EFFECT OF COVER CROP SYSTEMS ON THE CHARACTERISTICS OF SOIL HUMIC SUBSTANCES

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1 INTRODUCTION

Soil organic matter (SOM) is an important attribute of soil quality, influencing the productivity and physical well being of soils. Thus, it is important from economic and environmental standpoints to determine how changes in cover crop management will affect SOM and soil quality.

SOM contents and properties are functions of agricultural practices and the amounts and kinds of plant residues returned to the soil.[1-9] It is well established that the labile components of SOM change and reach a new steady state more quickly in response to various management practices than does total organic matter.[10-13] Wander and Traina[14] showed that SOM in crop rotation with cover crops was significantly higher than those rotations without cover crops. However, Lal et al.[15] reported no or minimal change of SOM content. The reason for not detecting any SOM change could be due to natural soil heterogeneity.[14] It is well known that continuous cultivation of cereal crops generally results in substantial losses of soil C and N.[16-18] However, studies of organic matter using ^{13}C nuclear magnetic resonance (NMR) spectroscopy have indicated that the chemical nature of the remaining C shows little change as a result of cultivation and that the stability of SOM appears to depend more on physical protection mechanisms than any inherent recalcitrance of the organic structures.[19-22]

In addition to SOM quantity, the quality (e.g., structure and composition) and distribution of individual fractions (e.g., humic acids, polysaccharides) are essential to the maintenance of soil productivity. Monreal et al.[23] observed a higher lignin dimer to lignin monomer ratio in continuous wheat rotation and this ratio decreased from large to small aggregate sizes, indicating the change in SOM quality. Wander and Traina[24] used diffuse reflectance Fourier transform infrared spectroscopy (DRIFTS) to examine functional groups of SOM fractions and reported that the ratios of reactive to recalcitrant fractions in humic acids best reflected overall SOM bioavailability.

DRIFTS detects molecular vibrations and is useful for functional group analysis and for identification of molecular structures of SOM.[25] But it cannot be used to quantify carbon contents of structural groups. By contrast, ^{13}C NMR spectroscopy provides quantitative data for structural components. NMR has been successfully used to characterize SOM by many scientists.[26-30] Thus, it would be advantageous to use both NMR and DRIFTS to characterize SOM and compare quantitative data of SOM under

different cover crop systems. The objective of this study is to determine quantitative, structural and compositional changes of humic substances (humic acid and fulvic acid fractions) caused by cover-crop systems using both NMR and DRIFTS.

2 MATERIALS AND METHODS

2.1 Site Description and Sampling

Since 1990, cover crop experiments have been conducted in the Connecticut River Valley at the Massachusetts Agricultural Experiment Station Farm in South Deerfield, Massachusetts. The soil at the University of Massachusetts at Amherst farm is a fine sandy loam (coarse, mixed, mesic Fluventic Dystrudept) and low in SOM (~2%). Its upper 0.6 m is homogeneous, overlaying inclined layers of coarse and fine material to a great depth. It is a typical soil in the intensively cropped Connecticut River Valley in Massachusetts. Three cover crop treatments with four nitrogen rates (applied to the corn crop after cover crop incorporation) were laid out in a complete factorial design in bordered 3 m x 7.5 m plots in four randomized blocks. Cover crop treatments and seeding rates were 1) check (no cover crop); 2) rye (125 kg/ha); 3) hairy vetch + rye (46+65 kg/ha). Nitrogen fertilizer rates were 0, 67, 135, 202 kg N/ha using NH_4NO_3. Detailed soil sample information is listed in Table1.

Table 1 *Soil samples (UMass South Deerfield Farm)*

Sample number	*Depth (cm)*	*Cover crops*	*Nitrogen rates (kg N ha^{-1})*
VR1	0-25	Vetch/Rye	0
VR4	0-25	Vetch/Rye	202
RA1	0-25	Rye alone	0
RA4	0-25	Rye alone	202
C1	0-25	No cover crop	0
C4	0-25	No cover crop	202

1 = No nitrogen fertilizer treatment, 4 = Nitrogen fertilizer treatment

2.2 Extraction, Fractionation and Purification of Humic Substances

2.2.1 Extraction. Soil was air-dried and passed through a 2 mm sieve. Air-dry soil (50 g) was weighed into a 1000 mL plastic flask, then 500 mL 0.1 M $Na_4P_2O_7$ was added. The air in the flask and solution was displaced with nitrogen (N_2) and the system was shaken for 24 hr at room temperature.

2.2.2 Fractionation. After separation from the $Na_4P_2O_7$ insoluble organic residues by centrifugation at 3000 rpm, the dark-colored supernatant solution was acidified to pH 1 with 6 M HCl and allowed to stand for 24 hr at room temperature for the coagulation of the HA fraction. The soluble material (FA) was separated from the HA by centrifuging at 10000 rpm.

2.2.3 Purification. HA was shaken 3 times for 24 hr at room temperature with 0.1 M HCl/0.3 M HF solution. The insoluble residue (HA) was separated from the supernatant by centrifuging at 10000 rpm, washed with deionized water until free of chloride ions and

then freeze-dried. The FA solution was passed through an XAD-8 resin column. The effluent was discarded and the XAD-8 column containing sorbed FA was washed 3 times with deionized water, then the sorbed FA was eluted with 0.1 M NaOH. The solution was immediately acidified with 6 M HCl to pH 1. The resulting solution volume was sufficient to maintain the FA in solution, which was then freeze-dried.

2.3 Diffuse Reflectance Fourier Transform Infrared (DRIFTS) Analysis

DRIFTS was performed in a Midac series M 2010 infrared spectrophotometer with a DRIFTS accessory (Spectros Instruments). All SOM fractions were ground with a sapphire mortar and pestle and stored over P_2O_5 in a dry box. Humic and fulvic acid concentrations for this determination ranged from 2 to 4 mg and were supplemented with KBr to a total weight per sample of 100 mg, then ground with an agate mortar and pestle. The milled sample was immediately transferred to a sample holder and its surface was smoothed with a glass microscope slide. Before analysis, the diffuse-reflectance cell containing the samples was flushed with N_2 gas to eliminate interference from carbon dioxide and moisture. A small jar (20 mL) containing anhydrous $Mg(ClO_4)_2$ was placed inside the sample compartment to further reduce atmospheric moisture.

To obtain DRIFTS spectra, 100 scans were collected at a resolution of 16 cm^{-1} and the spectra with numerical values for major peaks wave-numbers and intensities were recorded. The blank consisted of the powdered KBr stored under the same environmental conditions as the sample-KBr mixtures. Absorption spectra were converted to Kubelka-Munk functions using the Grams/32 software package (Galactic Corporation). Peak assignments and intensity (by height) ratio calculations were made with the methods of Wander and Traina,[24] Baes and Bloom[31] and Niemeyer et al.[32]

2.4 NMR Spectroscopy

CP-TOSS (Cross-Polarization and Total Sideband Suppression) was used.[30] Samples were run at 75 MHz (^{13}C) in a Bruker MSL-300 spectrometer. HA samples were packed in a 7-mm-diameter zirconia rotor with a Kel-F cap. The spinning speed was 4.5 kHz. A 90° ^1H pulse was followed by a contact time ($t_{c\rho}$) of 500 μs, and then a TOSS sequence was used to remove sidebands.[30,33] The 90° pulse length was 3.4 μs and the 180° pulse was 6.4 μs. The recycle delay was 1 s with the number of scans about 4096.

3 RESULTS AND DISCUSSION

3.1 Carbon-13 NMR Characteristics

For quantitative NMR measurements the conditions that must be met are $t_{c\rho} \ll T_{1\rho}$ (^1H). That is, the contact time must be much shorter than the time constant for proton spin lattice relaxation in the rotating frame.[34] In addition, the delay time between cross-polarization sequences must be long enough to allow for complete ^1H spin relaxation (i.e., at least five times $T_1(^1H)$). The ideal situation of $t_{c\rho} \ll T_{1\rho}$ (^1H) may be difficult to satisfy in practice, especially where $T_{1\rho}$ (^1H) is very short. Optimum acquisition parameters were therefore chosen after examination of the spin dynamics of the samples to avoid signal suppression by incomplete relaxation.

Although CP-TOSS spectra could not be used for absolute quantitation, they could be

compared because all the HAs samples were run under the same conditions and were from the same type of soil. Functional groups were assigned as follows: 0-50 ppm for aliphatic-C (C in straight-chain, branched and cyclic alkanes and alkanoic acids); 50-60 ppm methoxy-C including C from OCH_3 groups as well as C from amino acids; 60-96 ppm carbohydrate-C (aliphatic C bonded to OH groups, or ether oxygens, or occurring in saturated five- or six-membered rings bonded to oxygens); 96-108 ppm O-C-O; 108-145 ppm aromatic-C; 145-162 ppm phenolic-C (i.e., aromatic C bonded to OH groups); 162-190 ppm carboxylic-C; and 190-220 ppm carbonyl-C.[25,34,35]

The ^{13}C NMR data are summarized in Table 2. The HA from the rye alone system differs from other HAs in the aliphatic region (0-50 ppm) and the HA signal in this region is the smallest with no nitrogen fertilizer treatment. The most intense signal in this region appears under the vetch/rye system with no nitrogen fertilizer treatment. When compared to the total aliphatic region (0-108 ppm), the aliphatic-C of the vetch/rye HA without nitrogen fertilizer is 55.3%, and this figure decreases to 48.9% for rye alone without nitrogen fertilizer.

Table 2 *The ^{13}C NMR characteristics of HA samples*

Type of Materials	*Distribution of C Chemical shift (ppm) %*							
	0-50	*50-60*	*60-96*	*96-108*	*108-145*	*145-162*	*162-190*	*190-220*
VR1	26.8	8.4	15.9	4.2	22.6	6.5	14.4	3.2
VR4	26.4	8.4	16.9	4.2	21.6	6.6	14.4	1.5
RA1	20.8	6.7	17.1	4.2	25.4	7.5	16.4	1.9
RA4	21.2	7.1	18.2	4.3	23.5	7.2	16.3	2.3
C1	26.1	8.0	17.5	4.5	21.3	6.2	14.4	2.0

Types of Materials	*Aliphatic-C %*	*Aromatic-C %*	*(Aliphatic-C)/(Aromatic-C)*
VR1	55.3	29.1	1.9
VR4	55.9	28.2	2.0
RA1	48.9	32.8	1.5
RA4	50.7	30.7	1.7
C1	56.1	27.6	2.0

The phenolic-C (145-162 ppm) content is higher in the HA extracted from rye alone systems than the other two systems. Similarly, the HA extracted from the rye cover system is also relatively enriched in aromatic-C. An inspection of the data for HA with no cover crop shows that aromatic-C content (including 108-145 and 145-162 ppm regions) is the lowest (Table 2). The nonprotonated C signals (130 ppm) likely are from aromatic carbons, including C in polynuclear aromatic rings. Polymerization and polycondensation of HA macromolecules may have occurred on contact with soil minerals.[34,35] For both sites of rye systems (with or without nitrogen fertilizer), the relative increase in aromatic C and decrease in aliphatic C (Table 2) indicate that humification may be greater than in the other two treatments. These results may indicate a greater stability of HA from rye alone systems because 1) the HA appears to contain fewer biopolymers (proteinaceous materials and carbohydrates) and is therefore less biodegradable and 2) the greater aromaticity

implies a more stable chemical structure.

3.2 DRIFTS Spectroscopy of HA and FA

Wavenumbers and assignments for peaks in DRIFTS data are the same as in IR and FTIR spectroscopy.[25,31,32] The main absorbance bands and corresponding assignments obtained for the various HA and FA samples are listed in Table 3. All peaks observed in the HA and FA spectra are typical, as reported in the literature. However, more detailed information on the reactivity of HA and FA is provided by calculating O/R ratios.

The O/R ratios are computed by dividing the peak heights of oxygen-containing functional groups by those of aliphatic and aromatic (referred to as recalcitrant) groups.[24] The impact of cover crop systems on spectral composition is summarized by these ratios (Figures 1 and 2). The HA fraction isolated from vetch/rye plots with nitrogen fertilizer has the highest ratio

$$R_1 = (1727+1650+1160+1127+1050)/(2950+2924+2850+1530+1509+1457+1420+779))$$

and the lowest R_1 ratio appears at the vetch/rye without nitrogen fertilizer (Figure 1). The relatively high O/R ratios of the HA support the notion that SOM in the vetch/rye with nitrogen fertilizer is more biologically active. When compared with the R_1 values of the HA fractions with nitrogen fertilizer, the highest R_1 is for the vetch/rye system, followed by no cover crop and rye alone systems. For the treatments without fertilizer, R_1 values follow an order of no cover crop > rye along > vetch/rye.

R_2 is the ratio of the peak heights of ketonic and carboxyl (1720 cm^{-1}) groups divided by those of CH_2 and aromatic (1457+1420+779 cm^{-1}) groups. Its value is the highest in the HA spectrum of rye alone without fertilizer. This ratio is the lowest in the HA spectrum of rye alone with nitrogen fertilizer. We expect that since HA makes up the largest single SOM pool in mineral soils, HA O/R ratios would be a good indicator of overall SOM characteristics. However, the O/R ratio may not reflect the bioavailability of SOM.[24]

Fulvic acids have relatively low molecular weights and high oxygen contents, and as a result are more polar and therefore mobile than HAs. Thus, FA may be more representative of the available organic matter pool.[24,25] In contrast to the HA O/R ratios, the FA O/R ratio ((1850+1650+1400+1080+560)/(3340+2924+1535+1457)) is greater in the vetch/rye without fertilizer than with fertilizer treatment (Figure 2). Meanwhile, the O/R ratios of FA from both the vetch/rye treatments are significantly higher than rye treatments. This means that FA fractions from vetch/rye systems are more active than from the rye alone systems. The O/R ratios of FA do not differ much between the rye alone systems and no cover crop system with nitrogen fertilizer. This indicates that FA fractions may undergo a similar change either by chemical oxidation or as a microbial carbon source for those systems. When we compare the R_1 ratios of HA with O/R ratios of FA in different cover crop systems, the HA R_1 from both the vetch/rye and rye alone systems is higher with nitrogen fertilizer treatment than without nitrogen fertilizer. The reverse is true for the O/R ratios of FA. Both HA R_1 and FA O/R ratios of no cover crop system are higher without nitrogen fertilizer treatment than with nitrogen fertilizer. This suggests that cover crop systems (with or without nitrogen fertilizer) affect the HA and FA composition. However, even though FA O/R ratios reflect chemical reactivity, they are not positively correlated with SOM bioavailability (based on indirect measures of N and C mineralization).[8] It is generally assumed that the composition of humic substances, including HA and FA, remains relatively constant in a given soil.

Table 3 *Peak assignments of diffuse reflectance Fourier transform infrared spectra (DRIFTS) for HA and FA samples*[24,25,31]

Wavenumber (cm^{-1})	*Functional groups*	*HA*	*FA*
3279-3340	Phenol OH, amide N-H	X	X
2962-2950	CH_2, symmetric stretch	X	
2924-2930	CH_2, asymmetric stretch	X	X
2850	CH_2, symmetric stretch	X	
2500	CO-OH H bonded	X	X
1850	C=O stretch		
1735-1713	C=O ketonic, COOH	X	
1650	C=O, C=O-H bonded, amide H	X	X
1630-1608	C=C aromatic		
1550	Aromatic ring, amide		
1535-1520	C=C aromatic ring, amide	X	X
1509	Aromatic ring, amide	X	
1457	CH_3 asymmetric stretch, CH bond	X	X
1420	Aromatic ring stretch		X
1400	COO^- salt, COOH		X
1379-1327	COO^-, CH_3, symmetric stretch		
1260-1240	CO, COOH, COC, phenol OH	X	
1190-1127	Aliphatic, alcoholic OH	X	
1080-1050	CO aliphatic alcohol	X	X
1030	Aliphatic COC, aromatic ether, SiO		
918-912	OH, COOH, Al-OH		
850-830	CH aromatic bend, Al-O-Si	X	
799	Fe-O-Si		
779	CH aromatic out-of-plane bend	X	
750	Unknown mineral peak		
694	Unknown mineral peak		
560	COO salt, Mg/Si-O aliphatic	X	
530-520	Si-O		
480	Aromatic ring bending	X	

4 CONCLUSIONS

In this study, the chemically isolated HA and FA fractions display the quantitative and qualitative differences resulting from various cover crop treatments. These fractions are affected by agronomic and environmental factors. Although DRIFTS cannot be used to

quantify the absolute C contents of structural groups, it can be used to generate peak ratios from which we can assess the relative enrichment or depletion of specific functional groups. The HA O/R ratio is a good indicator of overall SOM characteristics, and cover crop systems and nitrogen fertilizer rates can change the O/R ratios of HA and FA. From CP-TOSS ^{13}C NMR data, the HA extracted from rye alone systems is more aromatic and less aliphatic than the HA from vetch/rye, indicative of the impact of cover crop systems on the structure and composition of humic substances. Future research needs to address how those changes of SOM affect soil productivity and sustainability.

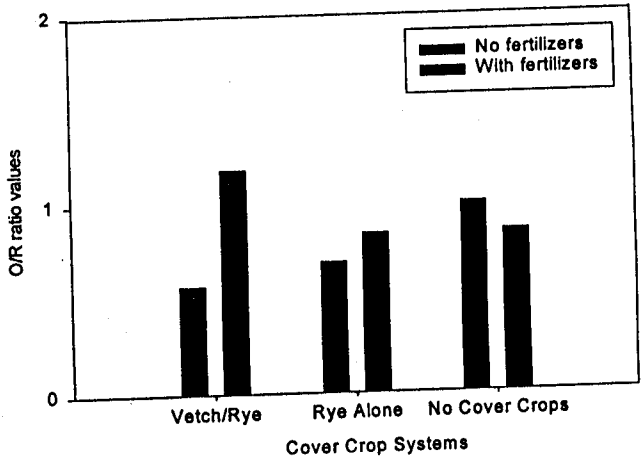

Figure 1 *Ratios of selected peak heights from DRIFTS spectra of HA samples under different cover crop systems*

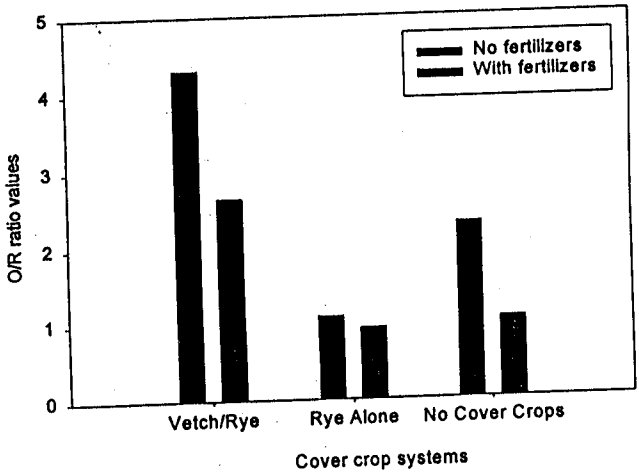

Figure 2 *Ratios of selected peak heights from DRIFTS spectra of FA samples under different cover crop systems*

ACKNOWLEDGEMENTS

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1 INTRODUCTION

Soil organic matter (SOM) is an important attribute of soil quality, influencing the productivity and physical well being of soils. Thus, it is important from economic and environmental standpoints to determine how changes in cover crop management will affect SOM and soil quality.

SOM contents and properties are functions of agricultural practices and the amounts and kinds of plant residues returned to the soil.[1-9] It is well established that the labile components of SOM change and reach a new steady state more quickly in response to various management practices than does total organic matter.[10-13] Wander and Traina[14] showed that SOM in crop rotation with cover crops was significantly higher than those rotations without cover crops. However, Lal et al.[15] reported no or minimal change of SOM content. The reason for not detecting any SOM change could be due to natural soil heterogeneity.[14] It is well known that continuous cultivation of cereal crops generally results in substantial losses of soil C and N.[16-18] However, studies of organic matter using ^{13}C nuclear magnetic resonance (NMR) spectroscopy have indicated that the chemical nature of the remaining C shows little change as a result of cultivation and that the stability of SOM appears to depend more on physical protection mechanisms than any inherent recalcitrance of the organic structures.[19-22]

In addition to SOM quantity, the quality (e.g., structure and composition) and distribution of individual fractions (e.g., humic acids, polysaccharides) are essential to the maintenance of soil productivity. Monreal et al.[23] observed a higher lignin dimer to lignin monomer ratio in continuous wheat rotation and this ratio decreased from large to small aggregate sizes, indicating the change in SOM quality. Wander and Traina[24] used diffuse reflectance Fourier transform infrared spectroscopy (DRIFTS) to examine functional groups of SOM fractions and reported that the ratios of reactive to recalcitrant fractions in humic acids best reflected overall SOM bioavailability.

DRIFTS detects molecular vibrations and is useful for functional group analysis and for identification of molecular structures of SOM.[25] But it cannot be used to quantify carbon contents of structural groups. By contrast, ^{13}C NMR spectroscopy provides quantitative data for structural components. NMR has been successfully used to characterize SOM by many scientists.[26-30] Thus, it would be advantageous to use both NMR and DRIFTS to characterize SOM and compare quantitative data of SOM under

different cover crop systems. The objective of this study is to determine quantitative, structural and compositional changes of humic substances (humic acid and fulvic acid fractions) caused by cover-crop systems using both NMR and DRIFTS.

2 MATERIALS AND METHODS

2.1 Site Description and Sampling

Since 1990, cover crop experiments have been conducted in the Connecticut River Valley at the Massachusetts Agricultural Experiment Station Farm in South Deerfield, Massachusetts. The soil at the University of Massachusetts at Amherst farm is a fine sandy loam (coarse, mixed, mesic Fluventic Dystrudept) and low in SOM (~2%). Its upper 0.6 m is homogeneous, overlaying inclined layers of coarse and fine material to a great depth. It is a typical soil in the intensively cropped Connecticut River Valley in Massachusetts. Three cover crop treatments with four nitrogen rates (applied to the corn crop after cover crop incorporation) were laid out in a complete factorial design in bordered 3 m x 7.5 m plots in four randomized blocks. Cover crop treatments and seeding rates were 1) check (no cover crop); 2) rye (125 kg/ha); 3) hairy vetch + rye (46+65 kg/ha). Nitrogen fertilizer rates were 0, 67, 135, 202 kg N/ha using NH_4NO_3. Detailed soil sample information is listed in Table1.

Table 1 *Soil samples (UMass South Deerfield Farm)*

Sample number	*Depth (cm)*	*Cover crops*	*Nitrogen rates (kg N ha^{-1})*
VR1	0-25	Vetch/Rye	0
VR4	0-25	Vetch/Rye	202
RA1	0-25	Rye alone	0
RA4	0-25	Rye alone	202
C1	0-25	No cover crop	0
C4	0-25	No cover crop	202

1 = No nitrogen fertilizer treatment, 4 = Nitrogen fertilizer treatment

2.2 Extraction, Fractionation and Purification of Humic Substances

2.2.1 Extraction. Soil was air-dried and passed through a 2 mm sieve. Air-dry soil (50 g) was weighed into a 1000 mL plastic flask, then 500 mL 0.1 M $Na_4P_2O_7$ was added. The air in the flask and solution was displaced with nitrogen (N_2) and the system was shaken for 24 hr at room temperature.

2.2.2 Fractionation. After separation from the $Na_4P_2O_7$ insoluble organic residues by centrifugation at 3000 rpm, the dark-colored supernatant solution was acidified to pH 1 with 6 M HCl and allowed to stand for 24 hr at room temperature for the coagulation of the HA fraction. The soluble material (FA) was separated from the HA by centrifuging at 10000 rpm.

2.2.3 Purification. HA was shaken 3 times for 24 hr at room temperature with 0.1 M HCl/0.3 M HF solution. The insoluble residue (HA) was separated from the supernatant by centrifuging at 10000 rpm, washed with deionized water until free of chloride ions and

then freeze-dried. The FA solution was passed through an XAD-8 resin column. The effluent was discarded and the XAD-8 column containing sorbed FA was washed 3 times with deionized water, then the sorbed FA was eluted with 0.1 M NaOH. The solution was immediately acidified with 6 M HCl to pH 1. The resulting solution volume was sufficient to maintain the FA in solution, which was then freeze-dried.

2.3 Diffuse Reflectance Fourier Transform Infrared (DRIFTS) Analysis

DRIFTS was performed in a Midac series M 2010 infrared spectrophotometer with a DRIFTS accessory (Spectros Instruments). All SOM fractions were ground with a sapphire mortar and pestle and stored over P_2O_5 in a dry box. Humic and fulvic acid concentrations for this determination ranged from 2 to 4 mg and were supplemented with KBr to a total weight per sample of 100 mg, then ground with an agate mortar and pestle. The milled sample was immediately transferred to a sample holder and its surface was smoothed with a glass microscope slide. Before analysis, the diffuse-reflectance cell containing the samples was flushed with N_2 gas to eliminate interference from carbon dioxide and moisture. A small jar (20 mL) containing anhydrous $Mg(ClO_4)_2$ was placed inside the sample compartment to further reduce atmospheric moisture.

To obtain DRIFTS spectra, 100 scans were collected at a resolution of 16 cm^{-1} and the spectra with numerical values for major peaks wave-numbers and intensities were recorded. The blank consisted of the powdered KBr stored under the same environmental conditions as the sample-KBr mixtures. Absorption spectra were converted to Kubelka-Munk functions using the Grams/32 software package (Galactic Corporation). Peak assignments and intensity (by height) ratio calculations were made with the methods of Wander and Traina,[24] Baes and Bloom[31] and Niemeyer et al.[32]

2.4 NMR Spectroscopy

CP-TOSS (Cross-Polarization and Total Sideband Suppression) was used.[30] Samples were run at 75 MHz (^{13}C) in a Bruker MSL-300 spectrometer. HA samples were packed in a 7-mm-diameter zirconia rotor with a Kel-F cap. The spinning speed was 4.5 kHz. A 90° ^1H pulse was followed by a contact time (t_{cp}) of 500 μs, and then a TOSS sequence was used to remove sidebands.[30,33] The 90° pulse length was 3.4 μs and the 180° pulse was 6.4 μs. The recycle delay was 1 s with the number of scans about 4096.

3 RESULTS AND DISCUSSION

3.1 Carbon-13 NMR Characteristics

For quantitative NMR measurements the conditions that must be met are $t_{cp} \ll T_{1\rho}$ (^1H). That is, the contact time must be much shorter than the time constant for proton spin lattice relaxation in the rotating frame.[34] In addition, the delay time between cross-polarization sequences must be long enough to allow for complete ^1H spin relaxation (i.e., at least five times $T_1(^1H)$). The ideal situation of $t_{cp} \ll T_{1\rho}$ (^1H) may be difficult to satisfy in practice, especially where $T_{1\rho}$ (^1H) is very short. Optimum acquisition parameters were therefore chosen after examination of the spin dynamics of the samples to avoid signal suppression by incomplete relaxation.

Although CP-TOSS spectra could not be used for absolute quantitation, they could be

compared because all the HAs samples were run under the same conditions and were from the same type of soil. Functional groups were assigned as follows: 0-50 ppm for aliphatic-C (C in straight-chain, branched and cyclic alkanes and alkanoic acids); 50-60 ppm methoxy-C including C from OCH_3 groups as well as C from amino acids; 60-96 ppm carbohydrate-C (aliphatic C bonded to OH groups, or ether oxygens, or occurring in saturated five- or six-membered rings bonded to oxygens); 96-108 ppm O-C-O; 108-145 ppm aromatic-C; 145-162 ppm phenolic-C (i.e., aromatic C bonded to OH groups); 162-190 ppm carboxylic-C; and 190-220 ppm carbonyl-C.[25,34,35]

The ^{13}C NMR data are summarized in Table 2. The HA from the rye alone system differs from other HAs in the aliphatic region (0-50 ppm) and the HA signal in this region is the smallest with no nitrogen fertilizer treatment. The most intense signal in this region appears under the vetch/rye system with no nitrogen fertilizer treatment. When compared to the total aliphatic region (0-108 ppm), the aliphatic-C of the vetch/rye HA without nitrogen fertilizer is 55.3%, and this figure decreases to 48.9% for rye alone without nitrogen fertilizer.

Table 2 *The ^{13}C NMR characteristics of HA samples*

Type of	*Distribution of C Chemical shift (ppm) %*							
Materials	*0-50*	*50-60*	*60-96*	*96-108*	*108-145*	*145-162*	*162-190*	*190-220*
VR1	26.8	8.4	15.9	4.2	22.6	6.5	14.4	3.2
VR4	26.4	8.4	16.9	4.2	21.6	6.6	14.4	1.5
RA1	20.8	6.7	17.1	4.2	25.4	7.5	16.4	1.9
RA4	21.2	7.1	18.2	4.3	23.5	7.2	16.3	2.3
C1	26.1	8.0	17.5	4.5	21.3	6.2	14.4	2.0

Types of Materials	*Aliphatic-C %*	*Aromatic-C %*	*(Aliphatic-C)/(Aromatic-C)*
VR1	55.3	29.1	1.9
VR4	55.9	28.2	2.0
RA1	48.9	32.8	1.5
RA4	50.7	30.7	1.7
C1	56.1	27.6	2.0

The phenolic-C (145-162 ppm) content is higher in the HA extracted from rye alone systems than the other two systems. Similarly, the HA extracted from the rye cover system is also relatively enriched in aromatic-C. An inspection of the data for HA with no cover crop shows that aromatic-C content (including 108-145 and 145-162 ppm regions) is the lowest (Table 2). The nonprotonated C signals (130 ppm) likely are from aromatic carbons, including C in polynuclear aromatic rings. Polymerization and polycondensation of HA macromolecules may have occurred on contact with soil minerals.[34,35] For both sites of rye systems (with or without nitrogen fertilizer), the relative increase in aromatic C and decrease in aliphatic C (Table 2) indicate that humification may be greater than in the other two treatments. These results may indicate a greater stability of HA from rye alone systems because 1) the HA appears to contain fewer biopolymers (proteinaceous materials and carbohydrates) and is therefore less biodegradable and 2) the greater aromaticity