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Quantitative ^{13}C NMR of whole and fractionated Iowa Mollisols for assessment of organic matter composition

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Abstract

Both the concentrations and the stocks of soil organic carbon vary across the landscape. Do the amounts of recalcitrant components of soil organic matter (SOM) vary with landscape position? To address this question, we studied four Mollisols in central Iowa, two developed in till and two developed in loess. Two of the soils were well drained and two were poorly drained. We collected surface-horizon samples and studied organic matter in the particulate organic matter (POM) fraction, the clay fractions, and the whole, unfractionated samples. We treated the soil samples with 5 M HF at ambient temperature or at 60 °C for 30 min to concentrate the SOM. To assess the composition of the SOM, we used solid-state nuclear magnetic resonance (NMR) spectroscopy, in particular, quantitative ^{13}C DP/MAS (direct-polarization/magic-angle spinning), with and without recoupled dipolar dephasing. Spin counting by correlation of the integral NMR intensity with the C concentration by elemental analysis showed that NMR was $\geqslant$85% quantitative for the majority of the samples studied. For untreated whole-soil samples with <2.5 wt.% C, which is considerably less than in most previous quantitative NMR analyses of SOM, useful spectra that reflected $\geqslant$65% of all C were obtained. The NMR analyses allowed us to conclude (1) that the HF treatment (with or without heat) had low impact on the organic C composition in the samples, except for protonating carboxylate anions to carboxylic acids, (2) that most organic C was observable by NMR even in untreated soil materials, (3) that esters were likely to compose only a minor fraction of SOM in these Mollisols, and (4) that the aromatic components of SOM were enriched to ~53% in the poorly drained soils, compared with ~48% in the well drained soils; in plant tissue and particulate organic matter (POM) the aromaticities were ~18% and ~32%, respectively. Nonpolar, nonprotonated aromatic C, interpreted as a proxy for charcoal C, dominated the aromatic C in all soil samples, composing 69–78% of aromatic C and 27–36% of total organic C in the whole-soil and clay-fraction samples.
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1. INTRODUCTION

Long-term stabilization of soil organic matter (SOM) has been attributed to (1) the intrinsic recalcitrance of components of plant tissues, (2) protection of plant residues from enzymatic attack by storage in micropores of natural aggregates, (3) sorption of organic compounds to solid surfaces so that cleavage points are sterically unavailable to enzymes, and (4) transformation of plant residues to black C by fire (Sollins et al., 1996; Krull et al., 2003; von Lützow et al., 2007). Understanding which mechanisms are important in different kinds of soils – with different mineralogies, types of diagnostic horizons, and management practices – is critical to improving the predictive power of models of terrestrial C dynamics. To further that understanding, many investigators have studied how soil organic matter components vary as a function of vegetation, cropping system, tillage, depth, and size of aggregates. In Iowa, the molecular-scale composition of humic substances isolated from aggregate size fractions and the occurrence of black C in prairie-derived soils have recently been examined (Mao et al., 2007; Laird et al., 2008).

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Both the concentration and the stock of SOM vary predictably across the landscape in Iowa. The greater concentrations of SOM in poorly drained soils compared with well drained soils may be attributed to (1) slow oxidation of C by anaerobic microorganisms under long periods of low redox conditions, (2) accumulation of eroded, organic-matter-rich sediments at the base of slopes, and (3) sorption of SOM to clay minerals that also accumulate in low-lying landscape positions (Walker and Ruhe, 1968; Malo et al., 1974; Schaetzl and Anderson, 2005).

In addition to the total amount of soil organic C, does the distribution of SOM components also vary with landscape position and (therefore) drainage regime? It would be useful to know if this were the case. Investigations that have looked for systematic differences in soil organic matter as a function of landscape position are rare. One is that of Novak and Bertsch (1991), who studied water-soluble organic carbon, fulvic acid, and humic acid in soils of an upland-to-bottomland transect in South Carolina. They reported that the *O*-alkyl content of fulvic acids extracted from the bottomland soils was greater than that from upland soils; but the native vegetation and the parent materials of the two sites differed considerably, making strict conclusions difficult.

There are several reasons that the components of SOM might vary with drainage regime: (1) Both the composition of vegetative species and primary productivity differ with soil water regime, at least in uncultivated landscapes. Since plant species differ in the abundance of the primary tissue components (e.g., polysaccharides, lignin, and lipids) and since the rates of decomposition of these components also differ, over long periods these components might be expected to differentially accumulate in soil organic matter. (2) Decomposition of lignin is slow in the absence of certain species of fungi that require aerobic conditions. Thus decomposition may be inhibited in poorly drained soils where O_2 levels in the soil solution are limited, thus enriching SOM in lignin residues (Benner et al., 1984; Gleixner et al., 2001; Kogel-Knabner, 2002). (3) The potential for lipids to be associated with clay fractions in soils has been noted by a number of authors (Mahieu et al., 1999; Schmidt et al., 2000; Mao et al., 2007). Trapped in clay quasicrystals, they may be physically protected from enzymatic degradation. Or they may be chemically protected as their hydrophilic moieties interact with the aqueous environment outside the quasicrystal, as suggested by Kleber et al. (2007).

On the other hand, the distribution of SOM components might not vary with landscape position, especially in the surface horizons of cultivated soils. The installation of drainage tile in cultivated soils may have equalized soil water regimes sufficiently to erase the signature of differential accumulations of plant biomass that would otherwise resist decay in low-redox conditions. And agricultural management across landscape positions (fertilization, tillage, and the monoculture of hybridized crop cultivars) may have homogenized the annual inputs of above- and below-ground biomass so that present-day SOM reflects primarily these most recent additions, instead of older organic matter from pre-cultivation periods.

Dynamic models of soil carbon typically differentiate three subtypes of SOM: active, slow, and passive. These terms refer to the rate of turnover or ease of microbial decomposition of the organic matter. There is considerable interest in identifying and measuring the SOM constituents that comprise these conceptual categories, because their integration into dynamic models such as Century (Parton et al., 2005) will improve long-term predictions of soil C management (e.g., Stewart et al., 2008). Such knowledge could lead to more accurate modeling of the landscape-scale distribution and fate of soil C. Most models of C dynamics in soils are particularly sensitive to the initial value chosen for passive SOM (e.g., Wang et al., 2005), and refining our understanding and measurements of C that is not readily susceptible to microbial oxidation (such as char) is a critical task.

In the present paper, we consider the hypothesis that components of soil organic matter in the surface horizons of Iowa Mollisols might vary as a function of landscape position. We have tested this hypothesis by investigating soils that occur in well and poorly drained catenary positions but that differ from one another primarily in parent material, that is, Wisconsinan till and Wisconsinan loess.

We have used quantitative, direct-polarization ^{13}C nuclear magnetic resonance (NMR) spectroscopy to characterize the types and amounts of organic C present in whole and HF-treated soil samples, their clay fractions, as well as plant and particulate organic matter. Direct-polarization NMR provides more quantitative spectra of highly aromatic soil organic matter than does the more commonly used cross-polarization (CP) method (Skjemstad et al., 1996, 1999; Smernik and Oades, 2000b; Mao et al., 2002a; Keeler and Maciel, 2003; Nelson and Baldock, 2005; Kaal et al., 2008). While direct polarization after a sufficiently long recycle delay, which can be determined in a simple auxiliary experiment (Mao et al., 2000), shows signals of all carbons in the right proportions, cross polarization requires transfer of magnetization from ^{1}H to ^{13}C through their strongly distance-dependent dipolar coupling. Thus, CP underrepresents the signals of carbons distant from hydrogen, for instance, in fused aromatic rings, which makes this method inherently nonquantitative. Indeed, the observability of soil carbon in CP NMR has been shown to be significantly reduced, typically to 60% (Smernik and Oades, 2000b; Keeler and Maciel, 2003), while that of char is even lower and quite variable, between 25% and 40%, precluding any reliable quantitative analysis (Knicker et al., 2005; Hammes et al., 2008). In addition, it has been extensively documented that CP significantly underrepresents the signal of aromatic carbons in soil organic matter, typically by a factor of two (Skjemstad et al., 1996, 1999; Smernik and Oades, 2000b; Mao et al., 2002a; Keeler and Maciel, 2003; Knicker et al., 2005; Nelson and Baldock, 2005; Kaal et al., 2008). For studies of charcoal, use of cross polarization, whether regular or ramped, is particularly ill advised given the large fraction of carbons that are in fused ring systems and thus far from the nearest cross-polarizing hydrogen nuclei.

In this study, the effects of HF extraction and of heating during extraction were also assessed by NMR. While most

previous quantitative ^{13}C NMR studies have been made of soil organic matter with C weight fractions exceeding 11% (Keeler and Maciel, 2003) or more typically 30% (Smernik and Oades, 2000a), several of the samples studied here had less than 2.5% C. Through spin counting by correlation of the total NMR signal with the C mass fraction from elemental analysis of the samples studied here and of model compounds (Botto et al., 1987; Smernik and Oades, 2000a,b; Keeler and Maciel, 2003), potential signal loss due to strong interactions between nuclei and unpaired electrons was quantified.

2. MATERIALS AND METHODS

2.1. Soils

This study followed the standard pedological research paradigm of a thorough reconnaissance survey followed by detailed investigations of samples selected to be representative of key soil mapping units (Soil_Survey_Staff, 1993). Two agriculturally managed fields in Iowa were chosen, one in Greene County, where the soils were developed in loam-textured Wisconsinan till, and the other, in Black Hawk County, where the soils were developed in silty clay loam Wisconsinan loess. Block diagrams that illustrate the landscapes and landscape positions of soils in the fields are presented in Fig. 1. Both fields were managed in alternate-year corn – soybean (*Zea mays*, *Glycine max*) rotations over many years before sampling. The Greene County field was managed with zero tillage and the Black Hawk County field was managed with reduced tillage (chisel plow and one disking per year). Mean annual precipitation at the sites is 750–900 mm.

At the Greene County field, 124 sites were sampled on a nested grid pattern over an area of ~17 ha by using a hydraulic probe (~9 cm in diameter) to a depth of 30 cm or by compositing three 2.5-cm-diameter cores collected to the 30-cm depth by hand. At the Black Hawk County site, 141 sites were sampled by the 9 cm hydraulic probe on a regular grid over an area of ~14 ha, also to a depth of 30 cm. All samples were divided into depth increments of 0–5, 5–15, and 15–30 cm, air dried, and ground to pass

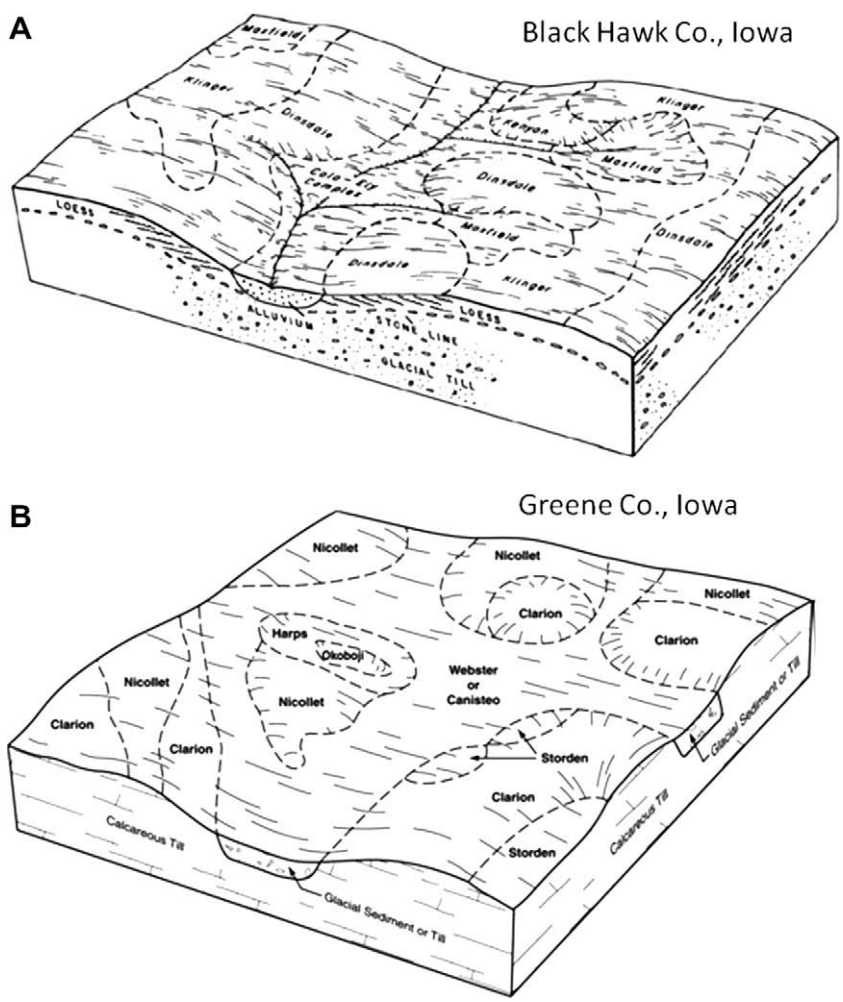

Fig. 1. Block diagrams illustrating landscape positions and topographic context of the soils investigated. (Not all soils indicated on these schematic diagrams were sampled. See Supplemental information for details.) The focus of the investigation reported here was on the well drained (Clarion and Dinsdale) and poorly drained (Webster and Maxfield) members of these classic topo- and hydrosequences (Fouts and Highland, 1978; Sherwood, 1985).

a 2-mm sieve. Total C and total N were determined for all samples by dry combustion of <0.15-mm (100 mesh), finely ground subsamples (Nelson and Sommers, 1996), and bulk density was determined by the hydraulic-probe core method (Grossman and Reinsch, 2002). For calcareous samples [only from one Greene Co. mapping unit (Canisteo)], inorganic C was determined by a pressure calcimeter method (Sherrod et al., 2002), and organic C was determined by difference from the total C values. All background data are available from the authors by request.

The organic C, N, and bulk density values were used to calculate areal stocks of organic C and N to a depth of 30 cm at each sampling location. The sampling locations were then grouped by their mapping units according to the second-order soil surveys of the fields (verified by intensive field surveys by the authors) (Fouts and Highland, 1978; Sherwood, 1985). Statistical details of the calculated C stocks of the mapping units at each of the two field sites are presented in the online Supplemental information.

From this suite of data, sampling sites were selected for the present study of SOM composition because they had C and N stocks and depth-weighted bulk density values (0–30 cm) near the means of sites that occurred in well drained and poorly drained mapping units of each field. The sites investigated are Mollisols with stocks of organic C of 6.5–10.5 kg m^{-2} to a depth of 30 cm (Table 1). The two well drained soils (Clarion and Dinsdale) have udic soil moisture regimes, and the poorly drained soils (Webster and Maxfield) have aquic soil moisture regimes (Table 1). These four soils are end members of two quintessential landscape – soil drainage sequences in Iowa (see Fig. 1 and the associated characteristics given in the online Supplemental information), and their properties fit well within the expected range of characteristics for their respective soil series. For the purposes of this study, we selected the 0–5 cm samples to extract particulate organic matter because POM was most concentrated near the soil surface and the larger sample would be more representative for inter-site comparisons. We chose the 5–15 cm samples for the detailed characterization of SOM because SOM from that larger depth increment would better represent humified organic matter in the surface horizons.

2.2. Particulate organic matter and clay fractionation

Following a method that is consistent with previous literature, we dispersed 50 g of air-dried soil (⩽2.0 mm) in 250 mL of distilled water at 22 J mL^{-1} (Leifeld and Kögel-Knabner, 2005) with a 1.2 cm-diameter probe sonifier (Sonicator 3000, Misonix Inc., Farmingdale, NY). When dispersed, the suspension was passed through a 53-μm sieve and the filtrate was collected in a tall 1-L beaker for further separation. The residue on the sieve (sand plus particulate organic matter, POM) was transferred quantitatively to a previously weighed 100-mL beaker and then dried at 50 °C. The dried residue was stirred and soaked overnight in 50 mL of Na polytungstate (Geoliquids Inc., Prospect Heights, IL) adjusted to a density of 1.85 g mL^{-1} with water. This allowed the light-weight POM to separate from the sand. The POM was siphoned into a tared beaker

Table 1
Location and properties of soil samples in this study.

Soil	Classification (USDA Soil Taxonomy; World Reference Base)	Drainage class	Landscape position, slope	Stock of organic C to 30 cm depth (kg m^{-2})	5–15 cm depth				POM[a] (0–5 cm)		
					Clay (g kg^{-1})	Sand (g kg^{-1})	Silt (g kg^{-1})	pH	TC[b] (g kg^{-1})	TN[c] (g kg^{-1})	C/N
Black Hawk County, Iowa											
Dinsdale	Fine-silty, mixed, superactive, mesic Typic Argiudoll; Phaeozem	Well	Side slope, ~5%	6.46	330	100	570	5.4	320	15	21
Maxfield	Fine-silty, mixed, superactive, mesic Typic Endoaquoll; Stagnosol	Poor	Concave drainageway, footslope, ~1%	8.44	360	90	550	6.2	296	17	17
Greene County, Iowa											
Clarion	Fine-loamy, mixed, superactive, mesic Typic Hapludoll; Phaeozem	Well	Convex shoulder, ~5%	6.78	250	400	350	4.8	241	14	17
Webster	Fine-loamy, mixed, superactive, mesic Typic Endoaquoll; Stagnosol	Poor	Slightly concave upland, 0%	10.47	280	340	380	4.9	244	13	19

[a] Particulate organic matter.
[b] Total carbon.
[c] Total nitrogen.

and then washed three times with distilled water. The beaker was dried at 50 °C for 72 h and then weighed.

The filtrate, on the other hand, was adjusted to a final volume of approximately 500 mL before it was dispersed at 450 J mL^{-1}. The clay fraction was collected by exhaustive sedimentation and decantation, coagulated with 0.5 M $MgCl_2$, and then dialyzed against water that was replaced until a minimal amount of chloride was present in the dialyzate. The clay fraction was freeze-dried and then weighed to determine yield for each fraction.

2.3. HF treatment

Six grams of the whole soil (ground to pass a 0.15-mm sieve) or the clay fraction were placed in a 50 mL polyethylene centrifuge tube and treated with 15 mL of 0.1 M HCl to remove Ca, which dominated the cation exchange complex in these soils (Swift, 1996). The sample was shaken end-to-end for 30 min and centrifuged at 1949*g* for 10 min. The wash was discarded and the HCl treatment was repeated. The residue after the HCl washes was treated with 15 mL of 5 M HF and shaken for 30 min before centrifugation at 1949*g* for 10 min. The HF treatment was performed four times, with the wash being discarded each time. The residue was washed with distilled water four times to remove excess acid and then freeze-dried.

We also explored the potential for additional heat to improve the effectiveness of the HF treatment. Samples in tubes (as above) were immersed in a 60 °C water bath for 30 min during each HF treatment. The tubes were stirred periodically by hand while heated, and the excess acid was removed before freeze drying as described above.

2.4. Nuclear magnetic resonance spectroscopy

Solid-state nuclear magnetic resonance (NMR) spectroscopy analyses were performed at room temperature using a Bruker DSX400 spectrometer at 400 MHz for ^{1}H and 100 MHz for ^{13}C. A Bruker 4-mm ^{1}H—^{13}C double-resonance magic-angle spinning (MAS) probe head was used in this study. Zirconium dioxide rotors of 4-mm diameter were used with 5-mm-long glass inserts at the bottom to keep the sample within the radio frequency coil. The mass of each sample was recorded during packing and ranged from 49 to 130 mg. The ^{13}C chemical shifts were referenced to tetramethylsilane, using the COO^- resonance of glycine at 176.49 ppm as a secondary reference for ^{13}C. The 90° pulses of both ^{13}C and ^{1}H were calibrated by using a ^{13}COO-labeled glycine sample. The 90° pulse length for both nuclei was 4 μs. Two-pulse phase modulation (TPPM) decoupling on the ^{1}H channel (Bennett et al., 1995), which is superior to the more standard continuous-wave decoupling, was turned on during the pulse sequences and detection.

2.4.1. High-speed quantitative ^{13}C DP/Echo/MAS NMR

To quantitatively observe carbon of soil organic samples, quantitative ^{13}C direct-polarization (DP)/Hahn echo/MAS NMR spectra were acquired at 14 kHz MAS. A sample-rotation synchronized Hahn echo was generated by a π-pulse applied after one rotation period to move the start of the detected time signal out of the pulse deadtime and thus avoid baseline distortions in the spectrum (Mao and Schmidt-Rohr, 2004). Appropriate recycle delays varied for samples, because the concentration and distribution of unpaired electrons affects the amount of time it takes samples to completely relax between scans. The recycle delays were 9 s for samples not treated with HF, 3–14 s for HF-treated clays, 5–25 s for HF-treated whole-soil samples, 12–55 s for HF- and heat-treated samples, 40–60 s for particulate organic matter, and 120–150 s for plant materials. These delay values were determined by means of the CP/T_1/MAS pulse sequence (Mao et al., 2000): The $T_{1,C}$ filtering time for which the remaining signal is less than 5% of the full intensity was set as the recycle delay of the quantitative DP/MAS experiments, in order to ensure that all carbon sites were fully relaxed. Measuring times generally ranged between 5.5 and 14.5 h per DP spectrum, except for the untreated samples, which were measured for about 38 h each.

In addition, for each sample, a corresponding quantitative spectrum of nonprotonated and methyl carbons, obtained after 68 μs of recoupled dipolar dephasing (Mao and Schmidt-Rohr, 2004) was also recorded. By combining dipolar dephasing with direct-polarization experiments, we can generate separate quantitative spectra of nonprotonated aromatic carbon, and those of protonated aromatics by difference. The concentration of nonpolar nonprotonated aromatic carbon (between 145 and 100 ppm) will be used as a proxy for soil charcoal content, given that this is the dominant type of carbon in chars, while typical plant material contains ⩽5% of such carbon. Since charcoal contains some protonated carbons along the periphery of the clusters of aromatic rings, our results may underestimate charcoal content, but this may be partly compensated by contributions from other aromatic species. The total aromatic-carbon content is a stringent upper limit for the aromatic fraction of charcoal in these samples.

DP spectra of alanine, NH_3^+—$CH(CH_3)$—COO^-, and of polystyrene, $(—CH_2—CH(C_6H_5)—)_n$, were recorded as spin-counting references. The quantitative spectrum of polystyrene (with a recycle delay of 200 s) also enabled a test of the aromaticity measurement. Gaussian broadening with $\sigma = 0.3$, 0.2, and 0.1 kHz was applied to spectra of soils, POM, and plant materials, respectively. This was less than the natural width of the two dominant resolved bands in the spectra of soils, but broadened lines of plant material significantly; spectral integrals were essentially unaffected.

2.4.2. CP/TOSS experiments

Although DP experiments remain the gold standard for NMR quantitation, CP experiments often generate peaks of alkyl carbons with better signal-to-noise ratio (albeit not quantitative). The location of these sharper peaks can be used to infer the presence of crystalline cellulose and polymethylene chains, among others. Therefore, we chose to conduct supplemental CP experiments. Because we were not using CP to quantitate, we applied the TOSS pulse sequence (Dixon, 1982) of four suitably timed 180° pulses before signal detection, which removes spinning sidebands

that can interfere with peak recognition. The CP contact time was 1 ms; the spinning frequency, 8 kHz; the recycle delay, 0.5 s.

3. RESULTS AND DISCUSSION

3.1. General soil properties

Consistent with expectations based on parent materials and landscape position, the till-derived soils had less clay than did the loess-derived soils, and the well drained soils had less clay than the poorly drained soils (Table 1). None of the soils was calcareous, so measurements of total C indicate organic C. The total C and total N values of the surface layers of these soils (Table 2, unfractionated samples in "before treatment" columns) displayed familiar patterns: C concentrations were greatest in the uppermost layer of each soil and greater in the poorly drained soils than in the well drained soils. Total C concentrations at the 0–5-cm depth were greater in the loess-derived soils than in the till-derived soils, but at the 5–15-cm depth there was no difference in C concentration based on parent material. The C content of the POM in the loess-derived soils was also greater than that of the till-derived soils (Table 1).

Total C of the clay fractions was about twice that of the unfractionated, whole-soil samples (Table 2). Like the whole-soil samples, clay-fraction C was greater in samples from the poorly drained than from the well drained soils; yet the differences between clay-fraction C in till-derived and loess-derived soils were slight. The C/N mass ratios of the clay-fraction SOM tended to be less than those of the whole-soil SOM, perhaps reflecting a greater degree of humification in the SOM associated with clay.

Table 2
Total C, total N, and C/N mass ratios before and after HF treatment to concentrate organic matter. In this table, the samples are grouped by drainage class, which is closely related to landscape position.

Soil	Depth (cm)	Before treatment			HF, no heat treatment			HF + heat treatment				
		TC (g kg^{-1})	TN (g kg^{-1})	C/N	TC (g kg^{-1})	TN (g kg^{-1})	C/N	TC (g kg^{-1})	TN (g kg^{-1})	C/N	CE[d]	TC recovery (%)
Unfractionated sample												
Dinsdale (W[b])	0–5	24.0	2.1	11	–[a]	–	–	62.2	4.5	14		
Dinsdale (W)	5–15	17.9	1.6	11	38.7	2.8	14	42.6	3.5	12	2.4	57
Clarion (W)	0–5	20.0	1.8	11	–	–	–	33.9	2.8	12		
Clarion (W)	5–15	18.6	1.8	11	22.7	1.6	14	22.6	1.7	13	1.2	39
Maxfield (P[c])	0–5	30.4	2.5	12	–	–	–	91.6	6.6	14		
Maxfield (P)	5–15	24.0	1.9	12	65.5	4.1	16	83.6	5.7	15	3.5	77
Webster (P)	0–5	25.6	2.1	12	–	–	–	32.7	3.3	10		
Webster (P)	5–15	24.3	1.9	13	46.4	3.1	15	62.0	4.2	15	2.6	77
Clay fraction (<2 μm)												
Dinsdale (W)	0–5	44.6	4.5	10	–	–	–	210	18.7	11		
Dinsdale (W)	5–15	39.9	4.3	9	158	14.1	11	227	20.0	11	5.7	63
Clarion (W)	0–5	47.8	4.7	10	–	–	–	240	22.3	11		
Clarion (W)	5–15	40.5	4.4	9	232	20.7	11	257	22.1	12	6.3	63
Maxfield (P)	0–5	53.3	5.0	11	–	–	–	351	28.6	12		
Maxfield (P)	5–15	48.8	4.1	12	171	12.5	14	233	18.0	13	4.8	81
Webster (P)	0–5	54.9	5.5	10	–	–	–	351	28.2	13		
Webster (P)	5–15	51.4	4.6	11	318	23.2	14	286	21.0	14	5.6	84

[a] Not determined.
[b] Well drained.
[c] Poorly drained.
[d] CE (carbon-enrichment) = [C (after treatment)]/[C (before treatment)] (modified from Schmidt et al., 1997).

3.2. Effects of HF treatment

Treatment of soil materials with HF is widely used to concentrate organic matter before NMR analyses (Preston et al., 1989; Skjemstad et al., 1994; Schmidt et al., 1997; Gonçalves et al., 2003; Schmidt and Gleixner, 2005; Eusterhues et al., 2007). Some investigators have used multiple treatments with 10% HF at room temperature and have reported little alteration of SOM, particularly in organic-rich samples. In the present study, heating the samples during HF treatment usually, but not always, increased the absolute concentration of organic C over that of HF treatment without heat (Table 2). For the whole soil samples, the average C enrichment value (i.e., the ratio of C concentration after treatment to that before treatment) was increased from 1.7 to 2.4 by adding heat to the HF treatment. For the clay-fraction samples, C enrichment values were ~5.5 with HF treatment, both with and without heat (complete data are presented in the online Supplemental information). The average recovery of C after the HF treatment without additional heat was 78% and 72% for the whole soil and clay fractions, respectively. The average recovery of C when heat was added to the HF treatment was 63% and 73% for the whole soil and clay fractions, respectively (complete data are presented in the online Supplemental information). The C/N mass ratios increased by an average of two units in treated samples compared with untreated samples, suggesting that there was loss of total N during the HF treatments. Loss on N could be partially attributed to loss of nonexchangeable ammonium that was "fixed" by layer-silicate clay minerals (which were destroyed by HF) (Stewart and Porter, 1963; Antisari and Sequi, 1988; Bremner, 1996). Nonexchangeable ammonium typically accounts for 7–8% of total N in the surface horizons of cultivated Iowa Mollisols (Yoo, 1990). This amount of inorganic N would account for a change in C/N ratio of about one unit upon HF treatment. It is also possible that some organic N was solubilized by the treatment. Generally, HF treatment is not expected to alter the structure of the fused aromatic rings that make up a large fraction of the organic matter in these soils.

Fig. 2 shows direct-polarization ^{13}C NMR spectra of the Webster whole-soil and clay-fraction SOM without and with HF treatments. Corresponding spectra of the nonprotonated C or C in mobile segments such as CH_3 groups are shown by thin lines. All the spectra are dominated by the band of aromatic C near 130 ppm and by a peak of COO carbons near 172 ppm. Given that most (>67%) of the aromatic carbons resonating near 130 ppm were not protonated, the aromatic fraction can be identified predominantly as oxidized charcoal residues (see further discussion of this point below). The alkyl region of the spectra exhibits resonances of O—CH groups near 72 ppm, of nonpolar methylenes, C—CH_2—C, near 30 ppm, and of NCH, OCH_3, or CCH groups near 55 ppm. The similarity of the spectra of a given soil material with or without HF treatment proves that the treatment did not result in significant alterations of the component moieties. Comparison of ^{13}C NMR spectra for clay fractions of the three other sites, without and with heating during HF treatment, also demonstrates that no major structural changes occurred as a result of heating during HF treatment (Fig. 3). Due to the higher concentration of carbon, the ^{13}C NMR spectra of HF-treated samples had a better signal-to-noise ratio.

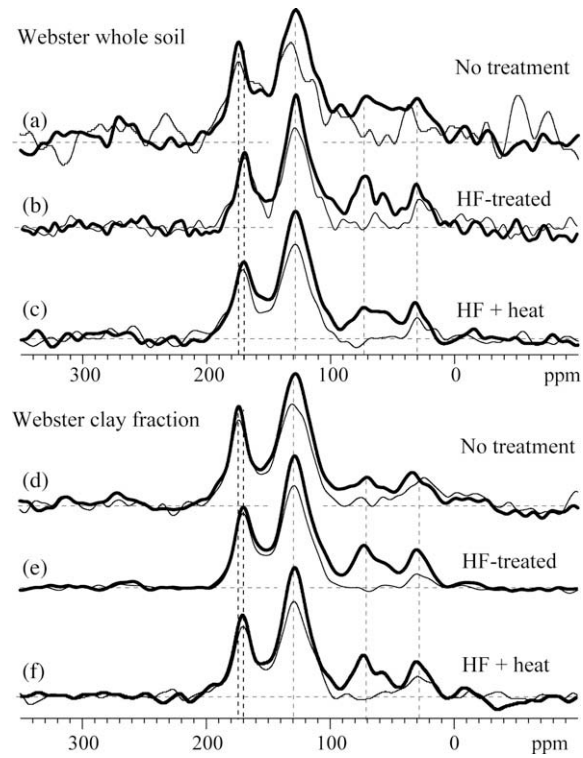

Fig. 2. Nearly quantitative, direct-polarization ^{13}C NMR spectra of: (a–c) Webster whole-soil and (d–f) Webster clay fraction, treated in three different ways: (a, and d) no chemical treatment, (b, and e) HF treatment at ambient temperature, (c, and f) heating at 60 °C during HF treatment plotted in each group. Thick lines are from all types of carbons, thin lines are from all quaternary carbons and CH_3 groups. A 3-ppm chemical shift difference is observed between the COO/COOH resonances of untreated and HF-treated samples for both soil materials. MAS frequency: 14 kHz.

The spectra in Fig. 2 show one interesting difference between untreated and HF-treated samples. First, the COO resonance has shifted from 174 ppm in the untreated sample to 171 ppm after acid treatment. This is indicative of protonation of COO^- to COOH groups due to acidification, which is, on average, accompanied by a (−4)-ppm shift (Duncan, 1997). Specifically, COO^- in the benzoate anion resonates between 179 and 171 ppm (depending on the degree of tight pair formation with cations) and the benzoic-acid COOH group between 173 and 167 ppm (Kosugi and Takeuchi, 1978). The bonding of many of these COO groups in Mollisols to aromatic rings has indeed been demonstrated by ^1H—^{13}C NMR of Mollisol humic acids (Mao et al., 2007).

Further, the 174-ppm position of the COO^- resonance in the untreated samples contains structural information not previously obtained. Its presence makes a dominant contribution from esters, COOC, unlikely, since these typically resonate around 169 ppm (Duncan, 1997). In particular, a significant fraction of the esters would have to be

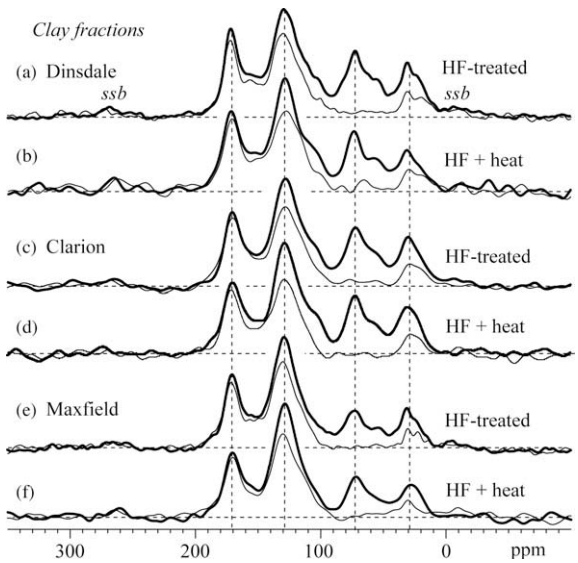

Fig. 3. Quantitative, DP MAS ^{13}C NMR spectra of clay fractions from three different soils extracted using two different methods. For each sample, the full spectrum (thick line) and quaternary carbon plus CH_3 spectrum (thin line) with regular HF treatments is plotted first, followed by the corresponding spectra obtained after HF treatment at 60 °C. Spinning sidebands (14 kHz MAS) are labeled "ssb." MAS frequency: 14 kHz.

bonded to aromatic rings and resonate between 160 and 171 ppm (Scott, 1972; Bovey, 1987), due to the high aromaticity and in light of the aforementioned $^{1}H—^{13}C$ NMR results (Mao et al., 2007). Even alkyl esters, which resonate typically between 165 and 174 ppm (Bovey, 1987) are only marginally consistent with the observed 174 ppm signal. In this analysis, the absence of chemical treatment of the samples is crucial: Acid can catalyze the hydrolysis of esters into carboxyl- and hydroxyl-bearing fragments; therefore studies of HF-extracted SOM or of soil humic acids (which precipitated by acidification during preparation) cannot reliably determine the abundance of esters in soil organic matter, since these may have been generated by the sample pretreatment. On the basis of the spectra of untreated samples in our study, we can conclude that esters, in particular aromatic esters, are likely to compose only a minor fraction of SOM in the Mollisols studied here. Amides (N—C═O), which also resonate around 173 ppm, cannot make a major contribution to the peak in question since the total abundance of N is <8% of C in several of the samples.

The aromatic-carbon band in our spectra was stronger than in most CP spectra of Mollisols (e.g., Skjemstad et al., 2002). The accuracy of our approach was confirmed by the aromatic:alkyl intensity ratio of 5.9:2 in the DP/MAS spectrum of polystyrene, which closely matches the expected 6:2 ratio.

3.3. NMR spin counting

Do the spectra of Fig. 2, three exhibit signals of most of the organic carbon in the samples, or could they be distorted due to differential loss of signal from organic matter near unpaired electrons of Fe, Cu, or Mn oxides? This can be established by spin counting (Botto et al., 1987; Smernik and Oades, 2000a; Keeler and Maciel, 2003). The spin-counting data shown in Fig. 4 prove that, in the samples studied here, most C is observed faithfully. The plot correlates the total (integrated) ^{13}C NMR signal, normalized per mg and per scan, with the atomic percentage of C obtained from elemental analysis. The methyl resonance of alanine, which has a short T_1 and therefore relaxes completely within 10 s, and the total signal of polystyrene were used to determine the scaling factor of the *y*-axis and thus provide the calibration line of slope one for spin counting.

The points from most samples lie close to the calibration line, showing that 65–100% of organic C is observed. These observability values are in a similar range as those determined by Smernik and Oades (2000b) for HF-extracted soil materials with >30 wt.% C. Surprisingly, we have found that even for untreated whole-soil samples with overwhelming (>95 wt.%) mineral fractions, most (~68%) of the organic C can be observable. In fact, the clay and POM samples tend to show more signal loss than do the whole-soil samples. The Webster samples show more than average signal loss, probably due to the presence of more para- (or ferri)magnetic minerals. Note that even for a point below the calibration line, the spectra could be undistorted if the signal loss is the same for all types of functional groups. Fig. 3 demonstrates that in spite of the reduced observability of carbon in clays treated with HF at ambient temperature, the relative intensities of the various bands are only slightly different from the quantitative spectra of the corresponding HF + heat-treated samples. Only a minor (~3%) reduction in the aromatic signals, mostly of the protonated aromatics, can be detected.

The majority of HF + heat-treated samples (open stars and pentagons in Fig. 4) show no signal loss, highlighting the superiority of this approach. For the clay fractions in particular, heating significantly improves observability. Therefore, the data from the HF + heat-treated samples were used in the quantitative analysis. For the whole soils, no significant difference in observability is seen; therefore, in the quantitative analyses their spectra with and without heating during HF treatment were averaged, improving the signal-to-noise ratio.

3.4. Structural differences related to fraction and landscape position

Having seen strong evidence that the HF treatments to concentrate SOM did not alter its chemical composition significantly and that our NMR approach was successful in observing C atoms over a large range of sample concentrations, we use the NMR-detected functional groups in the samples as indices of plant tissue components and the residues of those components in soil organic matter. For example, the *O*-alkyl signal is an index of polysaccharides, such as cellulose, the nonpolar alkyl signal is a proxy for lipids (including waxes and fatty acids), the OCH_3 and polar aromatic-carbon signals are indicators of lignin residues, and the NCH indexes peptides, including proteins. Finally, we identify nonpolar, nonprotonated aromatic C as an indicator of the condensed aromatic rings that dominate in charcoal residues. While perhaps none of these indices is

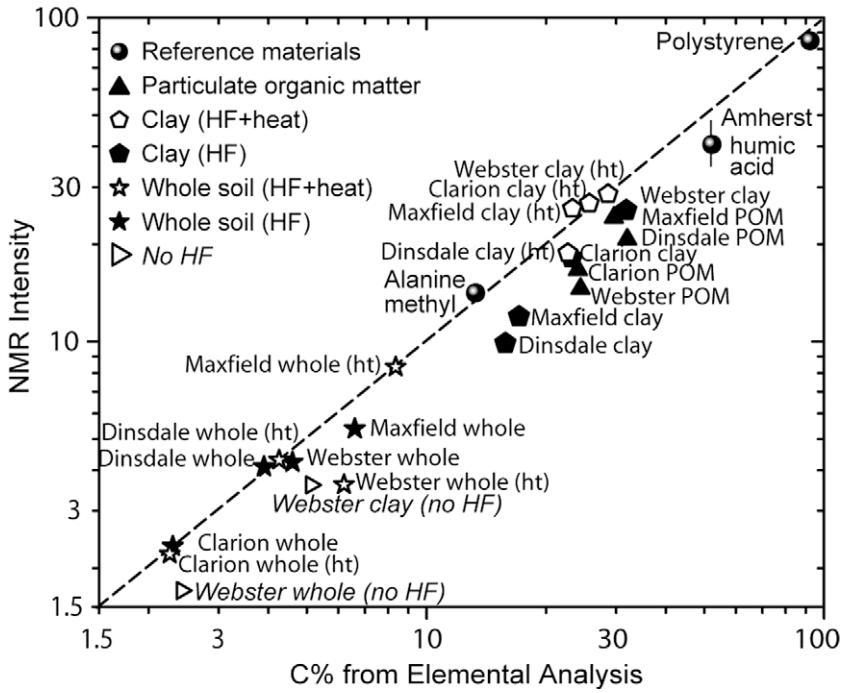

Fig. 4. Correlation plot of carbon percentages from DP MAS NMR measurements and from elemental analysis, for spin counting. The samples include clay fractions (pentagons), particulate organic matter (triangles), and whole-soils (stars). Filled pentagons and stars represent samples treated with HF at ambient temperature. Open pentagons and stars represent samples heated to 60 °C during HF treatment. Open triangles are for the clay fractions and whole-soils without any chemical treatment. The spin-counting NMR data are calibrated by measuring reference compounds (polystyrene, Amherst HA, and alanine) plotted as "spheres."

perfectly correlated with the organic matter components identified, they nevertheless afford consistent interpretations of the changes that plant materials undergo during decay and transformation to SOM.

Fig. 5 shows ^{13}C NMR spectra of HF-treated Dinsdale whole-soil samples and the Dinsdale clay and POM fractions. The relationships among these spectra are essentially repeated for the other soil materials. For reference, a quan-

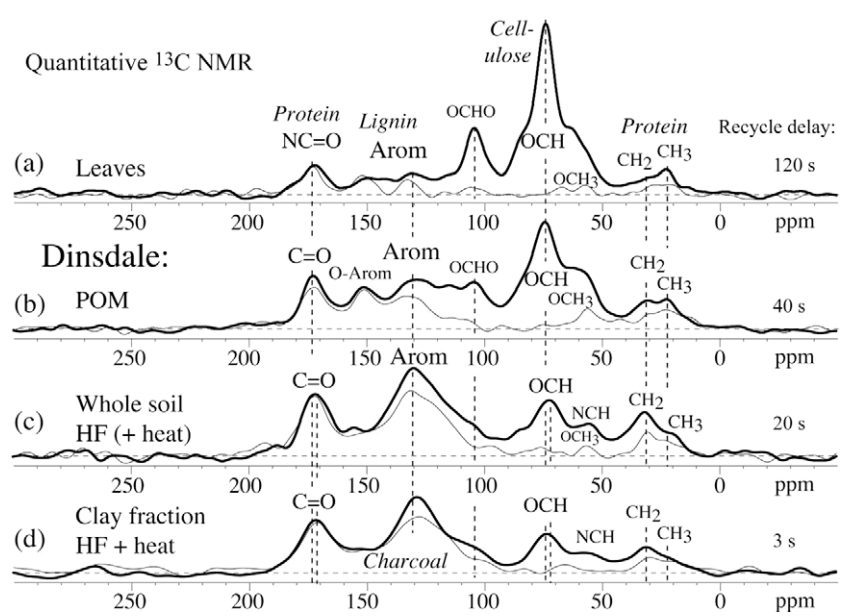

Fig. 5. Nearly quantitative, DP MAS ^{13}C NMR spectra of (a) corn leaves, pure plant material rich in cellulose and lignin, and (b–d) of organic matter from Dinsdale soil. (b) Particulate organic matter (POM), which shows lower intensities of OCH, and OCHO peaks compared to leaves. (c) Whole Dinsdale soil and (d) clay fraction (treated with HF at 60 °C), which have higher aromatic carbon and polymethylene intensities and lower polysaccharide intensities. The corresponding spectra of their quaternary carbons and CH_3 groups are plotted with thin lines. The recycle delays used are listed on the right-hand side. MAS frequency: 14 kHz.

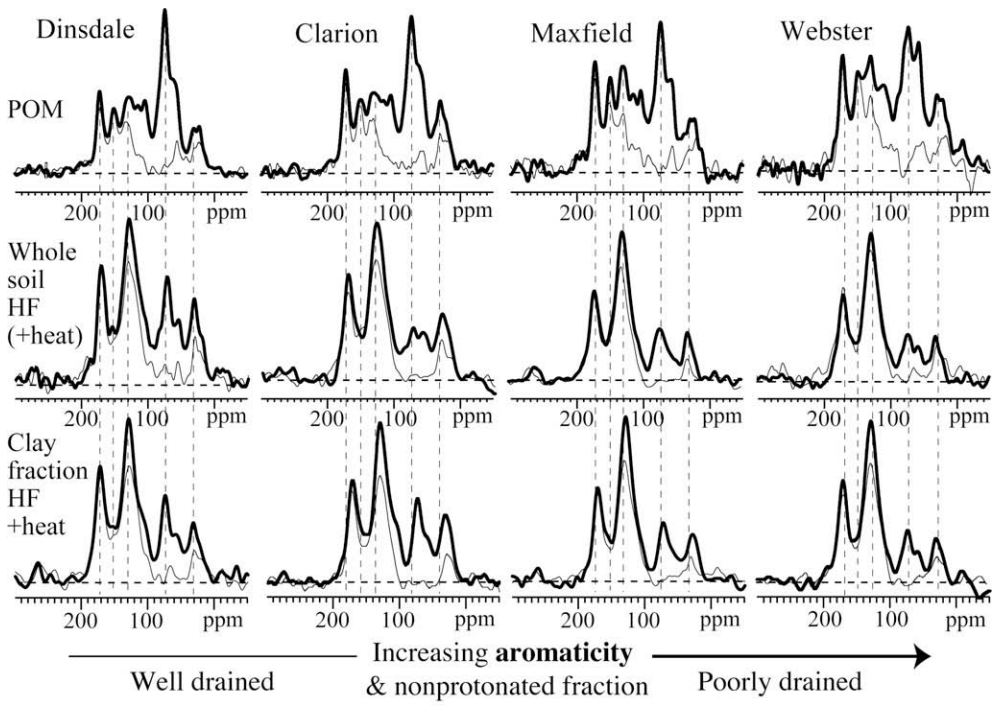

Fig. 6. Quantitative, DP MAS ^{13}C NMR spectra for samples from four different locations: Dinsdale, Clarion, Maxfield, and Webster, arranged from well drained to poorly drained, and three different fractions (top to bottom: particulate organic matter (POM), whole-soil, and clay fraction). The whole-soil spectra are averages of spectra obtained from samples after HF treatment at ambient temperature and at 60 °C. The clay fractions were treated with HF at 60 °C. Thick lines: full spectra; thin lines: corresponding spectra of quaternary carbons and CH_3 groups, after 68 μs of recoupled dipolar dephasing. MAS frequency: 14 kHz.

titative spectrum of corn leaves is also shown at the top of the figure. Corn stalks, corn kernels, and soybean residues give spectra that are similar to those of the leaves, with strong, sharp cellulose signals at 74 and 103 ppm, broad and low aromatic-C bands of lignin (Mao et al., 2006), and NC=O and alkyl resonances mostly due to peptides/proteins. Compared to leaves, POM spectra show a reduced OCH signal due to degradation of cellulose. The OCH_3 signal, an index of some common lignin monomers, is low in the POM, and it decreases further in both whole-soil and clay-fraction SOM, reflecting oxidation and loss of lignin C.

Spectral differences among the POM, whole soil, and clay fractions from all four locations are evident in Fig. 6. The functional group distributions of these spectra, with appropriate correction for aromatic-alkyl signal overlap (Mao and Schmidt-Rohr, 2004), are presented in Table 3. The spectra can be thought of as snapshots of the decomposition process. As the plant tissue decomposes to POM, the polysaccharide indicator (*O*-alkyl) declines, the lignin indicator (OCH_3) remains stable, and the lipid index (nonpolar alkyl) increases. At the same time, the abundance of carboxyl C roughly doubles, presumably a result of oxidative cleavage of ether groups as cellulose and lignin decompose. As POM decomposes further to SOM, the polysaccharide index declines considerably, carboxyl C increases slightly, the lignin fraction declines slightly, and the lipid index has no clear trend. Degradation of plant material to POM to whole-soil and clay-fraction SOM results in an increase in aromaticity, as shown by the aromaticity index [defined as (total aromatic C)/(total C)] in Table 3 (see further discussion below). The general plant decomposition index of Baldock and Preston (1995) (nonpolar alkyl/*O*-alkyl) also increased from plant to POM to SOM (Table 3). In general, clay-fraction SOM in these samples was quite similar to whole-soil SOM.

Some of the alkyl components in the samples studied here can be characterized with better sensitivity and resolution using nonquantitative cross polarization from ^{1}H to ^{13}C. The spectra in Fig. 7 show that sharp signals of crystalline cellulose in plant material in the top row convert to disordered saccharides in soil. Whole soil and even more prominently the corresponding clay fraction show a pronounced signal of polymethylene resonating near 30 ppm, which is partially mobile as proven by signal remaining after dipolar dephasing. This soft nonpolar alkyl fraction has been correlated with the capacity of the material for sorption of nonpolar aromatic contaminants (e.g., Chefetz et al., 2000; Mao et al., 2002b).

It is increasingly recognized that the fine-earth fraction of soils at today's land surface may contain considerable fire-derived black C, including both char (incompletely combusted plant residues) and soot (carbon particles condensed from the gas phase) (Schmidt and Noack, 2000; Preston and Schmidt, 2006). These compounds consist largely of fused aromatic moieties and are believed to resist microbial decomposition – although there is clearly a continuum of degradability that is related to the temperature and duration of heating during formation (Czimczik

Table 3
Quantitative ^{13}C NMR data of C in functional groups in plant tissues and three types of soil materials (POM, unfractionated soil samples and clay fractions). The unfractionated and clay-fraction samples were treated with HF to concentrate SOM (see text), except for the samples in the last two rows, which were not chemically treated. Values in italicized columns are subsets of adjacent columns to their left. Error margins: ±2%.

Soil	Ketone	COO	Total polar aromatic C	*Non-protonated, polar aromatic C*	Total nonpolar aromatic C	*Non-protonated, nonpolar aromatic C*	*O*-alkyl	NCH	O—CH_3	Nonpolar alkyl	Nonpolar alkyl/ *O*-alkyl	Aromaticity index[a]
	205–185	185–160	160–145	160–145	145–100	145–100	110–60	60–49	60–50	49–0		
	ppm[d] *% of total C*											
Plant tissue												
Corn leaves	0	7	3	*3*	14	*4*	59	5	2	10	0.17	0.17
Corn stalks	0	5	5	*5*	15	*5*	60	4	3	9	0.15	0.20
POM												
Dinsdale (W[b])	2	11	8	*7*	23	*12*	38	3	4	12	0.32	0.31
Clarion (W)	2	12	8	*7*	22	*11*	34	4	2	15	0.44	0.30
Maxfield (P[c])	3	13	9	*8*	25	*14*	32	3	2	12	0.38	0.34
Webster (P)	1	12	9	*8*	25	*13*	30	4	3	16	0.53	0.34
Unfractionated sample												
Dinsdale (W)	2	15	7	*5*	39	*27*	20	3	1	14	0.70	0.46
Clarion (W)	2	16	7	*7*	42	*32*	15	3	1	16	1.07	0.49
Maxfield (P)	3	17	7	*7*	45	*34*	15	3	1	11	0.73	0.52
Webster (P)	1	16	7	*6*	46	*36*	15	3	1	12	0.80	0.53
Clay (< 2 μm)												
Dinsdale (W)	1	17	7	*6*	41	*32*	17	4	0	13	0.76	0.48
Clarion (W)	1	16	7	*5*	40	*29*	17	4	0	14	0.82	0.47
Maxfield (P)	2	17	7	*5*	45	*34*	15	3	1	12	0.80	0.53
Webster (P)	3	16	7	*6*	45	*33*	14	2	1	11	0.79	0.52
Webster untreated samples												
Unfractionated	5	14	7	7	44	*31*	15	3	1	13	0.87	0.52
Clay	4	17	7	5	48	*36*	11	1	2	11	1.00	0.55

[a] Aromaticity index = [total polar aromatic C (%) + total nonpolar aromatic C (%)]/100%.
[b] W, well drained.
[c] P, poorly drained.
[d] ppm, chemical shift.

et al., 2002). Skjemstad et al. (2002) studied five modern soils in the US and reported that charcoal C made up 10–35% of the total organic C in the samples. The abundance of charcoal in most modern soils is unknown at present, yet geochemical models that predict the amount of carbon that can be sequestered in soils depend sensitively on quantification of C with turnover periods of millennia – such as charcoal C (Van Veen and Paul, 1981; Parton et al., 2005; Wang et al., 2005).

We earlier identified the nonpolar, nonprotonated aromatic C as an index of charcoal in the soils of the present study. The abundance of this type of C in undecomposed plant tissue was low (and originating from lignin, not charcoal), but it appeared to be much greater in POM (Table 3). The assignment to charcoal was supported by the increased ratio of nonprotonated: protonated nonpolar aromatic C in POM, around 1:1 compared to ~1:2 in the plant matter. We hypothesize that this signal of nonprotonated C resonating between 145 and 100 ppm in the POM samples primarily reflects those charcoal particles that were not incorporated in soil aggregates and were light enough and large enough to separate out with the POM. In the whole-soil and clay-fraction SOM, charcoal C accounted for most of the aromatics and increased greatly in abundance, as indicated by 27–36% nonprotonated nonpolar aromatic C (Table 3).

Previous NMR studies have indicated that Mollisols generally have higher aromaticities than other soils, with peaks at 130 ppm indicative of charcoal. In a CP NMR study of six arable European soils, Kiem et al. (2000) found that the aromatic component was refractory and particularly large in the studied Chernozems (comparable to Borolls in the US classification system). Since CP typically underestimates charcoal, the importance of charcoal in these samples was, if anything, underestimated. In their study of five US agricultural soils using CP NMR with an empirical correction for underrepresented char, Skjemstad et al. (2002) found the highest aromaticity in Mollisols, specifically 27% aryl C or 33% charcoal for HF-treated samples of a Mollisol in Minnesota. Our results are generally

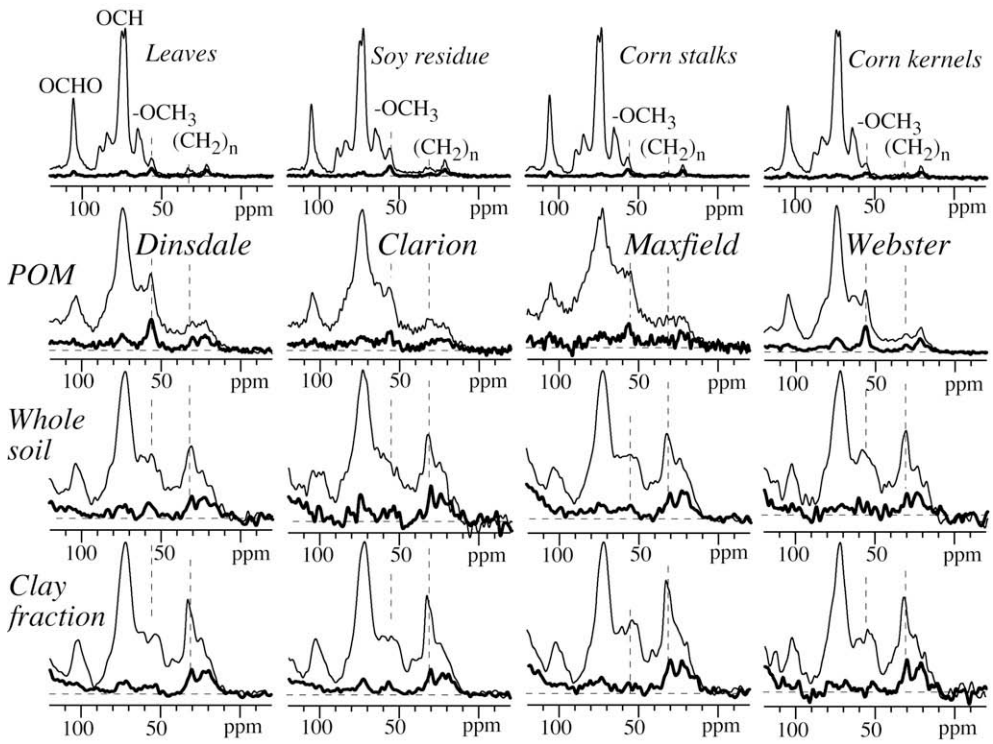

Fig. 7. $^{1}H—^{13}C$ cross-polarization ^{13}C NMR spectra (alkyl region). The spectra in the first row are all from plant materials: corn leaves, soy residue, corn stalks, corn kernels (from left to right). From the second to the bottom row, the samples are particulate organic matter, whole-soil, and clay fractions of Dinsdale, Clarion, Maxfield, and Webster soils. Thin lines: spectra of all types of carbons obtained by applying the CP/TOSS pulses sequence; thick lines: corresponding spectra of quaternary carbons and methyls after 40 μs of gated decoupling. MAS frequency: 8 kHz.

consistent with those findings, establishing even higher total nonpolar aromatic fractions of 39–48% in the four Mollisol samples studied here. The lower aromaticity found by Skjemstad et al. (2002) could be due to differences in the soils studied, but it is also typical of CP/MAS studies; due to the low and variable observability of charcoal in CP/MAS NMR (Knicker et al., 2005; Hammes et al., 2008), empirical corrections cannot be highly reliable. Skjemstad et al. (2002) themselves pointed out that "a Bloch decay" (i.e., direct-polarization as used in our study) "experiment is required to estimate charcoal-C accurately".

Our study shows that a large part of the aromaticity index for SOM of these soils must be attributed to the aromatic C in charcoal. Charcoal C was greater in the surface-horizon samples of the poorly drained soils than in those of the well drained soils (Table 3), probably the result of water or wind erosion from higher landscape positions and deposition in low spots on the landscape. The enrichment of organic C in depositional landscape positions of agricultural fields is widely appreciated (e.g., Lal et al., 2004; Oost et al., 2004). In studies of steeply sloping (46%) Ultisols and Alfisols under slash-and-burn cultivation and with a mean annual precipitation of 1400 mm, Rumpel et al. (2006a,b) have demonstrated that black carbon can be mobilized with eroded sediments. They attributed its preferential erosion to its low density and lack of interaction with mineral soil components. Our study suggests that steep slopes, low-SOM epipedons, slash-and-burn cultivation, and tropical precipitation patterns are not required for measurable accumulation of charcoal to occur in low-lying landscape positions. Indeed, recent studies of organic C transport by erosion indicate that carbon-enrichment ratios are greater in sediments moved at low erosion intensities than in those moved at high erosion intensities (Jacinthe et al., 2004; Roose and Barthès, 2006; Schiettecatte et al., 2008). Whether charcoal particles at the surface of a soil where the vegetation has recently burned are more (or less) susceptible to erosion than other SOM components is an interesting question that demands further empirical studies.

Another hypothesis to explain enrichment of charcoal in poorly drained vs. well drained soils is that charcoal may be degraded faster in oxidized environments than in reduced environments. In a laboratory simulation study, Nguyen and Lehmann (2009) recently reported that charcoal formed from corn residues was more susceptible to rapid decomposition in unsaturated conditions than in saturated conditions. It is difficult to compare these conclusions with those of the present study, however, since Nguyen's and Lehmann's charcoal samples were not protected from oxidation by association with mineral surfaces, other SOM, or aggregates of soil. Still, some charcoal components are likely to be relatively "labile" shortly after pyrolysis, and this mechanism for relative preservation in poorly drained

soils deserves further testing with naturally derived charcoal in field situations.

In the cultivated Mollisols of the present study, we found that charcoal accounted for more of the organic carbon in the SOM than in the POM (Table 3), probably reflecting both the dominance of recent, nonpyrolyzed crop residues in the POM as well as comminution of native charcoal since it was formed by pre-settlement fires. Charcoal carbon did not compose a greater proportion of SOM in the total clay fractions than it did in the whole-soil materials (Table 3). In contrast, Laird et al. (2008) examined SOM in the clay fraction of a suite of Iowa Mollisols similar to those of our study and found that char residues were concentrated in the coarse clay subfraction of the samples. That association could be attributed to differential preservation of charcoal residues in association with clay. Brodowski et al. (2006) reported that some char in soils may be protected from decomposition in microaggregates where its accessibility to enzymes is limited. However, it is useful to recall that the association of charcoal with particular size fractions depends on the procedure used to fractionate the soil samples. Like a number of other investigators, we used a gentle sonification method that probably left some aggregates intact, perhaps aggregates that were larger than those of Laird et al. (2008). Further size fractionation of our samples and more detailed studies are needed to explore the possible association of the charcoal with soil microaggregates more definitively.

4. CONCLUSIONS

In the surface horizons of four central Iowa Mollisols, we studied organic matter in the particulate organic matter fraction, the whole samples, and the clay fractions. To concentrate the SOM, we treated the soil samples with 5 M HF at 60 °C for 30 min. We used quantitative solid-state nuclear magnetic resonance spectroscopy to assess the composition of the SOM. Spin counting showed that NMR was >65% quantitative for the samples studied, including untreated whole soils with <2.5 wt.% C. The quantitative NMR analyses allowed us to conclude (1) that the HF plus heat treatment had little impact on determination of the organic C functional groups in the samples, (2) that organic C was observable even in untreated soil materials, (3) that esters were likely to compose only a minor fraction of SOM in these Mollisols, and (4) that the aromatic components of SOM were enriched in the poorly drained soils (compared with the well drained soils). Nonpolar, nonprotonated aromatic C, interpreted as a proxy for charcoal C, composed 27–36% of total organic C in these soil materials.

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APPENDIX A. SUPPLEMENTARY DATA

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.gca.2009.10.008.

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