

## Soil Organic Carbon Composition in a Northern Mixed-Grass Prairie: Effects of Grazing

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### ABSTRACT

Growing interest in the potential for soils to provide a sink for atmospheric C has prompted studies of effects of management on the amount and nature of soil organic C (SOC). In this study, we evaluated effects of different grazing management regimes (light grazing [LG], heavy grazing [HG], and non-grazed exclosures [EX]) on amount and composition of SOC at the USDA-ARS High Plains Grasslands Research Station (HPGRS), Cheyenne, WY. Soils (0–5 cm) from each treatment were analyzed for total C and N contents and lignin composition. Soil organic C and N contents were significantly greater in LG (SOC–13.8 Mg ha<sup>-1</sup>; total N–1.22 Mg ha<sup>-1</sup>) than HG (SOC–10.9 Mg ha<sup>-1</sup>; total N–0.94 Mg ha<sup>-1</sup>) or EX (SOC–10.8 Mg ha<sup>-1</sup>; total N–0.94 Mg ha<sup>-1</sup>). From CuO oxidation studies, significantly greater ( $P < 0.05$ ) total lignin (Vanillyl [V] + Syringyl [S] + Cinnamyl [C] compounds) contents were noted in EX (21 g kg<sup>-1</sup> SOC) than LG (12 g kg<sup>-1</sup> SOC) and HG (15 g kg<sup>-1</sup> SOC) soils. The lignin composition of humic (HA) and fulvic (FA) acids indicated that HA under LG contained significantly greater V and S than HG or EX. Fulvic acids contained S-depleted lignin compared with HAs and FAs from HG, which contained significantly greater V and C than FAs extracted from LG and EX. Nuclear magnetic resonance (NMR) spectra of HA and FA, however, did not vary significantly among the three grazing treatments. Results from CuO oxidation and NMR spectroscopy emphasized the familiar problem that determining the nature of soil organic matter (SOM) is a difficult task and sometimes different analytical techniques provide different information about the nature of SOM. Nonetheless, results of this study indicate that LG is the most sustainable grazing management system for northern mixed-grass prairies.

RANGELANDS ACCOUNT for approximately 160 million hectares in the USA, and are extensively used for grazing in the western parts of the country. On a global scale, nearly half of the world's land area is occupied by rangelands that contain approximately one-third of the above- and belowground C reserves. Carbon dynamics in these ecosystems is controlled by ecosystem processes such as photosynthesis, heterotrophic respiration (decomposition), and inherent soil C levels, all of which are affected by land-use practices including grazing (Povirk et al., 2000). Many grazing practices are aimed at ensur-

ing the sustainable use of rangelands for livestock production. However, many ecosystem components and processes like plant community structure, soil properties, and nutrient cycling are also affected by grazing management (Schuman et al., 1999). Density and duration of rangeland stocking can affect plant community composition through displacement of cool-season mixed grasses by warm-season short grasses in northern mixed-grass prairies (Dormaer and Willms, 1990).

Grazing can also influence the amount and composition of SOM (Dormaer and Willms, 1990; Frank et al., 1995) through impacts on litter accumulation and decomposition (Naeth et al., 1991; Shariff et al., 1994). The quantity and chemical composition of SOM is important to C and N cycling, as N is often the productivity limiting factor in rangeland ecosystems (Power, 1994). The extent to which different grazing practices alter C cycling and the composition of SOC is less understood (Milchunas and Lauenroth, 1993; Manley et al., 1995; Schuman et al., 2000). Lack of a clear relationship between grazing practices and SOC has been attributed to soil variations, depth of soil sampling, and insufficient evaluation of C distributions within the grazing system (Schuman et al., 1999). It is important that a clear understanding of grazing management effects on SOC accumulation and chemical composition be developed so that effective soil C management options are available for rangelands (Kaiser, 2000). Impacts of grazing on the distribution of soil C between labile and recalcitrant pools are also not well understood (Parton et al., 2000). If grazing results in greater incorporation of lignin into more recalcitrant soil C pools, then grazing may enhance long-term soil C storage.

Lignin is the second most abundant organic compound after cellulose (Kirk, 1984) and because of its complex and irregular ring polymeric structure it is more resistant to enzymatic attack. Lignin is believed to be the major source of aromatic C in soil humic substances (Stevenson, 1994; Zech et al., 1997; Kiem and Kögel-Knabner, 2003) and is composed of cross-linked phenylpropane units of vanillyl (V), syringyl (S), and cinnamyl (C) groups (Paul and Clark, 1996). The proportions in which these VSC groups occur in lignin depend on the source of lignin polymer (Kögel-Knabner et al., 1991). Thus, determining the composition of lignin and the relative distribution of phenylpropane units provides a bet-

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**Abbreviations:** CPMAS, cross polarization magic angle spinning; CuO, cupric oxide; DD, dipolar dephasing; EX, exclosures; FA, fulvic acid; HA, humic acid; HG, continuous heavy grazing; HPLC, high performance liquid chromatography; LG, continuous light grazing; NMR, nuclear magnetic resonance; SOC, soil organic carbon; SOM, soil organic matter; SPE, solid phase extraction; VSC, vanillyl-syringyl-cinnamyl groups.

ter understanding of sources contributing to the stable or recalcitrant C pool in rangeland soils. A combination of  $^{13}\text{C}$  NMR spectroscopy and CuO oxidation offer a powerful approach to study composition of SOC compounds at structural and molecular levels (Kögel et al., 1988; Dignac et al., 2002). The main objective of this study was to evaluate impacts of long-term grazing management practices on the composition of SOM. This was accomplished by using a combination of techniques such as  $^{13}\text{C}$  Cross Polarization and Magic Angle Spinning Nuclear Magnetic Resonance ( $^{13}\text{C}$  CPMAS NMR) spectroscopy, and alkaline CuO oxidation of lignin present in soil and the associated humic substances.

## MATERIALS AND METHODS

### Study Sites

Field study sites were located at the USDA-ARS High Plains Grasslands Research Station (HPGRS) near Cheyenne, WY. Vegetation in the area is dominated by grasses, forbs, and sedges. Plant community as a percentage of peak standing crop biomass is listed in Table 1. The study sites were characterized by semiarid climate with an average annual precipitation of 384 mm (average of period 1971–1994) and annual frost-free period of 127 d; however during 2000 to 2003 the area experienced a drought. Ascalon and Altvan sandy loams (mixed, mesic, aridic argiustoll) are the dominant soil series on the landscape, which is characterized as rolling topography at an elevations ranging from 1910 to 1950 m.

Grazing treatment pastures were established in 1982 in a randomized block design with two replicate blocks. Three treatments evaluated in this study were: (i) nongrazed enclosures (EX), (ii) light grazing (LG), pastures with continuous season long grazing at a light stocking rate of 0.16 to 0.23 steers  $\text{ha}^{-1}$  (mean of 20 steer-days  $\text{ha}^{-1}$ ), which is a stocking rate 35% below the rate recommended for this area by the NRCS, and (iii) heavy grazing (HG), pastures with continuous season-long grazing at a rate of 0.56 steers  $\text{ha}^{-1}$  (mean of 59 steer-days  $\text{ha}^{-1}$ ), which is 33% higher than the NRCS recommended rate (Hart et al., 1988). Land area of each block in EX, LG, and HG was 0.2, 40.5, and 8.9 ha, respectively.

In spring 2003, soil samples were collected to a depth of 60 cm at four depth intervals 0 to 5, 5 to 15, 15 to 30, and 30 to 60 cm. Five sample sites were located at 10-m intervals along 50-m permanent transects established in each replicate pasture in 1982 (before initiation of grazing treatments). At each location, two cores were collected and composited by depth. Samples were placed in plastic bags, which were sealed and then transported to the laboratory in coolers. Separate soil cores were collected at the second and fourth sampling sites along each transect to assess bulk density as described by Blake and Hartage (1986). Soil samples were air-dried, passed through a 2-mm screen to remove plant crowns, visible roots, and root fragments. Since most of the SOM is located in the surface 0 to 5 cm, results presented in this paper on SOM characterization represent the surface 5-cm increment.

Total soil C and N were determined using a Carlo-Erba automated combustion analyzer. Soil inorganic C was determined using the modified pressure calcimetric method (Sherrod et al., 2002). Total SOC was calculated using the difference between total C and inorganic C (Nelson and Sommers, 2001). The surface 5-cm soil layer contained very low inorganic C (average inorganic C in EX, LG, and HG were 80, 124, and 99  $\text{kg ha}^{-1}$ , respectively) and the pH of these soils ranged from 6.4 to 7.3.

**Table 1. Dominant vegetation types and composition (%) of peak standing crop biomass in the northern mixed-grass prairie study sites.†**

Plant name	Enclosure	Continuous light grazing	Continuous heavy grazing
Blue grama ( <i>Bouteloua gracilis</i> )	16	17	27
Western Wheatgrass ( <i>Pascopyrum smithii</i> )	29	45	21
Needleandthread ( <i>Stipa comata</i> )	13	7	11
Other grasses	3	5	12
Sedges	6	10	7
Forbs	33	16	22

† Schuman et al. (1999).

### Humic Acid and Fulvic Acid Fractionation

Separation of SOM fractions (HA and FA, humins were not studied) was carried out using the method of Calderoni and Schnitzer (1984). Eighty grams of air-dried soil from each of five sampling locations on the duplicate transects in each treatment was finely ground to pass a 250- $\mu\text{m}$  sieve and composited. Six composite samples of 400 g (each representing a replicate grazing treatment) were extracted with 4 L of 0.5 M NaOH under  $\text{N}_2$  at room temperature (10:1 NaOH/soil) for 24 h with intermittent shaking. The residue was sieved through a 53- $\mu\text{m}$  sieve and washed with distilled water. The HA in the supernatant was precipitated using 6 M HCl (pH = 2), with the precipitates washed four times with dilute acid (HCl/HF 1:20), rinsed until free of Cl, and freeze dried. The supernatant solution containing FA fraction was filtered through Whatman #42 filter paper, taken to dryness three times at 40°C to remove excess acid, dissolved in methanol three times, filtered to remove salt and evaporated. Further, FAs were dialysed against Spectrapor #1 membrane with a molecular cutoff of 6 to 8 kDa and freeze-dried (Preston et al., 1994). These extracts were used for lignin and NMR analysis as described below.

### Lignin Oxidation Products

Lignin characterization was performed on subsamples of <2-mm soil material, and on the soil HA and FA fractions. Alkaline CuO oxidation was conducted in a pressure bomb using a modified method developed by Kögel and Böchter (1985) and Kögel-Knabner et al. (1991). Each vessel contained 1.0 g of soil or 50 mg of HA or FA, 50 mg  $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$ , 250 mg of CuO, and 15 mL of 2 M NaOH, which was then sealed under  $\text{N}_2$ . The pressure bomb was heated to 170°C and the reaction temperature maintained for 2 h. After cooling, the suspension was centrifuged for 10 min at  $1510 \times g$ . The supernatant was saved, while sediments were washed with water and recentrifuged. After combining the supernatants the solution was acidified to pH 2 with 6 M HCl and held at room temperature for 1 h. The suspension was centrifuged, decanted and saved, and the residue washed with acidified water (1 mL 12 M HCl in 1 L distilled water) and centrifuged again. Lignin oxidation products were separated using C18 solid phase extraction (SPE) disposable columns (Alltech, Deerfield, IL). Before sample application, SPE columns were conditioned with methanol and 4% (v/v) acetic acid (Krzyszowska and Vance, 1994). The combined acidified supernatant was passed through the column using vacuum, allowed to dry for 10 min, and lignin oxidation products selectively eluted in four volumes of 1 mL of ethyl acetate. Ethyl acetate was removed from the eluent with a rotary evaporator; residue was dissolved in 1 mL of methanol and analyzed with high performance liquid chromatography (HPLC).

Lignin oxidation products were quantified using a Shimadzu HPLC system consisting of LC-10AS solvent delivery unit, SIL-9A auto injector (20  $\mu$ L of solution) unit and a SPD-M6A UV detector set at 280 nm. Separation was achieved using a 25 cm  $\times$  4 mm i.d. column filled with Nucleosil C18 (3  $\mu$ m) stationary phase in combination with a nucleosil C18 (5  $\mu$ m) cartridge placed immediately before the column. Gradient elution was performed at a flow rate of 0.8 mL min<sup>-1</sup>. Eluent A was 50 mM phosphate buffer (pH 2.1) containing 10% (v/v) acetonitrile and Eluent B contained acetonitrile with a 30% (v/v) phosphate buffer (pH 2.1). The gradient started with 97% Eluent A and 3% Eluent B, and was terminated after 60 min when Eluents A and B were 90 and 10%, respectively. The column was purged with pure Eluent B for 10 min and then reequilibrated with 97% Eluent A and 3% B for 10 min.

### <sup>13</sup>C CPMAS NMR Spectroscopy

Solid state <sup>13</sup>C Cross Polarization (CP) and Magic Angle Spinning (MAS) NMR spectra of HA and FA were run on a Bruker MSL 300 spectrometer (Bruker instruments Inc. Karlsruhe, Germany) operating at 75.47 MHz. Dry, powdered samples were packed into a zirconium oxide rotor of 4-mm o.d. and spun at 8500 Hz. Spectra were acquired with 1-ms contact time, 2-s recycle time, and 8000 to 21 000 scans. Spectra were also acquired with dipolar dephasing (DD) using a dephasing delay of 48 to 52  $\mu$ s to minimize intensity for the O-alkyl signal. Spectra were processed with 30 to 40 Hz line broadening and baseline correction. Chemical shifts were reported relative to tetramethyl silane (TMS) at 0 ppm, with the reference frequency set using adamantane. NMR spectra were divided into chemical shift regions corresponding to alkyl C (0–47 ppm); methoxyl and N-alkyl C (47–60 ppm); O-alkyl C (60–93 ppm); di-O-alkyl C (93–112 ppm); aromatic C (112–140 ppm); O-substituted aromatic C (phenolic C) including phenolics and aryl ethers (140–160 ppm) and carboxyl/amide/ester C (160–190 ppm) (Preston et al., 1994).

Areas of specific shift regions were determined from integrated areas and expressed as percentage of total area. It is well known that the intensity distribution in CP spectra may not be quantitative, due to under representation of C remote from protons, and C with high mobility in the solid state, such as methoxyl C, and the mobile fraction of long-chain CH<sub>2</sub> (Preston, 2001). Another well-known problem is that cross-polarization is weakened by high-speed spinning; this can be alleviated by ramped-amplitude cross-polarization, which was not available on our instrument. Signal is also reduced by the smaller sample size, albeit moderated by the greater efficiency of the smaller coil. However, high-speed spinning reduces the intensity distortions due to spinning sidebands (SSB), which can have substantial impact on samples high in carboxyl or

aromatic C. We used a spinning speed of 8500 Hz that minimized SSB while maintaining reasonable signal acquiring ability. Therefore, the NMR spectra are used qualitatively, to compare intensity distributions. This should be quite valid for comparisons among HA or FA. It should also be reasonable to demonstrate the large differences between HA and FA.

### Statistical Analyses

One way analysis of variance (ANOVA) was used to test impacts of different grazing practices on soil C and N concentrations, HA and FA compositions, lignin oxidation products in soil and humic substances, and relative intensities at different chemical shift regions in HA and FA NMR spectra. Least Significant Differences (LSD) procedures were used for treatment mean separation. All statistical evaluations are based on  $P < 0.05$ , unless otherwise noted.

## RESULTS AND DISCUSSION

### Carbon and Nitrogen

Soil organic C and N contents in LG sites were significantly greater than both HG and EX treatment (Table 2). Grazing can stimulate aboveground biomass (Detling et al., 1979) and increase incorporation of aboveground plant C and N components into the soil (Schuman et al., 1999). Increased cycling of C and N in grazed lands has been attributed to physical breakdown and greater soil incorporation of residues, enhanced root exudation and increased decomposition rates of plant residues. Immobilization of C and N in litter may also have resulted in significantly lower C and N observed in EX soils. Schuman et al. (1999) reported approximately 275 to 675 kg ha<sup>-1</sup> more C and 15 to 25 kg ha<sup>-1</sup> more N are immobilized in the litter materials of EX versus LG sites in research conducted within the same grazing systems in 1993. In heavily grazed land, significantly lower SOC and N may be due to physiological responses of grass to increased grazing pressure (Holland and Detling 1990). That is, heavy grazing has been reported to increase C allocation in grasses to aboveground biomass (new leaves) while decreasing allocation to roots (Detling et al., 1979). Simulation models also have predicted decreasing soil C levels with increased grazing rates (Parton et al., 1987) due to increased rates of SOM decomposition. However, others have also shown that heavy, season-long grazing can result in plant community shifts

**Table 2. Soil (surface 5 cm) C and N contents and C and N concentrations, C/N ratios in soil, humic acid, and fulvic acids in three grazing treatments on a mixed-grass prairie.**

	Soil (0–5 cm)	Soil (0–5 cm)	Humic acid	Fulvic acid
	%	Mg ha <sup>-1</sup>	%	Mg ha <sup>-1</sup>
<b>C</b>				
Exclosures	2.09 $\pm$ 0.26 <sup>ab†</sup>	10.8 $\pm$ 0.8 <sup>b</sup>	47.5 $\pm$ 3.3	32.2 $\pm$ 5.6
Light grazing	2.60 $\pm$ 0.19 <sup>a</sup>	13.8 $\pm$ 0.2 <sup>a</sup>	49.2 $\pm$ 0.7	40.2 $\pm$ 2.2
Heavy grazing	1.98 $\pm$ 0.05 <sup>b</sup>	10.9 $\pm$ 0.3 <sup>b</sup>	50.4 $\pm$ 5.4	42.7 $\pm$ 2.4
<b>N</b>				
Exclosures	0.18 $\pm$ 0.02 <sup>b</sup>	0.94 $\pm$ 0.04 <sup>b</sup>	4.8 $\pm$ 0.02 <sup>b</sup>	2.5 $\pm$ 0.35
Light grazing	0.23 $\pm$ 0.02 <sup>a</sup>	1.23 $\pm$ 0.03 <sup>a</sup>	5.6 $\pm$ 0.13 <sup>a</sup>	3.3 $\pm$ 0.05
Heavy grazing	0.17 $\pm$ 0.01 <sup>b</sup>	0.94 $\pm$ 0.04 <sup>b</sup>	4.7 $\pm$ 0.02 <sup>b</sup>	3.4 $\pm$ 0.07
<b>C/N ratios</b>				
Exclosures	11.7 $\pm$ 0.10		10.0 $\pm$ 1.08	12.8 $\pm$ 0.56
Light grazing	11.3 $\pm$ 0.04		8.9 $\pm$ 0.34	12.2 $\pm$ 0.46
Heavy grazing	11.7 $\pm$ 0.12		10.8 $\pm$ 0.75	12.7 $\pm$ 0.40

† Means followed by the same lowercase letter are not significantly different at the 0.05 probability level.

resulting in C4 grasses such as blue grama (*Bouteloua gracilis*) becoming more dominant, which results in a greater portion of C allocated to the root system than C3 grasses (Redetzke and Dyne, 1979). In accordance with previous field research and modeling efforts, our study shows that LG led to increased soil C and N. Continuous light grazing is therefore an important management tool that can ensure long-term sustainability of the northern mixed-grass prairie.

Carbon and N contents, and C/N ratios for HA and FA are given in Table 2. Whereas HA C content did not differ significantly among the three grazing treatments, N content of HA was significantly greater in the LG treatment than HG and EX treatments. A major proportion of N in HA occurs as structural components. Nitrogen in HA is biologically stable and not readily available for microbial decomposition. Recent studies indicate that most of HA N is in amide structures (Zang et al., 2000; Mahieu et al., 2002) and that amide N in organic matter is often not hydrolyzable in acid (Knicker and Hatcher, 2001). The amide N may become refractory due to the protection from the HA hydrophobic structure (Zang et al., 2000). The C/N ratio of soil and both humic and fulvic acids did not vary significantly among the three grazing treatments.

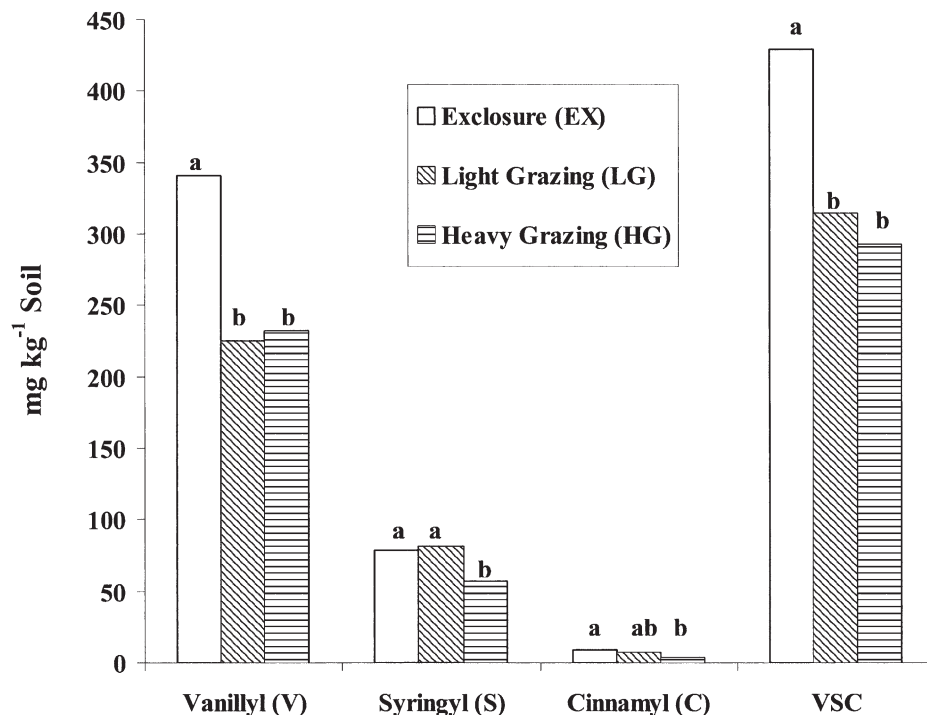
### Lignin Oxidation Products

Total lignin (VSC) was significantly greater in EX soils (429 mg kg<sup>-1</sup> soil or 21 g kg<sup>-1</sup> SOC) than LG (314 mg kg<sup>-1</sup> soil or 12 g kg<sup>-1</sup> SOC) and HG (292 mg kg<sup>-1</sup> soil or 15 g kg<sup>-1</sup> SOC) treatments (Fig. 1). These VSC values

are within the range of 13 to 22 g VSC kg<sup>-1</sup> SOC reported by Six et al. (2001) and the average value of 18 g VSC kg<sup>-1</sup> SOC reported by Amelung et al. (1999) for North American grassland soils. Guggenberger et al. (1994) also reported similar VSC value of 19 g kg<sup>-1</sup> SOC for soils under permanent grasslands in Germany. Knicker et al. (2000) observed that tussock grassland soils in New Zealand contained lower VSC contents that were in the range of 10 to 13 g kg<sup>-1</sup> SOC.

Vanillyl and S compounds were the major structural units of lignin in the SOM of all three treatments. Concentration of V units was significantly greater in EX (341 mg kg<sup>-1</sup>) than LG (225 mg kg<sup>-1</sup>) and HG (232 mg kg<sup>-1</sup>) treatments. Syringyl concentration was significantly greater in LG and EX than the HG treatment. Cinnamyl concentrations were significantly greater in EX than HG plots, with no significant differences between LG and HG treatments.

The complex and irregular ring polymeric nature of lignin makes it more resistant to enzyme attack than polysaccharides, and because of its slow degradation and turnover rates, lignin is believed to be a major precursor to soil humic substances (Radogna, 1990; Stevenson, 1994; Zech et al., 1997; Kiem and Kögel-Knabner, 2003; Krull et al., 2003). Phenolic products determined from CuO oxidation is the result of primary litter sources (plant derived) as phenylpropane structures are rarely produced as secondary metabolic products (Kögel and Böchter, 1985; Baldock et al., 1997; Ruhlmann, 1999). Therefore, the relative proportions of the three phenyl-



### Phenylpropanoid Moieties

Fig. 1. Concentrations of phenylpropanoid moieties (mg kg<sup>-1</sup> soil) in soils under three grazing treatments at the High Plains Grasslands Research Station, Cheyenne, WY.

propanoid moieties (i.e., VSC) within SOM or HA and FA is indicative of lignin precursors.

Vanillyl compounds are produced by oxidation of guaiacyl-based lignin (e.g., gymnosperms), syringyl compounds are produced by S-based lignin (e.g., angiosperm lignin oxidation yields similar proportions of V and S units), and cinnamyl compounds are mainly produced by lignins in nonwoody tissues such as grasses, needles, and tree leaves (Guggenberger et al., 1994; Sanger et al., 1996). As is evident from Table 1, grasses (angiosperms) are the dominant vegetation type in these three grazing treatment pastures. Therefore, it is not surprising that V and S were the major phenylpropanoid units in SOM and that minor amounts of cinnamyl compounds were present in these soils. Significantly greater concentrations of V, S, and C compounds in EX compared with LG and HG sites suggests that the rate of decomposition in EX may be lower than in the grazed sites. Schuman et al. (1999) observed that the annual aboveground biomass turnover rates in our EX sites were lower than the LG and HG sites. Thus the EX areas, which have not been grazed for the past 60 yr, contained significantly greater total lignin concentration (VSC) than either of the grazed pastures.

Total lignin concentration in HA extracted from LG soil was significantly greater than EX and HG HAs (Fig. 2). Concentrations of V and S units in LG HA were significantly greater than in those from EX and HG treatments. Vanillyl and cinnamyl concentrations in HG FA samples were significantly greater than that in EX and LG treatments (Fig. 2); however, no significant differences among treatments were observed with respect to total lignin (VSC) or S concentrations. The proportion of HA + FA in SOC were 40, 36, and 43% in EX, LG, and HG, respectively. Based on this, lignin contributed by HA + FA constitutes 24%, 43% and most of total soil lignin in EX, HG and LG treatments. The lignin contents of HAs and FAs were greater than bulk SOM. Similar results have been reported by Ertel and Hedges (1984), who found greater CuO oxidation product concentrations in HAs and FAs than bulk SOM in Klamath River Shelf sediments and Beaver Hill soils. Miikki et al. (1994) also reported an increase in CuO product concentrations due to humification processes. Greater lignin contents in humic substances are presumed due to greater preservation of original lignin or repolymerization of lignin monomers during humification (Waksman, 1932; Stevenson, 1994).

Results from CuO oxidation analysis of HA and FA suggests that S and V units comprised most of the lignin in humic fractions. While V compounds were predominant in HA, S compounds, with the exception of HG treatment, dominated in FA. This suggests that V units are more resistant to decomposition than S units. Similar results have been reported with other studies (Ander et al., 1984; Hedges et al., 1988; Preston et al., 1998), which have also indicated that S moieties are more susceptible to enzymatic attack as they are relatively more methoxylated than V and C rings. Due to rapid degradation, FA in HG pasture soils may have lost S compounds at a faster rate as they are more susceptible to degrada-

tion, resulting in significantly greater concentrations of V and C compounds in FA extracted from these pasture soils. Soil HAs are more aromatic than FAs (Kögel-Knabner et al., 1991; Stevenson, 1994; Preston et al., 1994; Eneji et al., 2003), therefore it is understandable that more recalcitrant units of lignin such as V and C compounds tend to dominate phenylpropanoid moieties in HA. As discussed later, the <sup>13</sup>C NMR spectra of HA and FA samples also confirmed higher aromatic + phenolic intensities for HA than for FA.

### Degree of Lignin Degradation

Oxidative degradation of lignin by soil microorganisms involves cleaving the  $\beta$ -O-4 linkages in lignin macromolecules and oxidation of lignin side chains (Hedges et al., 1988; Radogna, 1990). This results in an increase in carboxylic acids with respect to aldehydes (Kirk, 1984). Thus, the degree of lignin alteration by soil microorganisms is noted in the ratios of acids to aldehydes (Vanillic acid to Vanillin-Ac/ $Al_V$  or syringic acid to syringaldehyde-Ac/ $Al_S$ ), with increasing ratios indicating greater alteration or degradation of lignin (Hedges et al., 1988; Six et al., 2001; Kiem and Kögel-Knabner, 2003; Rumpel et al., 2004). An  $Ac/Al_V$  ratio of  $>0.2$  is attributed to degradation of non-woody vascular plant tissues of both angiosperms and gymnosperms (Hedges and Weliky, 1989). The C/V ratio is another measure used to estimate the extent of lignin decomposition and leaching of oxidation products (Hedges and Weliky, 1989). As degradation of lignin progresses and leaching of oxidation product occurs, C/V tends to decline and then remain constant once most soluble products are leached (Hedges and Weliky, 1989; Hadad et al., 1992).

In the present study,  $Ac/Al_V$  ratios for SOM in all three treatments were  $>0.2$  (Table 3) indicating that lignin in these soils was derived from non-woody plant tissues (Radogna, 1990). No significant differences were observed for bulk soils among treatments with respect to  $Ac/Al_V$ ,  $Ac/Al_S$ , and C/V ratios. The  $Ac/Al_V$  (0.39–0.51) for SOM were comparable with the range of 0.30 to 0.59 reported by Six et al. (2001) and 0.22 to 0.58 for permanent grasslands in Germany (Guggenberger et al., 1994) and 0.43 to 0.46 in tussock grasslands of New Zealand (Knicker et al., 2000). The  $Ac/Al_S$  values (0.36–0.44) in the present study compare well with the range of 0.54 to 0.88 reported by Six et al. (2001), 0.38 to 0.41 by Amelung et al. (1999), and 0.34 to 0.46 by Knicker et al. (2000). The published C/V value for soils (e.g., Hedges and Weliky, 1989) are in the range of 0.015 to 0.082 observed in the present study.

Humic acid  $Ac/Al_V$ ,  $Ac/Al_S$ , and C/V ratios are listed in Table 3. All the  $Ac/Al_V$  ratios are above 0.2 suggesting that phenylpropanoid moieties in these humic substances are derived from nonwoody plants. Ratios of  $Ac/Al_V$  and C/V for HA did not vary significantly among treatments; however, the  $Ac/Al_S$  ratio was significantly greater for HG compared with LG and EX. This suggests the preferential degradation of syringyl lignin in comparison with guaiacyl lignin in HG treatments

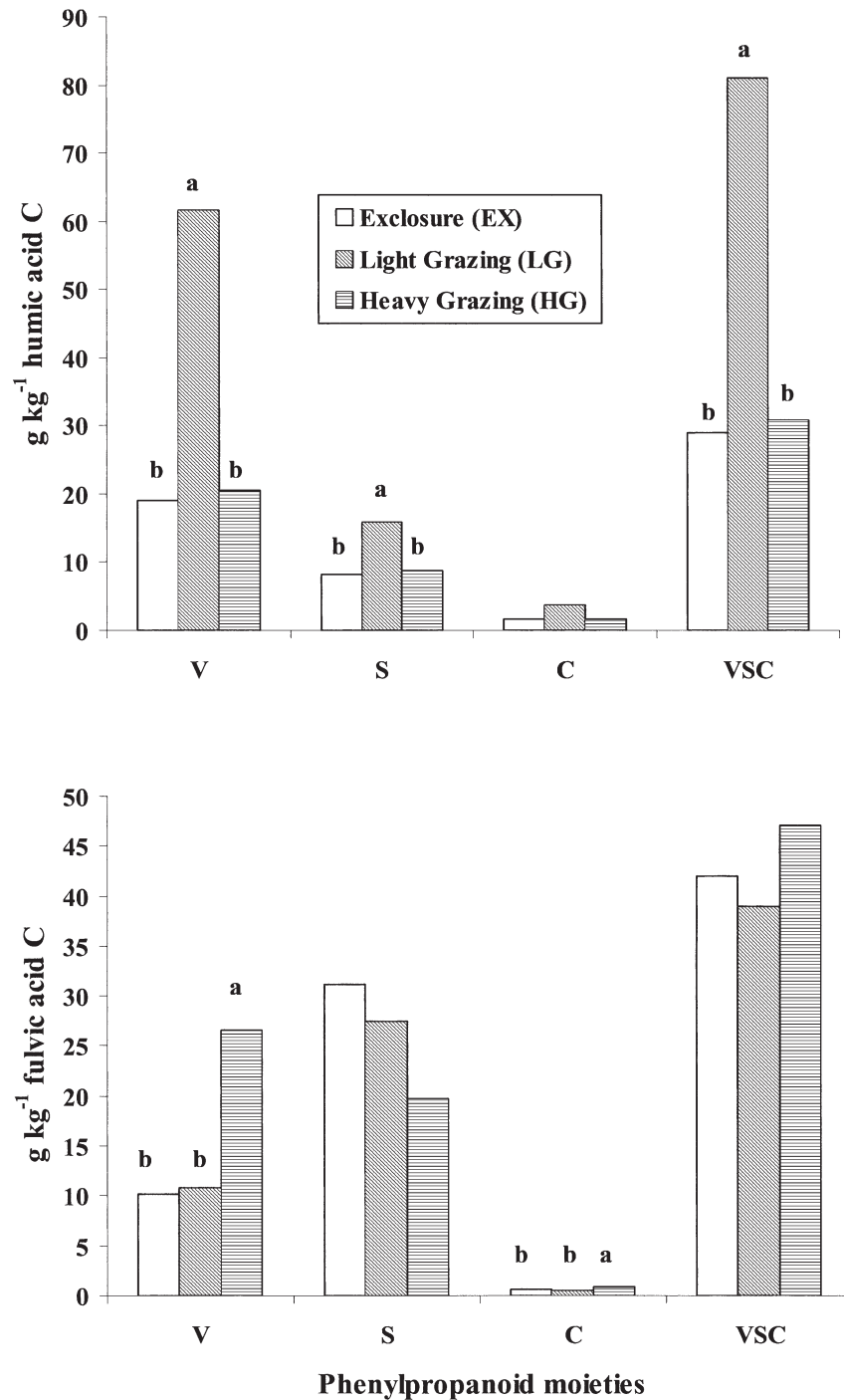


Fig. 2. Concentrations of phenylpropanoid moieties ( $\text{g VSC kg}^{-1}$  humic acid C or fulvic acid C) in humic acids and/or fulvic acid under three grazing treatments at the High Plains Grasslands Research Station, Cheyenne, WY.

(Yamaguchi et al., 1994; Chefetz et al., 2000). Fulvic acid  $\text{Ac}/\text{Al}_s$  ratios also did not differ significantly among treatments (Table 3), but unlike HA, both  $\text{Ac}/\text{Al}_v$  and  $\text{C}/\text{V}$  ratios for FA extracted from HG treatments were significantly lower than EX and LG treatments. This again confirms that lignin in FA of HG treatments has undergone considerable degradation compared with that extracted from EX and LG treatments.

The differences described above may be due to variations in microbial community structures in the three

grazing treatments. For example, Stahl et al. (2004) researching the same study sites reported significant differences in microbial community structure among the three grazing treatments. They also observed that continuously light grazed plots contained a significantly greater microbial biomass than HG or EX plots.

### <sup>13</sup>C NMR Spectra

The NMR spectra of HA and FA from the three grazing treatments contained similar features and signal

**Table 3. Indicators of extent of lignin alteration in bulk soil, humic and fulvic acids extracted from three grazing treatments.**

Grazing treatments	Ac/Al <sub>v</sub>	Ac/Al <sub>s</sub>	C/V
<b>Bulk soil (0–5 cm)</b>			
Exlosures	0.39 ± 0.02	0.38 ± 0.03	0.026 ± 0.001
Light grazing	0.51 ± 0.12	0.44 ± 0.03	0.032 ± 0.011
Heavy grazing	0.43 ± 0.01	0.36 ± 0.02	0.015 ± 0.006
<b>Humic acids</b>			
Exlosures	0.28 ± 0.071	0.33 ± 0.01 <sup>c</sup>	0.082 ± 0.013
Light grazing	0.27 ± 0.017	0.50 ± 0.04 <sup>b</sup>	0.060 ± 0.006
Heavy grazing	0.25 ± 0.044	0.62 ± 0.01 <sup>a</sup>	0.079 ± 0.013
<b>Fulvic acids</b>			
Exlosures	0.27 ± 0.03 <sup>ab†</sup>	0.52 ± 0.04	0.058 ± 0.008 <sup>a</sup>
Light grazing	0.23 ± 0.01 <sup>b</sup>	0.50 ± 0.01	0.051 ± 0.003 <sup>b</sup>
Heavy grazing	0.35 ± 0.06 <sup>a</sup>	0.49 ± 0.03	0.033 ± 0.003 <sup>c</sup>

† Means followed by the same lowercase letter are not significantly different at the 0.05 probability level.

intensity distributions (Fig. 3 and Table 4). The only signal intensity found to be significant among the treatments was the di-O alkyl C region for which LG HA was significantly greater than HG and EX HA. Although there were significantly greater lignin (VSC) concentrations in LG HA compared with EX or HG HAs (Fig. 2), this was not supported by NMR spectra of different treatments. Determining the nature of organic matter is fraught with problems and sometimes different analytical techniques provide information that is contradictory. It is well known that the intensity distribution in CP spectra may not be quantitative due to limitations

explained in the methods and materials section. In this study, we have used NMR spectra to evaluate qualitative differences among humic fractions among the three grazing treatments.

On average, alkyl C accounted for 38% of the total C followed by O-alkyl C (17%), aromatic (15%), carboxyl C (11%), N-alkyl C (11%), O-aryl and di-O alkyl C (both 5%) in the HA spectra. Fulvic acids NMR spectra on the other hand indicated that O-alkyl C accounted for the greatest signal intensity (36%) followed by alkyl C (21%), di-O-alkyl C, aromatic and carboxyl C (10%), N-alkyl C (9%), and O-substituted aromatic C (5%). There were significant differences between HA and FA spectra with respect to alkyl, N-alkyl/methoxyl, O-alkyl, di-O alkyl, and aromatic C regions. Humic acids contained significantly greater alkyl, N-alkyl/methoxyl, and aromatic C than FA, whereas FA contained significantly greater O-alkyl and di-O alkyl C than HA, which confirms observations by others (Preston et al., 1994; Mahieu et al., 1999).

A sharp peak at 30 ppm in the HA spectra from the three treatments indicated the presence of -CH<sub>2</sub> in long-chain aliphatics mainly derived from suberins and plant waxes (Jambu et al., 1991; Preston et al., 1994). These compounds are resistant to decomposition and the relative intensity within this region tends to increase with increasing degradation (Preston, 1996; Baldock et al., 1997; Preston et al., 1998; Lorenz et al., 2000; Ganje-

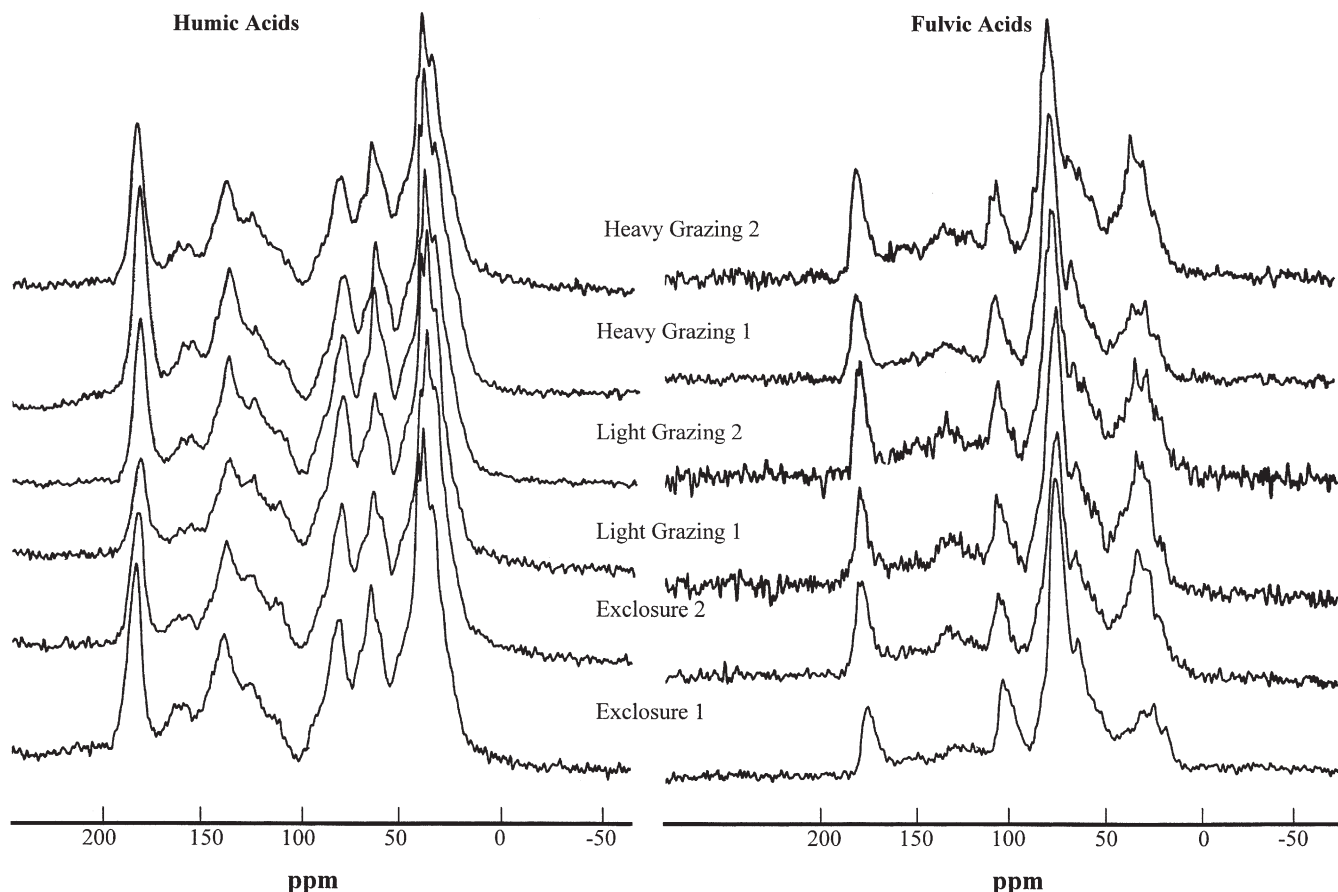


Fig. 3. Carbon-13 cross polarization magic angle spin nuclear magnetic resonance (<sup>13</sup>C CPMAS NMR) spectra of humic and fulvic acid extracted from three grazing treatments at the High Plains Grasslands Research Station, Cheyenne, WY.

**Table 4. The signal intensity distribution (%) of Carbon-13 cross polarization magnetic angle spinning (<sup>13</sup>C CPMAS NMR) spectra of humic (HA) and fulvic acids (FA) extracted from three grazing treatment soils (surface 5 cm).**

Grazing treatments	alkyl C	methoxy/N-alkyl C	O-alkyl C	di-O-alkyl C	aromatic C	phenolic C <sup>†</sup>	carboxyl C
<b>Humic acid</b>							
Exclosures	38.1 ± 3.0	11.2 ± 0.9	16.2 ± 0.5	3.8 ± 0.9 <sup>b</sup>	14.1 ± 1.1	5.1 ± 2.7	10.0 ± 1.9
Light grazing	38.2 ± 0.7	11.4 ± 0.2	19.7 ± 1.7	6.0 ± 0.5 <sup>a</sup>	14.3 ± 2.5	4.9 ± 1.0	9.3 ± 2.8
Heavy grazing	38.4 ± 1.1	8.9 ± 0.1	13.7 ± 1.4	4.2 ± 0.4 <sup>b</sup>	15.9 ± 1.2	5.5 ± 0.8	12.3 ± 0.7
<b>Fulvic acid</b>							
Exclosures	19.8 ± 3.7	9.0 ± 0.6	38.6 ± 6.2	10.9 ± 3.3	9.2 ± 0.9	4.9 ± 1.4	9.6 ± 1.4
Light grazing	22.4 ± 0.3	8.2 ± 0.8	33.6 ± 1.1	9.7 ± 0.3	10.4 ± 0.7	6.0 ± 0.2	9.3 ± 2.3
Heavy grazing	21.3 ± 3.2	8.6 ± 0.9	33.8 ± 7.4	9.6 ± 0.5	9.4 ± 1.9	5.0 ± 0.1	11.0 ± 1.4
Average HA	38.2 ± 1.5 <sup>‡</sup>	10.5 ± 1.3 <sup>a</sup>	16.5 ± 2.9 <sup>b</sup>	4.6 ± 1.1 <sup>b</sup>	14.8 ± 1.6 <sup>a</sup>	5.1 ± 1.4	10.5 ± 2.1
Average FA	21.1 ± 2.5 <sup>b</sup>	8.6 ± 0.7 <sup>b</sup>	35.3 ± 5.0 <sup>a</sup>	10.1 ± 1.6 <sup>a</sup>	9.7 ± 1.2 <sup>b</sup>	5.3 ± 0.8	10.0 ± 1.6

<sup>†</sup> O-substituted aromatic C including phenolics and aryl ethers.

<sup>‡</sup> Means followed by the same lowercase letter are not significantly different at the 0.05 probability level.

gunte et al., 2004). The alkyl C region in FA is not only small but also much broader than in HA spectra, suggesting short chain lengths and diversity of structures. The peak at 57 ppm, as discussed later for the DD spectra, is likely due to a combination of methoxyl C of lignin and N-alkyl C of protein.

Structures producing the O-alkyl signal at 74 ppm can include the ring C of carbohydrates, lignin side-chains, and structures derived from modified carbohydrates (Preston, 1996; Leifeld et al., 2002). For HA and FA, the latter are likely the most prominent. The yield of recognizable monosaccharides from organic matter is typically much lower than would be suggested by the proportion of O-alkyl intensity (Preston, 1996; Leifeld et al., 2002), and the HA O-alkyl region lacks the sharp features at 62 (C2) and 89 ppm (C4) of unmodified polysaccharides as found, for example, in fresh litter (Lorenz et al., 2000) and poorly decomposed forest floor (Kögel et al., 1988). The contribution of lignin side-chains was low based on the low O-aryl intensity. Similarly, the di-O alkyl C region likely represents anomeric C of carbohydrates within the HA structures as well as modified structures derived from carbohydrates. The typically sharp di-O-alkyl signal of carbohydrates is poorly resolved and partly superimposed on the broad aromatic C region. However, significantly greater di-O alkyl C in the LG HA (consistent with the nonsignificant trend for O-alkyl C) suggests the presence of greater amounts of carbohydrates or carbohydrate-derived structures in that treatment compared with the HA from other treatments.

The aromatic and O-substituted aromatic C regions of the HA spectra are consistent with structures derived from input and from the grazing treatment modification of lignin precursors (Chefetz et al., 2002; Leifeld et al., 2002). The HA spectra (Fig. 3) have weak signals at 119 ppm, characteristic of protonated aromatic C with the maximum intensity at 130 ppm, due to C-substituted aromatic C. The latter increasingly dominates as lignin is degraded in soil (Kögel-Knabner et al., 1991). The O-aryl region is generally broad and weak, as the characteristic signature of intact guaiacyl-dominated lignin. A peak at 147 ppm with a shoulder at 153 ppm (Preston et al., 1998; Chefetz et al., 2002) is reduced and modified due to increased lignin degradation and leaching in aerobic soils (Baldock et al., 1992; Preston et al., 1994).

Signal intensity between 160 and 190 ppm is largely from carboxyl C, including organic acids, esters, and amides. Degradation of lignin, and its incorporation into humic structures reduce the proportion of aromatic C that can be identified as lignin oxidation products (Kögel et al., 1988). However, we found that the much higher yields of lignin products from the LG HAs (which were reproducible) were not reflected in any significant differences in the NMR intensities or spectral features. The significantly greater alkyl, aromatic, and N-alkyl C in HA suggests that these humic substances are more stable compounds than FA (Preston and Blackwell, 1985; Mahieu et al., 1999).

Pyrogenic C could be another possible source for aromatic intensity in the HAs. Char produced from thermal alteration of organic matter has a broad aryl peak at 126 to 130 ppm (Skjemstad et al., 2002), which has also been found in HAs (Haumaier and Zech, 1995). Char is generally under represented in CP spectra because of low C protonation, so that assessing its possible contribution would require more detailed investigations (Skjemstad et al., 2002).

The DD spectra (Fig. 4) extend the results from the normal CP spectra. These results indicate the three grazing treatment HA are similar, as are those for the three grazing treatment FA. For the HA, the residual intensity in the alkyl region is due mainly to C with some mobility at the molecular level (i.e., methyl C and the more mobile fraction of long-chain CH<sub>2</sub>). The peak at 30 ppm is particular characteristic of long chains preserved from cutin and suberin. A sharp peak at 56 ppm is typical of methoxyl C from lignin, but the large loss of intensity from this region indicates a substantial contribution from protein. The peak for non-protonated aromatic C occurs at 130 ppm, with the phenolic peak more clearly seen with maxima at 148 and 152 ppm. A large carboxyl signal is consistent with carboxyl, amide, and ester C from both proteins and a variety of fatty acids, including those from cutin and suberin. Thus the DD spectra are consistent with the presence of proteins, lipids, and lignin structures as was found from CuO analysis.

The lower quality of the FA DD spectra is due to reduced proportions of mobile alkyl C and nonprotonated aromatic C. Residual signals around 73 ppm are due to the difficulty of adjusting the DD time with a dominant O-alkyl signal. The alkyl region is weaker and poorly re-



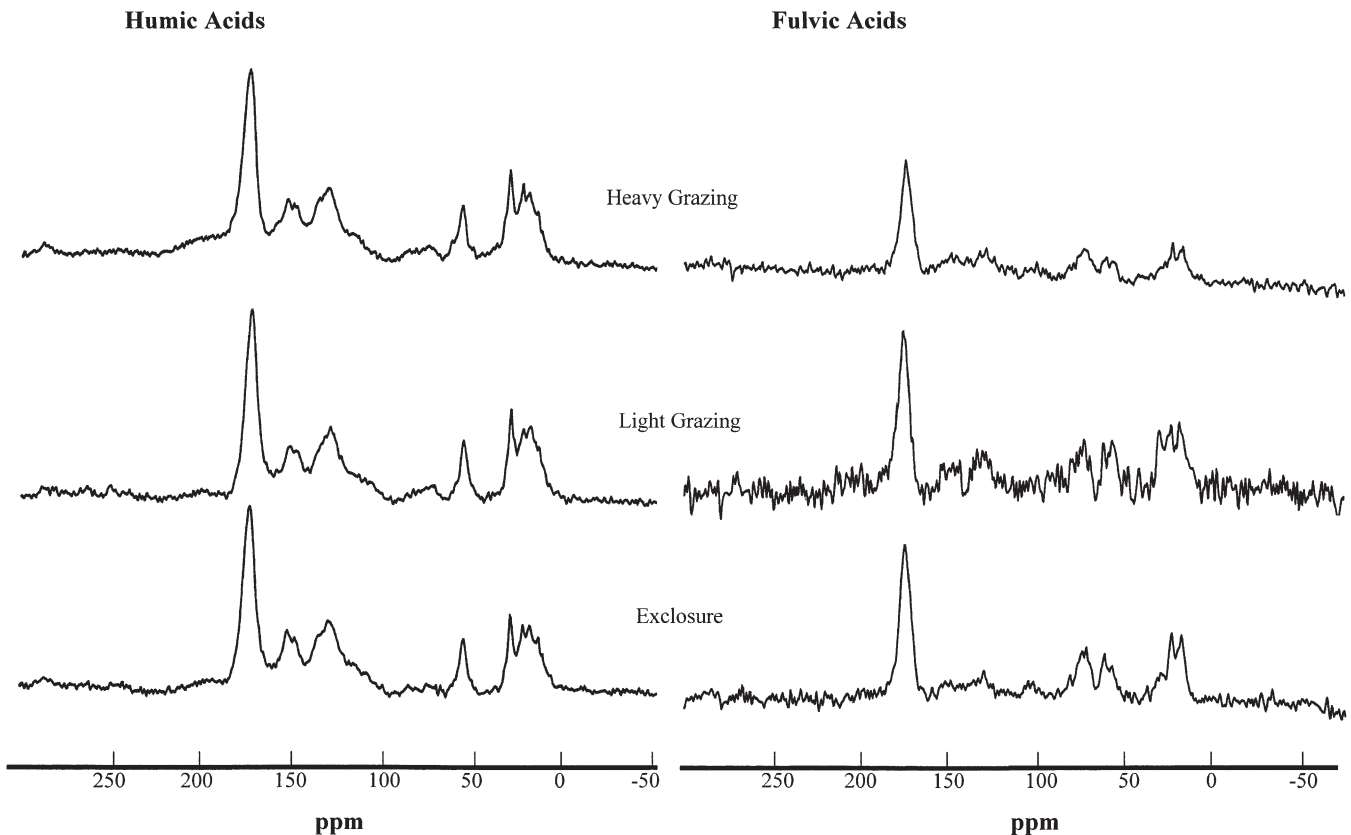


Fig. 4. Dipolar dephasing carbon-13 cross polarization magic angle spin nuclear magnetic resonance ( $^{13}\text{C}$  CPMAS NMR) spectra of humic and fulvic acids extracted from three grazing treatments (one of each replicate) at the High Plains Grasslands Research Station, Cheyenne, WY.

solved, consistent with shorter or more highly branched alkyl chains (Preston and Blackwell, 1985). Similarly, aromatic and phenolic signals for FA are also weaker than for HA, and the only strong signal is in the carboxyl region.

## CONCLUSIONS

Results of this study demonstrate that there are beneficial effects of light grazing compared with heavy grazing and nongrazing with respect to increased SOC and N contents. Despite recent drought conditions our results agree with past and present findings of studies conducted by Schuman and others on the same research sites. Stimulation of aboveground vegetation growth, better incorporation of aboveground plant residues, and increased decomposition rates of plant residues within the LG treatments resulted in increased SOM contents. The CuO oxidation analysis of soil and humic substances indicated that lignin in soil of all treatments was comprised primarily of V and S groups. The dominant impact of vegetation on SOM composition was demonstrated by presence of all three phenylpropanoid moieties (V, S, and C units), which are contributed by angiosperm (grasses) nonwoody residues. The NMR spectroscopy indicated qualitative differences between HA and FAs extracted from the three grazing treatments. While alkyl C comprised a major proportion of HAs, O-alkyl C constituted a greater proportion of FAs C. The CuO oxidation and NMR spectroscopy studies highlighted the problem of

interpreting different results commonly encountered in determining the nature of SOM. Grazing intensity appears to increase the breakdown of lignin and, in particular, that of S units that are more susceptible to decomposition than V or C groups. Enhanced lignin degradation in the grazed treatments is probably due to partial decomposition of plant material by livestock, as well as the resultant defecated material that is more susceptible to decomposition, physical breakdown of plant residues due to trampling, and increased contact with soil or partial burying of plant residues due to hoof action. Based on the results of this study it may be concluded that light grazing system would be the most sustainable grazing management system for northern mixed-grass prairies.

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