrifuge tubes and subjected to seven sequential fractionation steps. For each fractionation step, the samples were diluted to 33 mL with distilled water, dispersed by both mechanical agitation and sonication (30 s at 40 W), centrifuged for 20 min at the appropriate speed (Jackson, 1985), and decanted. The first five fractionation steps separated the <0.02 and 0.02- to 2.0-μm fractions, and the last two fractionation steps separated the 0.02- to 0.2- and 0.2- to 2.0-μm fractions.

Clay in the suspensions was flocculated by adding excess NaCl, allowed to settle, and the solution decanted. One portion of each size fraction was retained as the untreated sample, and another portion of each size fraction was treated with 30% (v/v) H_2O_2 for removal of OM (Kunze and Dixon, 1986). All samples were washed four times with 0.5 *M* $CaCl_2$, dialyzed against distilled water and freeze dried.

Total C and N were determined by dry combustion using a Carlo Erba[1] NA1500 NSC elemental analyzer (Haake Buchler Instruments, Paterson, NJ). Elemental analysis (Al, Ca, Fe, K, Mg, Mn, Si, Ti, and Zn) of the clay samples was performed with an ICP-AES using the suspension nebulization technique (Laird et al., 1991b). Cation-exchange capacities (CEC) were determined using the ICP-AES analysis of Ca, the index cation, relative to the sample oven dried weights. The mineralogy of the samples was determined by x-ray diffraction (XRD). Portions (100 mg) of the Ca-saturated samples were slurried in 95% (v/v) ethanol, oriented on glass slides by the paste method, air dried, and analyzed between 2 and 30° 2Θ using Cu Kα radiation. Percentages of mineral phases are based on elemental mass balance using the known chemistry of 2:1 phyllosilicates in the Webster soil clay (Laird et al., 1991a).

The chemical nature of OM associated with the untreated samples was determined using solid state ^{13}C-NMR. The ^{13}C-NMR spectra were obtained using a Bruker AMX-300 spectrometer (Bruker Instruments, Inc., Brillerica, MA) operating at a resonance frequency of 75.4 MHZ. Data were collected with cross polarization, using bilevel decoupling, together with magic angle spinning. Experimental data acquisition parameters included 1 ms contact times and 4 s recycle delays while samples were spun at 7 kHz. A total of 24 000 scans were used to produce each spectrum.

Acid extractable monosaccharides, amino acids, and amino sugars in the untreated samples were measured by ion chromatography with pulsed amperometric detection (Martens and Frankenberger, 1990, 1992). Monosaccharides were extracted by refluxing 50 mg of clay with 1 *M* H_2SO_4 at 80°C for 16 h, then the sample was titrated to pH 5 with 5 *M* KOH, centrifuged, and an aliquot of the supernatant was diluted for analysis. Amino acids and amino sugars were extracted by autoclaving 50 mg of clay for 16 h with 4 *M* methanesulfonic acid (2 mg tryptamine mL^{-1}), the sample was titrated to pH 5 with 5 *M* KOH, centrifuged, and an aliquot of the supernatant was diluted for analysis. The extracts were analyzed using a Dionex DX-500 (Dionex Corp., Sunnyvale, CA) ion chromatograph equipped with a CarboPac PA10 (2-mm i.d.) column for monosaccharide analysis and an AminoPac PA10 (2-mm i.d.) column for amino acid and amino sugar analysis. Separations were achieved with a NaOH gradient (5–80 m*M*) for monosaccharides and a NaOH-Na acetate gradient (30–80 mM NaOH; 0–500 m*M* Na acetate) for amino acids and amino sugars.

Phenolic acids were extracted from 50-mg clay samples with 5 mL of 4 *M* NaOH heated in Teflon microwave digestion bombs to 160°C in a CEM MDS-2000 microwave digester (CEM Corp., Matthews, NC) operated at 650 W (Provan et al., 1994). After cooling, the supernatant and solids were separated by centrifugation-decantation; then the solids were washed with water and the supernatants combined. The supernatant sample was titrated to <pH 2.0 with 4 *M* HCl, diluted to 14 mL, centrifuged to remove the formed precipitate, and an aliquot passed through a Varian (Varian Assoc., Harbor City, CA) Bond Elut PPL solid phase extraction tube. The tubes were dried under a stream of air and the phenolic compounds were eluted with 1 mL of ethyl acetate into GC autosampler vials. The phenolic compounds (1 μL; 10:1 split) were then analyzed with a Hewlett-Packard 6890 gas chromatograph (Hewlett Packard Co., Palo Alto, CA) equipped with a HP-5 (5% cross-linked phenylmethyl siloxane) capillary column (30-m length, 0.32-mm column i.d., 0.25-μm film thickness) and detected with a flame ionization detector. The following conditions were employed for phenolic acid separation:

[1] Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that might also be suitable.

injector temperature, 230°C; temperature ramp, 70°C for 2 min then ramped to 250°C at 10°C min^{-1}; detector temperature, 250°C.

Fatty acids were extracted from the clay samples (100 mg) with 10 mL of a monophasic mixture of methanol, chloroform, citrate buffer (2:1:0.8, v/v/v) for 2.5 h at 80°C (Frostegard et al., 1993). The solution and solids were separated by centrifugation and the supernatants were placed into glass test tubes with Teflon lined caps. The samples were split into two phases by adding chloroform and buffer; then the citrate-methanol phase was removed and chloroform phase dried under a stream of air. The sample was methylated by addition of 1 mL methanol, chloroform, concentrated HCl (10:1:1, v/v/v), and the mixture was heated overnight at 60°C (Zelles and Bai, 1993). The methylated fatty acids (FAMEs) formed were extracted by addition of 0.5 mL chloroform and 4 mL water. The chloroform phase was then removed after centrifugation for analysis with a Hewlett-Packard 6890 GC (Hewlett Packard Co., Palo Alto, CA) equipped with a HP-5 (5% cross-linked phenylmethyl siloxane) capillary column (30-m length, 0.32-mm column i.d., 0.25-μm film thickness) and detected with a flame ionization detector.

The identities of FAME and phenolic compounds were confirmed with a Hewlett-Packard 1800A GCD (Hewlett Packard Co., Palo Alto, CA) gas chromatograph equipped with a HP-Ultra 1 capillary column (25-m length, 0.2-mm column i.d., 0.33-μm film thickness) and a mass selective detector operated in the full scan (10–450 m/z) electron impact mode (70 eV, source temp. 170°C). The following conditions were employed for phenolic acid separation: 1-μL splitless injection, injector temperature of 250°C, initial column temperature of 70°C ramped to 250°C at 10°C min^{-1}, and detector temperature of 280°C. The temperature ramp used to separate the FAMEs was 70°C (2 min) then ramped to 140°C (10°C min^{-1}), to 180°C (3°C min^{-1}), and to 275°C (7°C min^{-1}) for 10 min. The mass spectra of standards and unknowns were compared with a NIST spectrum library.

RESULTS

The clay mineralogy of the Webster soil has been extensively studied (Laird et al., 1991a; Laird and Nater, 1993). Coarse-clay separates are dominated by quartz with lesser amounts of 10Å-illite, kaolinite, and feldspars. Fine-clay separates contain smectite and a low-charged interstratified phase. The smectite is a high-Fe montmorillonite with a moderate layer charge [0.482 (-) per formula unit] and 47% tetrahedral charge. The low-charged interstratified phase consists of randomly interstratified 10 and 15 Å 2:1 phyllosilicate layers with a layer charge of 0.473 e per formula unit and 87% tetrahedral charge (Laird et al., 1991a). Although, the low-charged interstratified phase cannot be classified using current nomenclature guidelines (Bailey et al., 1982), it has unofficially been referred to as "protoillite" (Laird and Dowdy, 1994) and that convention will be followed hereafter in the present manuscript.

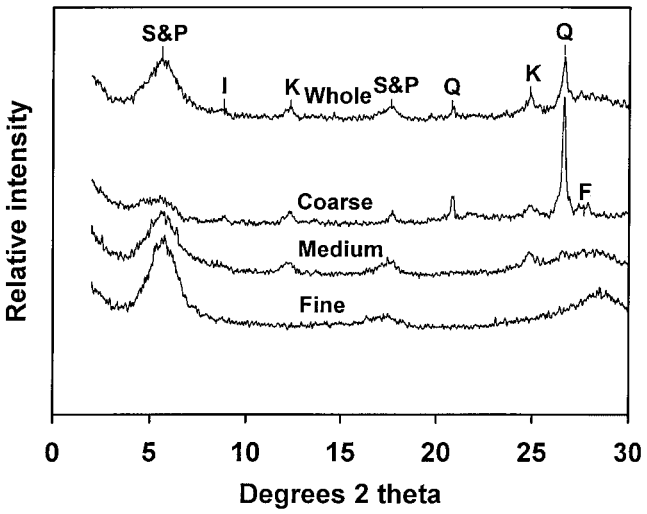

Fig. 1. X-ray diffraction (XRD) patterns of H_2O_2-treated clay fractions from the Webster soil. The samples were Ca-saturated, oriented on glass slides, air dried, and analyzed using Cu Kα radiation.

Table 1. Chemical composition of the H_2O_2-treated whole-, coarse-, medium-, and fine-clay fractions isolated from the Webster soil.

Fraction	Al	Ca	Fe	K	Mg	Si	Mn	Zn	Ti
	g kg^{-1} oxides								
Whole	104	14.6	63.6	15.9	12.0	301	0.498	0.301	4.45
Coarse	79.8	7.27	27.7	25.7	7.51	347	0.569	0.190	8.23
Medium	122	14.9	68.0	16.8	14.0	280	0.496	0.340	4.39
Fine	105	20.1	93.5	4.27	13.6	284	0.403	0.394	1.35

X-ray diffraction patterns for H_2O_2-treated samples of the whole-, coarse-, medium-, and fine-fractions of the Webster soil clay are presented in Fig. 1, and chemical analysis of the H_2O_2-treated samples are given in Table 1. The analyses revealed significantly different mineralogies for the coarse, medium, and fine clay fractions. The coarse clay fraction is dominated by quartz, but also contains kaolinite, protoillite, 10Å-illite, and feldspars. Because of the large number of mineral phases present, quantification of the clay mineralogy for the coarse clay fraction was not attempted. The medium clay fraction contains 70% (w/w) protoillite, 25% (w/w) smectite, and 5% (w/w) kaolinite; and the fine clay fraction contains 82% (w/w) smectite and 18% (w/w) protoillite.

Chemical analyses of OM in the untreated soil clay fractions are presented in Table 2. No effervescence was observed when the original soil sample was treated with acid, no carbonate minerals were identified in the XRD analysis, and the pH of the various Ca-saturated soil clay fractions in distilled water is 5.0 ± 0.1; hence, all of the C in the soil clay fractions is assumed to be organic. Total C determined by thermal combustion ranges from 69.8 g C kg^{-1} clay in the coarse clay fraction

Table 2. Chemical properties of soil clay fractions from the Webster soil. Standard deviations are given in parentheses. Data originally presented in Laird et al. (1994).

	Soil clay fractions			
	Whole	Coarse	Medium	Fine
	g kg^{-1} clay			
C untreated	63.2 (2.0)	69.8 (1.5)	67.2 (2.1)	51.7 (0.3)
N untreated	5.0 (0.3)	4.1 (0.5)	6.4 (0.5)	5.3 (0.1)
	mol(+) kg^{-1} clay			
CEC untreated	0.86†	0.65	0.86	1.02
CEC H_2O_2-treated	0.65	0.32	0.68	0.91

† Cation-exchange capacity (CEC) values for untreated samples are based on the weight of the untreated samples and the CEC values for the H_2O_2-treated samples are based on the weight of the H_2O_2-treated samples.

Table 3. Biochemistry of organic compounds extracted from the Webster clay fractions.

Sample	SUM	Mono-saccharides	Amino sugars	Amino acids	Phenolic acids	Fatty acids
			% C (w/w)			
Whole	31.5†	2.3	2.4	14.4	0.2	12.2
Coarse	29.9	1.0	1.1	9.3	0.3	18.3
Medium	33.1	2.2	2.5	13.7	0.2	14.5
Fine	52.0	3.0	3.1	17.1	0.3	28.3
			% N (w/w)			
Whole	103.2	–	5.9	97.3	–	–
Coarse	92.9	–	3.6	89.4	–	–
Medium	81.5	–	5.1	76.4	–	–
Fine	97.9	–	6.0	91.9	–	–

† Data are expressed as weight percent C or N relative to the total C or N determined by thermal combustion (Table 2).

to 51.7 g C kg^{-1} clay in the fine clay fraction. Total N ranges from 6.4 g N kg^{-1} clay in the medium clay fraction to 4.1 g N kg^{-1} clay in the coarse clay fraction. The coarse clay has a C/N ratio of 17 while the medium and fine clay fractions have C/N ratios of 10. The CEC of the clay–humic complexes range from 0.65 mol(+) kg^{-1} for the coarse clay to 1.02 mol(+) kg^{-1} for the fine clay. The CEC's decreased following the H_2O_2 treatment, reflecting the contribution of the humic substances to the CEC of the clay–humic complexes. The largest decrease in CEC was for the coarse clay fraction [0.33 mol(+) kg^{-1}] and the smallest decrease in CEC was for the fine clay fraction [0.11 mol(+) kg^{-1}].

Results of the biochemical analyses of the various soil-clay fractions are presented in Table 3. The sum of extractable C ranged from 19.9 g C kg^{-1} clay for the whole clay to 26.9 g C kg^{-1} clay for the fine clay. The sum of extractable C for the coarse and fine clay fractions account for 29.9 and 52.0%, respectively, of the total C as determined by thermal combustion. Most of the extractable C was in unsaturated C18 fatty acids (12.2–28.3%) and amino acids (9.3–17.1%). Less than 3% of the C was identified as monosaccharides and amino sugars and <0.3% was identified as phenolic acids. The N in amino acids and amino sugars accounts for nearly all (82–103%) of the N in the samples as determined by thermal combustion. Arginine, by far the most abundant amino acid, accounts for 50 to 66% of the total N in the soil clay extracts. Lysine is the second most abundant amino acid and contributes 3 to 8% of the total N in the samples.

Solid-state ^{13}C-NMR analysis was used to further evaluate the chemical nature of the humic substances associated with the various soil-clay fractions. The NMR analysis was conducted using the untreated clay–humic complexes in an effort to avoid bias caused by both selective extraction and chemical alteration which may occur when humic substances are extracted from soil and clay samples. The disadvantage of the approach, however, is that the clay–humic complexes have relatively low levels of ^{13}C and relatively high levels of paramagnetic elements (principally Fe^{3+}). Paramagnetic elements inhibit cross polarization thereby masking the NMR signal for ^{13}C nuclei that are proximal to paramagnetic elements. Because of the low signal-to-noise ratio, the spectra are only amenable to qualitative comparisons. The spectra

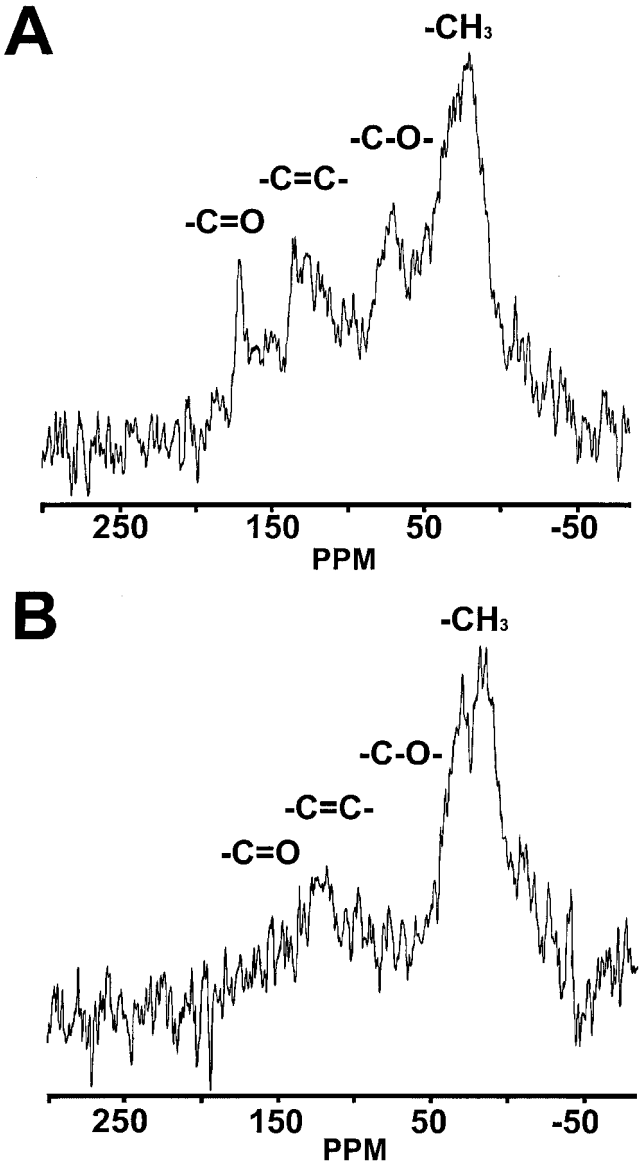

Fig. 2. Solid state-magic angle spinning ^{13}C-NMR spectra of the (a) untreated coarse and (b) fine clay fractions from the Webster soil.

for the coarse clay fraction (Fig. 2a) exhibits broad but clearly identifiable peaks in the alkyl (0–50 ppm), O-alkyl (50–100 ppm), alkene-aromatic (110–150 ppm), and carboxyl (160–190 ppm) regions. By contrast, the spectra for the fine clay (Fig. 2b) has a diffuse peak in the alkene-aromatic region and two prominent peaks in the alkyl region. The spectra for the whole and medium clay fractions (data not shown) exhibited characteristics intermediate between those of the coarse and fine clay fractions.

DISCUSSION

The initial noninvasive fractionation procedure used in this study separated clay–humic complexes (the whole soil-clay fraction) from biological materials. The efficacy of the separation in removing biological materials was verified by scanning electron microscopy (Laird, 2000).

The second invasive fractionation procedure separated the whole soil clay into coarse, medium, and fine clay fractions which are dominated by quartz, protoillite, and smectite, respectively. These mineralogically distinct fractions contained similar amounts of OC (Table 2), which indicates that the associated humic substances are either chemically bonded to the mineral surfaces or present in discrete particles with similar size and density to that of the mineral particles. If the humic substances had been present as discrete low-density particles, the OC would have been discarded with the aqueous supernatant during sample preparation or concentrated in the fine clay fraction.

Chemical, biochemical, and spectroscopic analysis of the various soil-clay fractions revealed unique characteristics for the OM associated with each of the mineralogically significant fractions. The OM associated with the coarse clay fraction has a higher C/N ratio, lower levels of extractable amino acids, fatty acids, monosaccharides, amino sugars, and stronger carboxyl and O-alkyl ^{13}C-NMR peaks relative to the OM associated with the fine and medium clay fractions. If humic substances had been randomly associated with the different clay minerals, the chemical nature of the humic substances associated with the coarse, medium, and fine clay fractions would have been similar. Thus, the observed differences demonstrate specificity for the relationship between soil-clay mineralogy and humic substances.

The biochemical analyses of the clay–humic complexes determined that arginine and lysine are the most abundant amino acids followed by aspartic acid and glutamic acid. Glucosamine which derived from chitin found in fungal hyphae is the most abundant amino sugar in the clay–humic complexes. Arabinose is the most abundant monosaccharide measured followed by galactose and xylose. Oades (1984) suggested that a ratio <0.5 of galactose plus mannose/arabinose plus xylose is typical of plant derived monosaccharides and a ratio of >2 is characteristic of microbial carbohydrates. The ratio decreases from 1.03, 0.81, to 0.62 for the coarse, medium, and fine clay, respectively, suggesting more of a plant source for the monosaccharides in the clay–humic complexes than a microbial source. The low levels of glucose in the extracts suggest that this monosaccharide is predominately decomposed by soil organisms and not sequestered on clay surfaces. Fatty acids are a major component of the C from the clay fractions (Table 3) and 67 to 76% of the FAMEs isolated from the clay fractions were unsaturated C18 (octadecene fatty acids) as confirmed by mass spectrum analysis. Low levels of C14:0, C15:0, and C16:0 FAME's were also identified. Arao (1999) reported that the unsaturated C18 FAME's are predominately found in fungi while the saturated FAME's are biomarkers for bacteria. *P*-hydroxycinnamic acid was the predominate phenolic acid extracted with lower levels of syringic acid and syringaldehyde confirmed by mass spectrum analysis. Hydroxycinnamic acids are important components of plant cell walls (Provan et al., 1994) suggesting that the clay associated compounds reflect both a microbial and a plant source.

Although the biochemical analysis accounted for $<52\%$ of the total C in the clay–humic complexes, the results are generally consistent with the ^{13}C NMR analysis. The relatively strong alkyl peaks in the ^{13}C NMR spectra are consistent with the high levels of extractable fatty acids and amino acids found in the samples. The small and even absent O-alkyl ^{13}C NMR peaks are consistent with the very low levels of extractable monosaccharides and amino sugars in the clay–humic complexes. Even the absence of extractable phenolic acids is not necessarily inconsistent with the ^{13}C NMR spectra. Alkene and aromatic C are not resolved in the ^{13}C NMR spectra, hence the relatively strong peaks in the 110 to 150 ppm region (Fig. 2) could be because of alkene C in the unsaturated fatty acids rather than aromatic C. On the other hand, the results of the ^{13}C NMR and biochemical analyses contrast with published analyses for whole soils. Analysis of biochemicals extracted from whole soils generally indicate 5 to 25% carbohydrates, 2 to 6% fats, waxes, and resins, and 2 to 30% phenolic acids (Stevenson, 1994). And ^{13}C NMR analyses of whole soils and humic acids extracted from whole soils typically indicate much higher levels of O-alkyl and aromatic C (Stevenson, 1994; Hayes, 1991). The discrepancy between published results for whole soils and the results of this study is attributed to the fact that whole soils contain both clay–humic complexes and biological materials, whereas the samples used in this study contain only clay–humic complexes. Thus the results suggest that both monosaccharides and phenolic acids are dominantly associated with biological materials rather than clay–humic complexes.

The large enrichment of arginine in the clay–humic complexes relative to levels of arginine in whole soils (4 to 5.5% of total amino acids; Senwo and Tabatabai, 1998) suggest that arginine actively accumulates in the clay–humic complexes. Arginine is the most basic of the 20 α-amino acids in biological systems. Thus the positively charge guanidinium group (pKa = 12.48) on arginine may bond directly to negative charge sites on basal surfaces of 2:1 phyllosilicates. This interpretation is consistent with the relatively small difference in CEC (Table 2) between the untreated and H_2O_2-treated fine clay fraction [0.11 mol(+) kg^{-1}], which is dominated by smectite, and the much larger difference in CEC between the untreated and H_2O_2-treated coarse clay [0.33 mol(+) kg^{-1}], which is dominated by quartz and other minerals that lack permanent charge sites. The interpretation is also consistent with the trend of increasing N content and decreasing C/N ratios with decreasing particle size (i.e., increasing smectite content) observed in this and several other studies (Anderson et al., 1981; Tiessen and Stewart, 1983; Catroux and Schnitzer, 1987).

Previously, both adsorption and desorption of atrazine were quantified for the same soil clay fractions (Laird et al., 1994). The study revealed a substantial decrease in the affinity of the coarse clay fraction for atrazine following the H_2O_2-treatment and only a minor decrease in the affinity of the fine clay fraction for atrazine following the H_2O_2-treatment. The results indicate that humic substances associated with the coarse clay

have a much higher affinity for atrazine than the humic substances associated with the fine clay. Wu et al. (1999), using similar procedures, found that the humic substances associated with the coarse clay fraction of a Zook soil (fine, smectitic, mesic Cumulic Vertic Endoaquolls) have a much higher affinity for Cu than the humic substances associated with medium or fine clay fractions. The mineralogy of the clay fractions separated from the Zook soil is similar to the mineralogy of the clay fractions separated from the Webster soil. Thus, considerable evidence indicates that the humic substances which are physically separated with the different soil clay minerals are functionally different as well as chemically, biochemically, and spectroscopically different.

The observed relationship between clay mineralogy and the chemical nature of the associated humic substances indicates that either soil clay mineralogy strongly influences the humification process or that humic substances with different properties are selectively adsorbed on different clay mineral species. Based on the present study it is not possible to distinguish which of the two processes (or both) are responsible for the observed differences in the clay associated humic substances. Regardless of which process is involved, it is apparent that a full understanding of humification and C-sequestration mechanisms cannot be obtained by a priori extraction of humic and fulvic acids from soils, but must include an understanding of the influence of clay mineralogy on humification. In the second paper of this series (Laird, 2001), scanning electron microscopy is used to further elucidate the relationships between humic substances and soil clay mineralogy.

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