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## **Soil organic matter fractions of no-tilled and tilled soils and their reactivity with herbicides**

Gordon Kim Stearman

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To the Graduate Council:

I am submitting herewith a dissertation written by Gordon Kim Stearman entitled "Soil organic matter fractions of no-tilled and tilled soils and their reactivity with herbicides." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Plant, Soil and Environmental Sciences.

Russel J. Lewis, Major Professor

We have read this dissertation and recommend its acceptance:

Jeff Wolt, Don Tyler, Milton Lietzke

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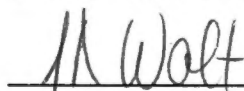
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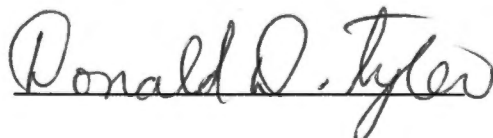
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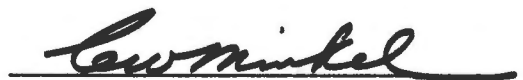
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SOIL ORGANIC MATTER FRACTIONS OF NO-TILLED AND TILLED SOILS  
AND THEIR REACTIVITY WITH HERBICIDES

A Dissertation  
Presented for the  
Doctor of Philosophy  
Degree  
The University of Tennessee, Knoxville

Gordon Kim Stearman  
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## ABSTRACT

Properties of soil humic fractions were determined on surface and 7.5-15.0 cm soil samples of continuously (7-year) no-tilled and tilled cotton, corn, and soybean plots in West Tennessee. Soil humic and fulvic acid were extracted by standard methods and the humic acid was characterized by  $^{13}\text{C}$ -NMR spectroscopy, titration of total acidity and carboxyl groups, and infrared and elemental analysis. Humic acid NMR spectra were divided into six regions (0-40, 40-62, 62-105, 105-150, 150-170, 170-190 ppm) and peak areas were compared. Humic acid composition differed by depth and crop. Small differences were observed between tillage systems. Humic acid aliphatic and aromatic carbons ranged from 48 to 65% and 25 to 40% of total peak area, respectively. The humic acids extracted from soils with larger amounts of carbon (surface no-tilled treatments) had larger aliphatic to aromatic ratios, indicating less decomposed organic matter. Carboxyl groups of the humic acids ranged from 9 to 13% and samples from tilled soil had slightly greater amounts of carboxyl and aromatic groups. Carboxyl group determinations by  $^{13}\text{C}$ -NMR, compared more closely with total acidity determinations by titration than with carboxyl determinations by titration. All infrared spectra were similar. Elemental composition of humic acid averaged C, 52.7%; H, 5.6%; N, 4.8%; and O, 36.9%.





A greenhouse herbicide bioassay was conducted on a cotton-cropped tilled soil and three no-tilled soils, where cotton was cropped with vetch, rye, and crimson clover cover crops. Total C ranged from 9.9 g kg<sup>-1</sup> in the tilled soil to 13.5, 16.6, and 23.5 g kg<sup>-1</sup> in the no-tilled soil with rye, vetch, and crimson clover covers, respectively. The tilled soil had the largest fraction of extractable C and fulvic acid, relative to total soil C. Sorghum growth was measured to indicate soil effects on activity of herbicides, metribuzin and oxyfluorfen. Herbicide activity was inversely related to soil C, extractable C, carboxyl groups of humic acid, and fulvic acid C of soils. Fulvic acid C best predicted herbicide phytotoxicity. Results indicated that C was more reactive in tilled than in no-tilled soils.



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## I. INTRODUCTION

No-till agriculture has become a popular system of farming in Tennessee. No-till farming is possible because of better weed control with herbicides and planters that operate in almost any kind of soil cover. Farmers practicing no-till techniques may save money because of reduced fuel, labor, and machinery costs. Topsoil is conserved since the soil is covered and protected from wind and water erosion. No-tilled soils are not plowed or cultivated, but some disturbance of top soil occurs when seeds are drill planted into the crop residue. Long term no-tilled soils differ from conventionally tilled soils in chemical, physical, and biological properties. Differences between tillage systems can be accounted for by the following no-till characteristics: (1) soil is not moved, (2) fertilizer is generally applied to the surface, and (3) the surface mulch increases water infiltration and reduces the evaporation rate from the soil surface. Major differences occur in the surface layer of the soil profile.

Organic matter decomposes on top of soil in no-till systems, opposed to more rapid decomposition due to uniform mixing of crop residues in tilled operations. The increased rate of decomposition in tilled soils is due to a high soil temperature and to aeration which increases oxidation. Also, the mixing of organic residues allows for greater surface reactions and a more uniform distribution of organic matter in the plow layer.

The distribution of organic matter in the soil profile and slower rates of organic matter decomposition cause the no-till system to develop two distinct characteristics: (1) higher concentrations of organic matter in the surface layer, and (2) a concentrated humus layer below the crop residue. These characteristics contrast with the more uniform distribution and lower soil organic matter in tilled systems.

Although soil organic matter generally comprises a relatively small portion of the soil, (0.5-6.0%), its importance to agricultural systems cannot be overstated. Organic matter has been termed the "life blood" of soils. It has a tremendous impact upon the chemical, physical, and biological properties of the soil. Organic matter is usually composed mainly of carbon (50-60%), oxygen (30-40%), hydrogen (2-4%), nitrogen (3-6%), and phosphorus and sulfur (0.1-1.5%). Nitrogen, P, and S are essential macronutrients for plant growth and are continually supplied as organic matter decomposes. Organic matter increases water holding capacity of soils. The cation exchange capacity of soils increases with larger amounts of soil organic matter, allowing more nutrient retention. The physical properties of soils are generally improved by increasing the soil organic matter content, which causes soil clay, silt, and sand particles to aggregate, enhancing soil structural properties and improving root channelling and plant growth. Bulk density of soil is also decreased with increased amounts of organic matter, allowing for



easier penetration of roots, thus enabling them to find additional sources of water and nutrients. Finally, studies have shown that from 50-98% of clay particles are coated with organic matter, (Greenland, 1965). Therefore, soil organic matter is an integral and beneficial component of soil because it dominates surfaces, increases water holding capacity, adds nutrients, and improves soil structure. Humus is the most active fraction of the soil organic matter. For agricultural soils, it is defined as the well-decayed, stabilized portion of the soil organic matter. Humus consists of fulvic acid (FA), soluble in both alkali and acid, humic acid (HA), soluble in alkali but insoluble in acid, and humin, insoluble in both alkali and acid.

In soils that have been under no-till for several years the dark humus layer, although often less than one centimeter in depth, can be differentiated. It is important to characterize soil organic matter fractions of soils, since inputs such as herbicides and fertilizers react with functional groups of soil organic matter. Therefore, characterization of the topmost layers in various tillage systems is necessary to determine differences in composition and reactive sites.

It is preferable to compare no-till and conventionally tilled systems that have received the same management schemes for several years. Soil organic matter properties can be contrasted, with tillage being the main variable. Differences in soil properties between the two tillage systems can be quantitatively



and qualitatively enumerated. A comprehensive understanding of humus functional groups as they change with tillage operations and soil inputs may result in better use of herbicides and fertilizers. This leads to the theoretical framework of this study, which is based on the belief that quality as well as quantity of soil organic matter is important in herbicide-humus interactions. Quality of soil organic matter is defined as the proportions of carbon, nitrogen, hydrogen, and functional groups present, as well as ratios of HA and FA and aromaticity of HA.

Soil organic matter is an important soil property controlling herbicide phytotoxicity, (Upchurch et al., 1966, and Peter and Weber, 1985a,b,c). Therefore, if both the quantity and quality of soil organic matter are measured, their relative importance in herbicide control may be evaluated. The theory will be tested by fractionation and characterization of humus and relating these properties to herbicide activity. Organic matter fractions, specifically, HA and FA, have not been characterized in different tillage systems, nor have the functional group contents of HA been directly related with herbicide phytotoxicity. A goal of this study is to determine if the increased surface soil organic matter in the no-tilled system is different in reactivity and chemical composition from that of tilled systems.

The primary objectives of this research project are (1) to extract soil organic matter fractions and characterize HA of no-tilled and tilled cotton, corn, and soybean plots that have the

same seven-year management scheme and (2), to demonstrate how soil organic matter fractions affect herbicide activity.

## II. LITERATURE REVIEW

### A. TILLAGE AND SOIL ORGANIC MATTER

Five and ten year tillage studies with corn have indicated that soil organic matter significantly increases in no-tilled soils compared to conventionally tilled soils (Blevins et al., 1977; 1983). Soil organic matter in no-tilled soil is approximately twice that of tilled soil receiving the same inputs (Phillips and Phillips, 1984). It has been clearly established that plowing increases rates of organic matter decomposition in soils (Giddens, 1957).

Not only is the increased content of organic matter an important consideration in soil management decisions, but also the characterization of soil organic matter may result in a more comprehensive understanding of reactive groups and their effects on soil inputs. Organic matter is generally the most reactive portion of soils (Stevenson, 1982; Schnitzer and Khan, 1978). Due to this reactivity, it is closely correlated with herbicide phytotoxicity (Upchurch et al., 1966; Peter and Weber, 1985a,b,c). Although it is known that tillage decreases the concentration of organic matter, investigators have not published characterization of soil organic matter based on tillage differences. It is natural to assume tilled soil organic matter is thoroughly mixed and more oxidized, and therefore, more reactive than no-tilled soil organic



matter. The possibility of significant differences occurring in soil organic matter between tilled and no-tilled soils poses the interesting proposition that the quality of humus might influence soil management decisions in different tillage systems.

## B. SOIL ORGANIC MATTER: DEFINITION, EXTRACTION, AND CHARACTERIZATION

### 1. Humus: Definition and Historical Perspective

"Soil organic matter chemistry is undoubtedly the least understood field of soil science, and the most perplexing" (Stevenson, 1972). Humus has attracted the attention of scientists for two hundred years. In fact, the first isolation of humic substances from soil appears to have been made by Achard in 1786, as noted by Stevenson (1982). By extracting peat with alkali, he obtained a dark amorphous precipitate upon acidification. This alkali-soluble, acid-insoluble material later came to be known as humic acid (Stevenson, 1982). Humus is defined as "the organic portion of soil, brown or black in color, consisting of partially or wholly decayed plant and animal matter, that provides nutrients to plants and increases the ability of soil to retain water" (Aiken et al., 1985, p.651). Humic substances are a broad category of naturally occurring biogenic, heterogeneous organic substances that can generally be characterized as being yellow to black in color and having high molecular weight (500 to



1,000,000; Steelink, 1985). Humic substances are not pure compounds, rather they are a heterogeneous mixture of many compounds with generally similar chemical properties. This heterogeneity places important constraints on humus characterization methods. Soil humus consists of polymerized phenolic units with linked peptides, amino acids, and other organic structures in various arrangements. The humus structure results in an unusually high density of reactive functional groups, such as carboxyl and hydroxyl groups. Many properties of HA are due to the nature of the functional groups.

As humus is not a unique chemical entity, it must be defined operationally. Based on solubilities, humus is divided into three major fractions, HA, FA, and humin. All of these materials are generally regarded as polymers of aromatic compounds containing aliphatic side chains though the extent of aromaticity and degree of cross-linking have only recently been established through the use of  $^{13}\text{C}$ -NMR spectroscopy (Schnitzer and Preston, 1986; 1987; Hatcher et al., 1981a,b; 1983; Wilson et al., 1986). Differences among the three categories of humic materials appear to be at least partly attributable to the degree of polymerization and thus molecular weight. These differences were evidenced by a more highly acidic and oxidized FA compared to HA from the same sample (Stevenson, 1982; Schnitzer and Khan, 1978).

Humus is highly colloidal and amorphous rather than crystalline, with cation exchange capacities reportedly between





150 to 300  $\text{cmol}(+)\text{kg}^{-1}$ . Humus absorbs 80 to 90% of its weight in water while clay adsorbs only 15 or 20%. Humus generally is more reactive and retains more water than does clay.

Organic colloids have a strongly pH-dependent charge. In a typical soil, the contribution of soil organic matter to the cation exchange capacity may range from 50% at pH 7.0 to only 25% at pH 5.0 (Stevenson, 1972). The negatively charged binding sites on soil organic matter result predominately from dissociation of hydrogen from carboxyl and hydroxyl groups. Therefore, pH dictates the degree of ionization of acidic groups and the number of sites available for binding (Stevenson and Fitch, 1986). As the pH increases, hydrogen dissociates and functional groups develop negative charges, adsorbing cations to replace the ionized hydrogen.

## 2. Humus Fractionation and Extraction

Schnitzer, at Ottawa, Canada and Stevenson, at the University of Illinois, have attempted the complex task of characterizing humus fractions in soil. Much of the literature is devoted to fractionation schemes, rather than enumerating HA differences based on soil, crop, management, and climatic variables. Only recently (Malcolm, 1985, personal communication), has there been an effort to standardize extraction procedures for humic substances by the recently formed (1983) International Humic Substances Society.



The characterization of humus based upon the previously mentioned alkali-acid fractionation scheme has been accepted by many researchers. However, a wide array of alkali extractants and concentrations have been used, (Levesque and Schnitzer, 1966; Hayes, 1985). Most researchers have used sodium hydroxide or sodium pyrophosphate as the alkali extractant. Hydrochloric acid is commonly used to precipitate HA. Procedures recommended by the International Humic Substances Society are now being used by many researchers in an effort to uniformly extract HA and FA (Malcolm, 1985, personal communication). These standardized procedures include acid pretreatment, to eliminate carbonates, extraction with base (NaOH), acidification with HCL to precipitate HA, purification with KOH-KCL solution, purification with HCL-HF solution, dialysis to eliminate salts, and lyophilysis to dry the HA in powder form.

Calderoni and Schnitzer (1984) extracted HA and FA from six paleosols and reported 8.8 to 32.0% of total carbon extracted as purified HA. The ash content of purified HA varied from 0 to 3.4%. Compared to HA, yields of FA were small.

### 3. Purification of Humic Acids

In earlier work many humic and fulvic acids contained significant amounts of ash and therefore characterization was limited by impurities contained within the sample. Purification of both HA and FA fractions has been recommended in order to

accurately characterize functional groups (Malcolm, 1976). Extracting HA with KOH-KCl solution has been recommended to eliminate clays, which tightly bind humus. Repeated washing with HCL-HF solution eliminates any remaining silicates. Washing with deionized water to eliminate chloride ions from the fractions is commonly used (Schnitzer, 1982). Dialysis has also been recommended, but extensive dialysis has caused some loss of aromaticity of humic substances in certain cases (Wilson and Goh, 1977).

#### 4. Characterization of Humic Acids

Once fractionated and purified, HA and FA are characterized by various methods, including nuclear magnetic resonance, infrared spectroscopy, elemental composition, and titration of functional groups. The following sections examine characterization techniques of humic substances with major emphasis on the relatively new, powerful spectroscopic method, nuclear magnetic resonance (NMR).

##### a. Elemental Composition of Humic Acid

The elemental composition of HA indicates purity and provides information concerning the molecular formula. Low levels of carbon in the sample indicate ash or moisture is present. Elemental composition combined with NMR spectra or titration of functional groups can also aid in the formulation of a hypothetical



structure of humic substances. Atom ratios (H/C, O/C, and N/C) are helpful in proposing chemical structures for humic and fulvic acids. HA elemental composition is usually in the range carbon, 53.8 to 58.7%; hydrogen, 3.2 to 6.2%; oxygen, 32.8 to 38.3%; nitrogen, 0.8 to 4.3%; and sulfur, 0.1 to 1.5% (Schnitzer and Khan, 1978). The O/C ratio for soil HAs is usually about 0.5, while the H/C ratio is approximately 1.0 (Steelink, 1985). Lower O/C and H/C ratios indicate more aromatic compounds present, while higher values indicate an abundance of aliphatic structures. High nitrogen and hydrogen contents have been attributed to a predominantly aliphatic nature of humic substances and to the contribution of nitrogen-rich proteinaceous materials (Hatcher et al., 1983). This elemental composition is not unique to humic substances, but is characteristic of many other organic compounds. Clearly, humic substances are a complex mixture and no two are exact replicas. Therefore, elemental composition does not define humic substances. It does, however, set boundaries for probable chemical configurations, which can be confirmed by magnetic resonance spectra and other analytical properties.

The most serious problem in the study of humic substances is the lack of reproducibility of analytical results. Elemental composition varies between samples, depending on extraction and fractionation procedures. There are cases where the same authors have used the same source and the same extraction procedure and

have obtained significantly different elemental concentration (Steelink, 1985).

b. Potentiometric Determination of Humic Acid Functional Groups

Griffith and Schnitzer (1975) Chen et al. (1978) and Ortiz de Serra and Schnitzer (1973) have characterized humus fractions of soils from widely different geographic locations, and found that functional group content, as determined by titrations, varies between HA and FA. Fulvic acid generally has a higher concentration of functional groups, from 900 to 1400  $\text{cmol kg}^{-1}$ , while HA functional group content ranges from 500 to 870  $\text{cmol kg}^{-1}$  (Stevenson, 1972). Humic acids of soils from different regions of the world have similar contents of functional groups, (Griffith and Schnitzer, 1975; Chen et al., 1978; Ortiz de Serra and Schnitzer; 1973). Stevenson (1982) has also reported similar ranges of functional group content of HA from different soils.

There have been conflicting opinions expressed concerning the validity and accuracy of functional group concentrations based on potentiometric methods (Perdue, 1985). Total acidity determination by the barium hydroxide method (Schnitzer and Gupta, 1965) and carboxyl group determination by the calcium acetate method (Wright and Schnitzer, 1959) have frequently been used to determine functional groups. Schnitzer and Gupta (1965), checked the accuracy of total acidity determination by discontinuous titrations and found good agreement. Wright and

Schnitzer (1960) checked the accuracy of calcium acetate for determining carboxyl groups and found agreement between four independent methods. Phenolic hydroxyl groups have been computed by difference between the total acidity and carboxyl group titrations. There have been discrepancies between NMR computations of phenolic hydroxyl groups (usually very small) and the determinations by difference. Schnitzer and Preston (1986) attempted to explain the difference by demonstrating that many phenolic carbon signals occur in the 160-175 ppm region but are being computed as part of the carboxyl region. Computation of carboxyl groups from NMR spectra were closer to total acidity values than to carboxyl group values by titration. This could indicate that carboxyl groups might be overestimated by the NMR data due to overlap of the phenolic carbon group with the carboxyl group. Thurman and Malcolm (1983) reported values for carboxyl and phenolic-hydroxyl groups by titration and solid and liquid state  $^{13}\text{C}$ -NMR that were in close agreement for a HA and a FA. However, several researchers have reported problems when comparing functional group by titration with NMR spectral integration (Perdue et al., 1980, and Perdue, 1984). Perdue (1985) stated that because humic substances represent a complex mixture of organic compounds the acidity of humic substances can only be attributed to a complex mixture of nonidentical functional groups. He further stated that since potentiometric methods of functional group analysis are based on  $\text{pK}_a$  values of the functional



groups they could only yield operationally defined estimates of the concentration of a particular class of acidic functional groups. He concluded that only total acidity, as determined by the barium hydroxide method, appeared to be a potentially accurate potentiometric method of analysis. "For this reason, an increased reliance on spectroscopic methods of functional groups analysis is recommended", (Perdue, 1985).

#### c. Infrared Adsorption of Humic Acids

Most infrared studies with HA have used the dried solid pressed-pellet technique. Generally, due to the complexity of the mixture, the IR spectra consists of broad bands. Where there have been differences in absorbance, the overall similarity in the spectra of humic substance of diverse origin has been more noteworthy than differences (MacCarthy and Rice, 1985). However, the humic substances might not have similar structures due to lack of resolution of spectral bands. Therefore, application of IR spectroscopy has been limited in the quest to elucidate either detailed or gross structural differences between humic substances.

#### d. Nuclear Magnetic Resonance Spectroscopy

##### i. History and Theory

Nuclear magnetic resonance (NMR) spectroscopy has become the method of choice for determining functional group content in



humic substances. Although NMR has only been effective since 1976 for determining functionality of humic substances, it has provided more information than all other methods combined (Wershaw, 1985).

To properly interpret NMR spectra, NMR theory must be understood. Any attempt to quantify NMR spectra must account for nuclear couplings and relaxation times. The term NMR describes observation of resonating nuclei in a magnetic field. Resonance in this application is the absorption of energy.

NMR came into being accidentally in the mid 1940's when two groups of workers simultaneously discovered resonant absorption in bulk matter (Levy et al., 1980). It had been known for several decades that certain nuclei have a spinning electric charge and NMR at this stage was purely an experimental method to determine nuclear magnetic momentum of nuclei. Around 1949, it was discovered that nuclei absorb energy (resonate) at different frequencies depending on their chemical environment. Therefore, it became possible to determine the structure surrounding a nucleus and NMR was born. The first commercial NMR spectrometer was built in 1953.

Briefly, the theory of NMR spectroscopy is that certain nuclei (not all) have spin angular momentum. Only nuclei with odd numbers of protons and neutrons have a nuclear magnetic moment. Because nuclei, such as carbon 12, have 6 protons and 6 neutrons that are paired, the nuclear magnetic moment is zero. When placed



in a magnetic field the nuclei may have a positive or negative spin state which align themselves either parallel or antiparallel to the field vector, with a slight energy difference between spin states. Another magnetic field is applied perpendicular to the steady field, while frequency is varied until nuclei resonant or absorb energy from the oscillating field and undergo transition to the higher state. Detection of energy absorption is recorded and the range of frequencies is compared to a known reference compound. The difference in resonance from the standard is termed the chemical shift and is defined as the observed chemical shift from the chemical shift of the standard. Resonance is quantified and the signal output becomes the NMR spectra. Consequently, it is possible to identify a particular functional group from the frequency required to induce resonance. Functional groups can then be quantified based on peak area of the signal (Stothers, 1972).

Basic components of an NMR spectrometer include (1) a magnet, which produces the splitting of energy levels, (2) a source of radiation, which transmits the appropriate radio frequency for the particular field, (3) a receiver coil to detect absorption of energy, and (4) a recorder to plot the absorption curve or signal.

Two nuclei of importance to this discussion are  $^1\text{H}$  and  $^{13}\text{C}$ . Until the 1970's most NMR work was conducted using protons, ( $^1\text{H}$ ), because of the prohibitive amount of time required to obtain  $^{13}\text{C}$  spectra. Protons were easily detected because of their natural

abundance and their large gyromagnetic ratio. The gyromagnetic ratio is the proportionality constant between the magnetic moment, and the spin number. The proton has four times the magnetic moment of the  $^{13}\text{C}$  atom and this along with its abundance makes it easier to detect than the  $^{13}\text{C}$  nuclei. The  $^{13}\text{C}$  isotope naturally occurs at only 1.1%. It was not until the late 1960's that NMR underwent a revolutionary change in technique, moving from continuous wave to pulsed excitation. This was possible because of major advances in computer technology and the use of Fourier transformation, which transforms time domain into frequency domain by using algorithms.

In the 1970's NMR spectrometers were built with digital computers and pulse excitation to utilize Fourier transformation. This allowed the carbon nuclei to be scanned and functional groups to be computed semiquantitatively. The application of  $^{13}\text{C}$ -NMR (as well as other isotopes with spin) became feasible and enabled formulation of chemical structures and the ability to quantify carbon groups (Shaw, 1984).

## ii. Methodology: Proton, Solid and Liquid State Carbon-13 and Methylation Analysis

Proton-NMR and  $^{13}\text{C}$ -NMR have become powerful tools to elucidate gross chemical structures and functional groups of humic substances. Generally, there has been agreement among researchers as to the basic character and types of functional

groups present. Some differences have been expressed as to aromatic content of humic substances (Wilson and Goh, 1981; Ruggiero et al., 1979a). However, there is widespread agreement that humic substances consist of a mixture of many different compounds arranged in a variety of similar structures.

Several types of NMR studies have been reported including proton-NMR, liquid and solid state  $^{13}\text{C}$ -NMR, and methylation followed by  $^{13}\text{C}$ -NMR. Each of the methods has a place in defining functional groups and gross chemical structure of humic substances. However, both liquid and solid state  $^{13}\text{C}$ -NMR have recently been the method of choice (Schnitzer and Preston, 1987; Wilson et al., 1987; Preston et al., 1987). The purpose of this section is to compare NMR methods as to spectral range, resolution, and to demonstrate applications of each.

Early NMR work with humic substances was performed using  $^1\text{H}$ -NMR (Schnitzer and Barton, 1963; Schnitzer and Skinner, 1968). According to Wershaw (1985) H. Oka and coworkers, in 1969, were the first to use proton NMR on an underivitized HA. Results were useful only in a qualitative sense because of poor quality of spectra. Proton-NMR examines protons, which are abundant and have a large gyromagnetic ratio. However, the spectra occurs over a much narrower range (0-20 ppm) than do the  $^{13}\text{C}$  spectra (0-220 ppm). There have been problems reported with exchangeable protons in certain peak areas (Wilson et al., 1978; Ruggiero et al., 1979b; 1981). The main application of  $^1\text{H}$ -NMR is to estimate





proton aromaticity of the sample by integrating the 6-8 ppm region and comparing it with the rest of the spectra, and secondly to measure the amount of branching in the aliphatic structures.

The first definitive report using  $^{13}\text{C}$ -NMR was by Vila et al. (1976). They divided the spectra into three broad regions and integrated peak areas. Since then several groups of researchers have provided detailed structural and functional group information utilizing  $^{13}\text{C}$ -NMR on both terrestrial and marine humic substances, (Schnitzer and Preston, 1986; 1987; Preston and Schnitzer, 1984; Hatcher et al., 1980a,b; 1981a,b; 1983; Wilson et al., 1983; 1986). Carbon-13 NMR has the advantage of directly measuring the carbon atoms in humic substances. The larger chemical shift range of  $^{13}\text{C}$ -NMR allows for separation of peaks, so that detailed structural determination is possible. The disadvantages of  $^{13}\text{C}$ -NMR, low abundance of  $^{13}\text{C}$  and low sensitivity of carbon relative to protons, have largely been overcome through the use of Fourier transformation. A disadvantage of liquid state  $^{13}\text{C}$ -NMR is the possibility that artifacts might be introduced from the solvent (usually NaOD, deuterated sodium hydroxide) used for dissolving the substance, although this has not been reported to the knowledge of the author.

Solid state  $^{13}\text{C}$ -NMR has the advantage of examining the carbon atoms, without possible artifacts present due to the solvent solution. However, with solid state  $^{13}\text{C}$ -NMR there is extreme line broadening due to static dipolar interaction of  $^{13}\text{C}$



atoms with adjacent protons. In solid samples nuclei are fixed in space and  $^{13}\text{C}$ - $^1\text{H}$  vectors have all possible orientations. In liquid samples this does not occur because the rapid movement of molecules results in an averaging of dipolar interactions to zero (Wershaw, 1985). The dipolar coupling in solid state can be eliminated by irradiating the protons with a strong signal at their resonant frequency. This double resonance decoupling is similar to the double resonance technique used to remove similar  $^{13}\text{C}$ - $^1\text{H}$  coupling in liquid samples except much higher energy levels are required. Also, solid state has chemical shift anisotropy. Anisotropy broadening arises from the fact that the chemical shift of a given  $^{13}\text{C}$  atom in a molecule will vary to some extent as a function of its orientation in the magnetic field. In liquid samples this variation is averaged out to a single isotropic value. The effect can be eliminated in solid state by spinning the sample at the so called magic angle ( $54.7^\circ$ ).

Hatcher et al. (1983) reviewed solid state  $^{13}\text{C}$ -NMR, and concluded that peaks are poorly resolved and that there is substantial overlap of some peaks. However, they quantified carbon groups based on peak area. Preston and Ripmeester (1982) pointed out that aromatic peaks in both liquid and solid state spectra may be reduced in intensity by line broadening caused by coordination of paramagnetic ions to aromatic or phenolic structures. Consequently, they have been more cautious than



Hatcher and coworkers in using peak areas as a quantitative measure of functional group content.

Preston et al. (1987) reported solid state  $^{13}\text{C}$ -NMR spectra from organic soils that showed cultivated sites had less carbohydrates and more lipid and methoxyl carbon than did virgin sites. However, results might be different on soils with less organic matter.

Wilson et al. (1983), examined aquatic HAs by  $^1\text{H}$ -NMR and liquid and solid state  $^{13}\text{C}$ -NMR. They found agreement between the three NMR techniques used on the same sample. Hatcher et al. (1980a,b; 1981a; 1983) have examined humic substances from both aquatic and terrestrial origins by  $^1\text{H}$ -NMR and liquid and solid state  $^{13}\text{C}$ -NMR and found that terrestrial and aquatic HAs were similar except terrestrial HA had more aromatic C than aquatic HA (20-35% versus 10-15%). Stuermer and Payne (1976) also found aromatic signals in  $^{13}\text{C}$ -NMR spectra of terrestrial FA but not in spectra of FA isolated from sea water. This is a good indication that precursors such as lignin from plants in soil and the dominantly aliphatic algae in the sea determine the chemical structures of humic substances.

Schnitzer and Preston (1986) compared liquid and solid state  $^{13}\text{C}$ -NMR spectra of HAs as to aliphatic, aromatic and carboxyl regions and concluded that the solution spectra had much higher resolution and provided more detailed information than the



solid-state spectra. Recently, Gillam and Wilson (1986) compared solution versus solid state techniques and proton versus  $^{13}\text{C}$ -NMR in aquatic humic substances. They concluded that solution NMR has the advantage of extra resolution and that quantitative aspects are more fully understood. Solid state NMR has increased sensitivity due to signal enhancement techniques, rapid relaxation, and a higher concentration of nuclei between the poles of the magnet.

Several recent studies have compared liquid and solid state  $^{13}\text{C}$ -NMR on the same sample. Results were encouraging, since spectra were comparable. Schnitzer and Preston (1986) reported both liquid and solid state  $^{13}\text{C}$ -NMR spectra on eighteen HAs. They pointed out the possibility that phenolic OH groups overlap with the carboxyl groups in the 170 ppm region of the spectra. They integrated carboxyl peak areas and compared the liquid and solid results with chemical titrations of total acidity and carboxyl groups. The solid state carboxyl group calculation compared more closely with the total acidity measurement than did the carboxyl measurement by titration. Carboxyl determination by liquid state was slightly lower than that by solid state determination and compared closely with total acidity by titration. Hatcher et al. (1983) examined one HA by three NMR techniques and found that solid state closely approximated solution state NMR; however, the resolution appeared slightly better in solution. They computed 24% COOH by solution versus 19% COOH by solid state NMR. Wilson, et al. (1986) reported results from three NMR techniques and found





that COOH groups were 12% for liquid state versus 16% for solid state. Wilson also reported aromatic groups computed from solution state as 16% versus 19% by solid state. Therefore, it appears that although liquid and solid spectra are similar, NMR spectra of the liquid or solid form have to be interpreted cautiously as a quantitative tool. At present, NMR at best, is semiquantitative and elucidates gross chemical structures.

Ogner (1979) showed that a methylated HA gave a simpler spectrum than did the original HA dissolved in deuterated NaOH. Mikita et al. (1981) permethylated HA and FA with  $^{13}\text{C}$ -enriched reagents and measured peak intensities between 45 and 62 ppm. They reported the first direct measurements of aliphatic hydroxyl functionality in humic substances. The previous determination was calculated by difference. Preston and Schnitzer (1984) also methylated HA and FA to determine functional groups and reported that almost all carboxyl groups were attached to aromatic structures, and that phenolic OH's were significant functional groups. Preston and Schnitzer (1984) reported that 75 to 80% of methylated preparations were soluble in deuteriochloroform. The advantage of not using strong base, which might produce artifacts, becomes apparent. Steelink et al. (1983) permethylated humic substances and found bands in the 50 to 60 ppm region of the spectrum, representing carboxyl, aromatic and aliphatic groups, which revealed the identity and approximate abundance of OH functionality in the sample. Methylation techniques are valuable,

however, there are problems dissolving the methylated substances in organic solvents. If this obstacle can be overcome it allows one to identify and estimate the relative abundance of the various OH groups, including carboxyl, phenolic, hydroxyl, and saccharide groups. Schnitzer and Preston (1986) proposed methylation as the technique to assist in quantification of functional groups in HA and FA.

### iii. Use in Gross Structural Characterization and Functional Group Content

In the past ten years, nuclear magnetic resonance (NMR) spectroscopy has been used for structural group analysis of humic substances, especially determinations of aliphatic, aromatic, and carboxyl groups of humic substances. The theory and various methodologies of NMR have been explained, and interpretation of peak areas and specific peaks of spectra of liquid state  $^{13}\text{C}$ -NMR follow. The purpose of this section is to define specific peaks and chemical shift regions of liquid state  $^{13}\text{C}$ -NMR in an effort to depict the gross chemical structure and functional group content of HAs. Also, some effort must be expended to explain quantification of functional groups by use of  $^{13}\text{C}$ -NMR.

Liquid state  $^{13}\text{C}$ -NMR has been used successfully to determine gross chemical structural differences among HAs since Vila et al. (1976) divided the spectra into three broad groups, 10-100 ppm, aliphatic C; 100-160 ppm, aromatic C; and 160-200 ppm, carboxyl



C. Since then several groups of researchers, notably Schnitzer and Preston (1986; 1987), Preston and Schnitzer (1984), Hatcher et al. (1980a,b; 1981a,b; 1983), Wilson (1981) and Wilson et al. (1983; 1986), have quantified  $^{13}\text{C}$ -NMR spectra by integrating peak areas from 0-105 ppm, aliphatic carbons; 105-160 ppm, aromatic carbons; and 160-200 ppm, carboxyl carbons. Recently, Schnitzer and Preston (1987) divided the spectra into six distinct areas; 0-40, 40-62, 62-105, 105-150, 150-170, and 170-190 ppm. Due to HAs being complicated, ill-defined mixtures, many peaks occur in each area rather than one well-defined peak corresponding to a well-defined structure. Therefore, a distinct chemical structure is not responsible for carboxyl peaks, but several peaks exist in this area of the spectra corresponding to several types of carboxyl compounds. Hatcher et al. (1983) divided the solid state  $^{13}\text{C}$ -NMR spectra into five areas, 0-50 ppm, carbons bonded to other carbons; 50-110 ppm, oxygen and nitrogen substituted carbons as in carbohydrates, ethers, alcohols, and amines; 110-160 ppm, aromatic carbons; 160-190 ppm, carboxyl, amide, and ester carbons; and 190-220 ppm, aldehyde and ketone carbons.

Some care must be taken when quantifying functional groups. Problems of quantification of functional groups have been reported due to variable relaxation times among different carbon nuclei and the variable nuclear Overhauser enhancement (NOE) (Newman et al., 1980; Wilson, 1981 Newman and Tate, 1984). Therefore, preliminary experiments must determine relaxation times and NOE

different rates from very fast (microseconds) to slow (10 seconds) depending on their immediate environment, quantitative determinations must account for variable relaxation times and allow for sufficient time between pulses for relaxation to occur (Newman et al., 1980; Wilson, 1981; Newman and Tate, 1984). Thurman and Malcolm (1983) have reported differences in peak areas with the same FA depending on the method of isolation. This indicated that caution must be used when interpreting or comparing data quantitatively. Differences in peak areas have also been reported with aromatic and carboxyl groups of FA when NaOD was the solvent as compared to the neutral solvent, D<sub>2</sub>O (Steelink et al., 1983).

For purposes of this review it is important to examine quantification of functional groups such as carboxyl and phenolic-hydroxyl. Carboxyl peaks are usually centered between 170 and 180 ppm, with a peak comprising from 5-25% of the total spectral area. Phenolic hydroxyl peaks are present at 150 to 170 ppm (Schnitzer and Preston, 1986) and are usually very small. Spectra generally have several large peaks between 20 and 40 ppm. Also, prominent peaks at 58, 63, 75, 105, 130, and 175 ppm have been reported. These represent alkyl carbon groups, methoxyl, carbohydrates, acetal, aryl and carboxyl groups, respectively (Schnitzer and Preston, 1986; 1987). Wilson et al. (1983) assigned areas of 0-64 ppm, alkyl carbons; 64-95 ppm, oxygenated alkyl C



of ethers and alcohols; 95-110 ppm, dioxygenated C; 110-160 ppm, aromatic C; 160-190 ppm, COOH, ester, salt and amide C.

Clearly, NMR has come a long way since the early 1950's and especially in the last 15 years. The continued advancement of spectrometers and computers, together with liquid and solid state enhancement of NMR parameters could enable scientists to establish gross chemical structures and quantify functional groups present in humic substances in the near future.

### C. HUMUS - HERBICIDE INTERACTIONS

The initial source of humus is largely from decomposing plant material. Therefore, difference in HA functional groups may be related to cropping system variables. Components of cropping systems include, crop, cover crop, fertilizer, and herbicide. Soil texture and climatic regimes are also important parameters that must be considered in humus-herbicide reactions. Studies have focused on differences between HA and FA, neglecting differences in HA between distinct soil management systems. The content of functional groups of HAs might indicate reactivity of different types of organic matter. These HA properties can then be related to an applied soil management problem such as herbicide effectiveness. The paucity of published data indicates that research to examine HA fractions from controlled tillage and crop systems has been neglected.

## 1. Relationship Between Humus and Herbicides

It has been well established that phytotoxicity of many herbicides is inversely correlated with organic matter in many soils, (Harrison et al., 1976; Upchurch and Mason, 1962; Upchurch et al., 1966; Peter and Weber, 1985a,b,c). Upchurch et al. (1966) examined 5 herbicides at 17 locations in North Carolina. Even though organic matter varied from 0.7-49.0% and proved to be the most important single soil property from which herbicide activity could be related, less than 66% of total variation could be accounted for by using organic matter levels. These results suggest that other soil and environmental factors play an important role in herbicide phytotoxicity. Some herbicides must be applied to soils high in organic matter at up to 20 times the normal rate in order to compensate for their adsorption by humus and achieve adequate weed control (Vaughan and Malcolm, 1985). Weber (1987) conducted herbicide tests with 201 soils and found soil organic matter, as determined by the chromic acid test and soil humic matter content, as determined by NaOH/DPTA/alcohol extraction to be correlated with each other ( $r=0.89$ ). However, soil humic matter correlated with herbicide activity more closely than soil organic matter ( $r=0.89$  to  $0.97$  versus  $r=0.87$  to  $0.92$ , respectively). Hance et al. (1968) observed that climatic variables exerted greater control of herbicide phytotoxicity than did soil properties in field situations. Therefore, greenhouse experiments have the advantage of controlling weather variables, allowing for





more controlled conditions to study organic matter interactions with herbicides. Best et al. (1975) and Ladlie et al. (1976) minimized environmental effects by conducting greenhouse experiments, as well as field studies, and studied tillage, organic matter, and pH effects on herbicide activity. They found that field, greenhouse, and nutrient-sand cultures were not always similar in plant response to herbicides.

## 2. Importance of Characterization of Soil Organic Matter

Stevenson (1972) observed that in herbicide reactions, not only is the amount of organic matter important, but also the nature of the organic matter. Stevenson (1972) states that

the fact that soils differ greatly in their organic matter contents is well-known, but it is not generally appreciated that major qualitative differences also exist, both with respect to the known classes of organic compounds (lipids, carbohydrates, proteins) but with the so-called humic substances (humic acid, fulvic acid, etc.).

Also, information on the nature of organic matter-herbicide reactions may provide a more rational basis for their effective use, thereby reducing undesirable side effects due to carryover and contamination of the environment. As Hayes (1970) pointed out, mechanisms of herbicide-organic matter interactions will remain obscure until more is known about the chemistry of humic substances, particularly HA. The type of material being decomposed and the stage of decomposition were important in adsorption of four triazine herbicides in a study by Walker and



Crawford (1968). Hayes et al. (1968) obtained results which indicated that FAs were much less effective in reducing the activity of s-triazine herbicides than was HA. For soils with similar textures and organic matter levels, the contribution of organic matter to sorption of herbicides was greatest where the clay fraction was dominated by kaolinite (Stevenson, 1972). This would be expected since kaolinitic clays have relatively low cation exchange capacities compared to 2:1 clays.

Because humus is highly reactive and able to adsorb ionic and non-ionic molecules by ion exchange and Van der Waals attraction, hydrophobic bonding, hydrogen bonding, charge transfer, and ligand exchange, it often controls activity of herbicides in soil. Khan (1978) pointed out advantages of using humic and fulvic acids to study soil organic matter reactions including,

(1) they can be readily extracted from soil organic matter in relatively pure form; (2) they have been thoroughly characterized by various techniques; and (3) they are the major and common constituents of soil organic matter.

### 3. Properties of the Pesticide

The nature of the pesticide as well as the nature of the organic matter are important in these reactions. The chemical character, shape and configuration, its acidity ( $pK_a$ ), or basicity ( $pK_b$ ), its water solubility, the charge distribution on the cation, the polarity of the molecule, its molecular size and polarizability all affect the adsorption-desorption of pesticides by soil colloids.



Vaughan and Malcolm (1985) state that herbicides are generally low molecular weight materials and can combine with soil organic matter so that herbicide activity and mobility is inhibited. The major limitations in understanding these interactions have been the complex nature of soil organic matter coupled with the numerous processes in the soil environment. The simultaneous occurrence of these limitations complicates the process of analysis and comprehension of the interactions. Therefore, utilizing relatively simplified greenhouse studies and well defined humus fractions to examine status of herbicide-humus interactions may increase the understanding of these interactions.

Herbicide phytotoxicity has commonly been measured using bioassays. A major advantage of biological assays, is the assurance that the phytotoxic portion of the herbicide molecule is being measured (Lavy and Santelmann, 1986; Appleby, 1985).



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### III. CHARACTERIZATION OF HUMIC ACID FROM NO-TILLED AND TILLED SOILS USING $^{13}\text{C}$ -NMR

#### A. ABSTRACT

Humic acids from continuously (seven-year) no-tilled and tilled cotton, corn, and soybean plots in West Tennessee were characterized by  $^{13}\text{C}$ -NMR from soils sampled at various depths. Humic acids were extracted and purified by standard methods. NMR spectra were divided into six regions, (0-40, 40-62, 62-105, 105-150, 150-170, and 170-190 ppm) and peak areas compared. Humic acid composition differed by depth and crop. Small differences were observed between tillage systems. Aliphatic and aromatic carbons ranged from 48 to 65% and 25 to 40% of total peak area, respectively. Humic acids extracted from those soils containing larger amounts of C (surface no-tilled treatments) had greater aliphatic to aromatic ratios, indicating earlier stages of decomposition. Carboxyl groups of humic acids ranged from 9 to 13% and samples from tilled soil had slightly greater amounts of carboxyl and aromatic groups than did no-tilled soil.



## B. INTRODUCTION

Nuclear magnetic resonance spectroscopy has enabled the comparison of soil organic matter structure and gross composition. In the last decade,  $^{13}\text{C}$ -NMR has become a powerful method of characterizing soil organic matter fractions, providing more information than other methods. The possibility of significant differences occurring in soil organic matter composition between tilled and no-tilled soils poses the interesting proposition that the quality of soil organic matter might influence soil management decisions in different tillage systems.

Five and ten year tillage studies with corn indicated that soil organic matter increased significantly in no-tilled soil compared to conventionally tilled soils (Blevins et al., 1977 and 1983). After two years Tyler et al. (1983) found no-till organic matter levels in soybean plots were significantly higher in the top 5 cm depths than in conventional tillage treatments. Organic matter in no-tilled soils is approximately twice that of tilled soils receiving the same inputs (Phillips and Phillips, 1984). It has been clearly established that plowing increases rates of organic matter decomposition in soils, (Giddens, 1957).

Soil organic matter is extracted and fractionated based on solubilities of fulvic acid (FA), soluble in both alkali and acid, humic acid (HA), soluble in alkali and insoluble in acid, and humin, insoluble in both alkali and acid. Recently, there has been an effort



to standardize extraction procedures for humic substances by the International Humic Substances Society, (R. L. Malcolm, 1985, personal communication). Much of the research effort has been devoted to fractionation schemes, rather than enumerating differences between soil organic matter fractions. The importance of working with purified fractions was expressed by Malcolm (1976).

Humic and fulvic acids have been characterized by numerous methods, including elemental analysis, titration of functional groups, and infrared and nuclear magnetic resonance spectroscopy. However, because of the heterogeneous nature of HA, an exact structure has yet to be formulated. Schnitzer and Preston (1986) compared titrations of total acidity and carboxyl groups of HA with both solid and liquid state  $^{13}\text{C}$ -NMR spectra. They reported NMR determinations of carboxyl groups that agreed more closely with total acidity titrations than with carboxyl group titrations. Solid state NMR carboxyl values were greater than those of liquid state NMR. Therefore, NMR spectra of liquid or solid forms have to be interpreted cautiously as a quantitative tool. At present, NMR at best is semiquantitative and elucidates gross chemical structures, (Newman et al., 1980; Wilson, 1981; Newman and Tate, 1984). However, if NMR instrumental parameters are constant for all samples, NMR can offer structural information that is useful when comparing HAs.



Humic acid elemental composition is usually in the range; C, 53.8 to 58.7%, H, 3.2 to 6.2%, O, 32.8 to 38.3%, N, 0.8 to 4.3%, and S, 0.1 to 1.5%, (Schnitzer and Khan, 1978). The O/C ratio for soil HAs is usually about 0.5, while the H/C ratio is approximately 1.0, (Steelink, 1985).

Perdue (1985; 1984) stated that because humic substances represent a complex mixture of organic compounds, the acidity of humic substances can only be attributed to a complex mixture of nonidentical functional groups. Therefore, since potentiometric determination of functional groups by titration is based on  $pK_a$  values of the functional groups, only operationally defined estimates of the concentration of a particular class of acidic functional groups is determined. On the other hand, Schnitzer and Gupta (1965) have evaluated the total acidity and carboxyl group titrations by discontinuous titrations and decarboxylation, respectively, and found the titrations to be accurate.

Calderoni and Schnitzer (1984) reported yields of purified HA-C from 8.9 to 32.9% of total carbon of six paleosols in Southern Italy, while FA yields were very small. The aromaticity of the paleosol HAs was large (68 to 88%) when compared to aromaticities of soil HAs as determined by liquid state  $^{13}\text{C}$ -NMR by Hatcher et al., 1981, who reported aromaticities between 43 and 92% for nine HAs from soils in Japan, Argentina, and Italy.

Preston et al. (1987) reported differences in composition of organic matter of virgin and cultivated organic soils using





solid-state  $^{13}\text{C}$  CPMAS (Cross Polarization Magic Angle Spinning) NMR. Cultivated sites had less carbohydrate and increased lipid and methoxyl carbon than did the virgin sites. Wilson et al. (1986) demonstrated that lignin was not a prerequisite for aromatic components in soil and that due to slow rates of decomposition in Antarctic soil, the FA fraction was almost entirely carbohydrate. They attempted to explain variations in soil aromaticity and carbohydrate concentrations and found climate and vegetation to be important factors. Schnitzer and Preston (1987) divided solution state  $^{13}\text{C}$ -NMR spectra into six regions and compared aliphatic C (0-105 ppm) to aromatic C (105-170 ppm) using various HA extractants. The carboxyl region was reported as the area between 170 and 190 ppm.

The objectives of this study were to compare soil organic matter fractions and characterize HA of no-tilled and tilled cotton (*Gossypium hirsutum* L.), corn (*Zea mays* L.), and soybean (*Glycine max* (L.) Merr.) surface soils that had the same seven-year management scheme.

## C. MATERIALS AND METHODS

### 1. Origin and Extraction of Soil Organic Matter

Soil samples were collected from surface horizons of no-tilled and tilled continuous seven-year soybean and cotton plots at the West Tennessee Agricultural Experiment Station in Jackson,



Tennessee and corn plots at the Ames Plantation near Bolivar, Tennessee. The soil at Jackson was Lexington silt loam (fine, silty, mixed, thermic, Typic Paleudalfs) and at Ames the soil was Loring silt loam (fine, silty, mixed, thermic, Typic Fragiudalfs). Soil ranged from 5.7 to 47.2 g kg<sup>-1</sup> C and pH 4.8 to 7.2. Soil was sampled at 0 to 1, 1 to 2, and 7.5 to 15.0 cm depths. A flat bottom ash shovel, marked at one cm depth, was used to sample 0 to 1 and 1 to 2 cm depths of soil after stubble mulch was removed. A soil sampling tube was used to sample 7.5 to 15.0 cm depths. Humic and fulvic acids were extracted from air dried soil ground to pass a 0.25 mm sieve, based on the procedure recommended by the International Humic Substances Society, (R. L. Malcolm, 1985, personal communication). Humic acid was purified based on the previously cited methods and characterized.

Ninety g of air dry soil was shaken for 30 minutes after adding 50 ml of 1.0 mol L<sup>-1</sup> HCl and 850 mL of 0.1 mol L<sup>-1</sup> HCl, allowed to settle for two hours and centrifuged (1800 x g) for 10 minutes. An aliquot of the supernatant was saved for analysis of soluble fulvic acid C. The supernatant was decanted, and 70 mL of 1.0 mol L<sup>-1</sup> NaOH was added to the soil followed by the addition of 830 mL of 0.1 mol L<sup>-1</sup> NaOH. Nitrogen gas was bubbled into the bottles for two minutes and the soil shaken for eight hours and allowed to settle overnight. The soil was centrifuged for ten minutes at 1800 x g, and the supernatant decanted into another 1 L centrifuge bottle and acidified to pH 1 with 6.0 mol L<sup>-1</sup> HCl. A sample of extracted



soil was saved and acidified with  $0.1 \text{ mol L}^{-1}$  HCl, then dried in an oven at  $105^{\circ} \text{C}$  and analyzed for nonextracted carbon. The extracted HA and FA was centrifuged and an aliquot of the supernatant saved for carbon (FA) analysis. Suspended clays were removed by dissolving HA in a minimum volume of  $0.1 \text{ mol L}^{-1}$  KOH under  $\text{N}_2$  and adding KCl to make the system  $0.3 \text{ mol L}^{-1}$  with respect to K. Nitrogen gas was bubbled into the containers and they were allowed to stand for 4 h, and suspended solids removed by centrifugation. The clear HA solution was acidified to pH 1 and the HA allowed to coagulate. The latter was separated by centrifugation and shaken repeatedly for 24 h at room temperature with  $0.1 \text{ mol L}^{-1}$  HCl- $0.3 \text{ mol L}^{-1}$  HF solution. The HA was washed with small aliquots of deionized water to remove chloride ions, freeze dried, and stored in a desiccator over  $\text{P}_2\text{O}_5$  at room temperature.

## 2. Analytical Methods

Soil pH was determined with a glass electrode pH meter and 1:1 soil to water ratio by volume, (McLean, 1982, p.208-09). Cation exchange capacity was done by summation of exchangeable cations using the ammonium acetate method, (Thomas, 1982, p.160-161, and Yuan, 1959). Calcium, Mg, and K were determined by atomic absorption. Carbon in the soil and HA was determined with a Leco Carbon Determinator CR12 by dry combustion (Nelson and Sommers, 1982, p.549-50). The amount of C in fulvic acid extracted was measured using a Beckman Total Organic Carbon Analyzer, Model



915-B. Nitrogen and H were determined on HA by dry combustion using a Perkin Elmer 240 elemental analyzer (Galbraith Laboratories, Inc., Knoxville, Tennessee). Oxygen was calculated by difference. Moisture content of HA was determined by heating at 105<sup>o</sup> C for 24 h and ash by ignition at 750<sup>o</sup> C for 4 h. Total acidity and carboxyl groups were determined by methods described by Schnitzer and Gupta (1965). Briefly, total acidity was determined by titration with 0.5 mol L<sup>-1</sup> HCl after treatment with 0.1 mol L<sup>-1</sup> Ba(OH)<sub>2</sub> solution. Carboxyl groups were determined by titration with 0.1 mol L<sup>-1</sup> NaOH after reaction with 0.5 mol L<sup>-1</sup> calcium acetate. All titrations were replicated two to four times. Infrared (IR) spectra were recorded on KBr pellets (5.0 mg of HA and 200 mg of dry KBr) from 4000 to 700 cm<sup>-1</sup> on a Beckman IR 4260 spectrophotometer.

The <sup>13</sup>C-NMR solution spectra for HA's were determined on a JEOL FX 90Q spectrometer. The HA sample (100mg) was dissolved in 2 ml 0.5 mol L<sup>-1</sup> NaOD. The HA solution was mixed on a vortex mixer for 3 minutes and filtered through a coarse sintered filter. Chemical shifts were measured relative to sodium 3-(trimethylsilyl)propionic acid (TSP). Solution <sup>13</sup>C-NMR spectra were obtained at 22.5 MHz using double precision 16k data points with a 15015 Hz spectral width. A 45<sup>o</sup> pulse width with a 0.9 sec pulse delay was used to give an acquisition time of 0.534 sec. Optimum conditions were chosen to maximize signal to noise ratio. Between 95,000 and 110,000 scans were collected in the FT mode





requiring 36 to 45 h total acquisition time. The spectra were divided into six areas 0 to 40, 40 to 62, 62 to 105, 105 to 150, 150 to 170, and 170 to 190 ppm, and broadly divided from 0 to 105 ppm, aliphatic carbons; 105 to 170 ppm, aromatic carbons; and 170 to 190 ppm carboxyl carbons. Areas were computed by razor cutting the spectra and weighing on a balance to determine total of each group. Areas were also measured with a VT area meter using a video screen. Area measurements agreed  $\pm 5\%$  between the two methods employed and results averaged.

#### D. RESULTS AND DISCUSSION

The chemical characteristics and amounts of extractable C of the thirteen soil samples are presented in Table 1. Soils cropped to soybean had a more neutral pH than did those cropped to cotton and corn. The no-tilled cotton treatments with vetch and wheat covers, had the largest levels of total C. The type of soil organic matter in surface no-tilled soils was closer to that of plant materials, and it was not as decomposed as the mixed tilled soils or the deeper soil samples. The cation exchange capacity and the soil organic matter content of the soil samples decreased with depth, indicating they were directly related. The 0-1 cm samples had the largest values for both soil organic matter and cation exchange capacity. Therefore, soil organic matter contributed to the reactive properties of soils. The extractable C ranged from 25 to 40% with



Table 1. Soil chemical characteristics and extractable carbon fractions.

<u>Depth cm</u>	<u>pH</u>	<u>CEC</u> <u>cmol(+)</u> <u>kg<sup>-1</sup></u>	<u>Total</u> <u>Carbon</u> <u>g kg<sup>-1</sup></u>	<u>% of Total</u> <u>Carbon</u> <u>Extracted</u>	<u>% of Soil Carbon</u> <u>FA</u> <u>HA</u> <u>HA-clay</u>		
<u>Corn. No-Till(NT)</u>							
0-2	6.1	11.5	21.7	38.6	14.3	15.4	8.9
<u>Corn. Till(T)</u>							
0-2	6.3		6.1	29.3	13.8	5.2	10.3
7.5-15	7.2		5.7	28.4	14.8	5.3	8.3
<u>Soybeans. NT</u>							
0-2	7.2	13.8	19.4	32.5	15.1	15.5	1.9
7.5-15	7.1	10.5	5.8	40.5	20.4	19.1	0.0
<u>Soybeans. T</u>							
0-2	7.2	9.4	7.3	28.4	11.0	9.9	7.5
<u>Cotton. NT, Vetch Cover</u>							
0-1	5.6	14.6	47.2	26.0	9.1	12.4	4.5
1-2	5.1	8.3	22.1	32.3	12.1	9.1	11.1
7.5-15	6.1	8.7	7.0	36.6	13.7	7.7	15.2
<u>Cotton. NT, Wheat Cover</u>							
0-1	6.2	15.4	29.7	24.7	10.5	10.8	3.4
1-2	4.8	10.5	14.1	28.4	13.0	8.6	6.8
<u>Cotton. NT</u>							
0-2	5.3	10.7	15.7	31.0	11.9	8.4	10.7
<u>Cotton. T</u>							
0-2	5.1		10.8	30.8	13.5	8.2	9.1



little difference between tillage treatments or crops. In all samples, extracted FA (9 to 20% of total soil C) increased with depth, and small differences were observed in quantities between tillage treatments. Humic acid extracted ranged from 5 to 19% of total carbon with less HA extracted from the tilled corn and soybean treatments than from the no-tilled treatments, although there was no difference between tillage treatments for cotton.

Humic acid-clay associations increased with depth of sample. In most cases percent extractable C increased with depth except for the corn 7.5 to 15.0 cm sample. The difference between extractable C and the sum FA and HA was the HA associated with clay, separated in the KOH-KCl purification procedure, (Table 1). The soybean, 7.5 to 15.0 cm sample, had all extractable C associated with FA and purified HA, whereas in corn and cotton samples from comparable depth, 30-40% of extractable C was HA-clay mixtures.

Hydrogen and N of the purified humic acids (Table 2) were higher than those reported by Schnitzer and Preston (1986). The larger ratios of H/C and N/C indicated that the HAs were more aliphatic than those characterized by Schnitzer and Preston (1986). This was further supported in that these humic acids were 56% aliphatic, compared to Schnitzer and Preston's which averaged 46%.

Carboxyl groups of HA, as determined by  $^{13}\text{C}$ -NMR, were closer in value to total acidity titrations than carboxyl group titrations (Table 3). Schnitzer and Preston (1986) reported similar findings



Table 2. Elemental analyses and atomic ratios of purified humic acids on a moisture and ash-free basis.

<u>Soil Treatment</u>	<u>(wt/wt%)</u>				<u>Atomic ratios</u>		
	C	H	N	O	H/C	O/C	N/C
Corn, NT	50.1	5.7	4.6	39.6	1.37	.59	.08
Corn, T	53.3	5.6	4.5	36.6	1.25	.52	.07
Corn, 7.5-15 cm	50.5	5.1	3.1	41.4	1.21	.62	.05
Soybeans,NT	51.9	5.6	4.9	37.6	1.29	.72	.08
Soybeans,T	52.3	5.2	5.1	37.4	1.19	.54	.08
Soybeans 7.5 -15.0 cm	58.8	5.4	4.4	31.4	1.10	.40	.06
Cotton, NT	53.1	5.6	5.2	36.1	1.26	.51	.08
Cotton, T	56.9	5.5	5.2	32.4	1.16	.43	.08
Cotton,NT Vetch 0-1cm	54.9	6.1	5.5	33.5	1.33	.61	.09
Cotton,NT Vetch 1-2cm	53.7	5.8	5.7	34.8	1.29	.49	.09
Cotton,NT Wheat 0-1cm	45.3	6.0	4.9	43.8	1.59	.73	.09
Cotton, NT Wheat 1-2cm	51.8	5.4	4.8	38.0	1.25	.55	.08
Means	52.7 ±3.4	5.6 ±0.3	4.8 ±0.7	36.7 ±3.6	1.27 ±0.12	.56 ±0.10	.08 ±0.01





Table 3. Concentration of humic acid COOH groups by solution-state  $^{13}\text{C}$ -NMR and titration, and measurements of total acidity.

Soil Humic acid	COOH groups		
	$^{13}\text{C}$ -NMR Solution-state	Titration	Total acidity
	mol kg <sup>-1</sup>		
<u>Depth 0-2 cm</u>			
Corn, NT	5.5	3.6	6.6
Corn, T	6.9	3.3	8.0
Soybeans, NT	7.3	4.0	6.3
Soybeans, T	6.6	4.1	7.5
Cotton, NT	6.7	3.9	6.0
Cotton, T	6.6	3.5	6.4
<u>Depth 7.5-15.0 cm</u>			
Corn	5.3	3.9	8.8
Soybeans	8.9	5.0	9.4
Cotton	7.9	3.7	9.1
<u>Depth 0-1 cm</u>			
Cotton, vetch cover	6.6	3.1	6.0
Cotton, wheat cover	5.7	2.9	7.6
<u>Depth 1-2 cm</u>			
Cotton, vetch cover	7.3	3.4	6.5
Cotton, wheat cover	7.6	3.3	9.8
Means	6.8 ± 1.0	3.7 ± 0.5	7.5 ± 1.4

on eighteen HAs. The total acidity determinations were larger in tilled compared to no-tilled treatments and with depth. There was no apparent pattern of carboxyl group content based on tillage or crop variables.

Infrared spectra for all HAs were similar. Because of broad overlapping peaks, spectra were not quantified.

The  $^{13}\text{C}$ -NMR spectra of HA from no-tilled and tilled corn, cotton, and soybean soils are displayed in Fig. 1. The gross similarity between no-tilled and tilled treatments was apparent between spectra. Differences were more pronounced between crops than tillage. However, the corn tilled sample was more aromatic than the corn no-tilled sample, which was more aliphatic. There was a large peak at 58.1 ppm in corn and cotton while it was smaller in soybean. This resonance is associated with C in methoxyl groups and possibly C in amino acids and peptides. The integrated peak areas (Table 4) demonstrated that in all cases the 105 to 150 ppm region was less in no-tilled than in tilled treatments, indicating that the tilled HA was more aromatic.

The NMR spectra of HA from cotton no-tilled plots with vetch or wheat cover crops (Fig. 2) indicated the overall similarity between samples, although peak areas (Table 5) demonstrated the more aromatic nature of the 7.5 to 15.0 cm sample. The cotton no-tilled vetch cover treatment at 0 to 1 cm depth had the largest amount of total carbon ( $47.2 \text{ g kg}^{-1}$ , Table 1). The HA from this treatment had the largest value for aliphatic carbons, indicating



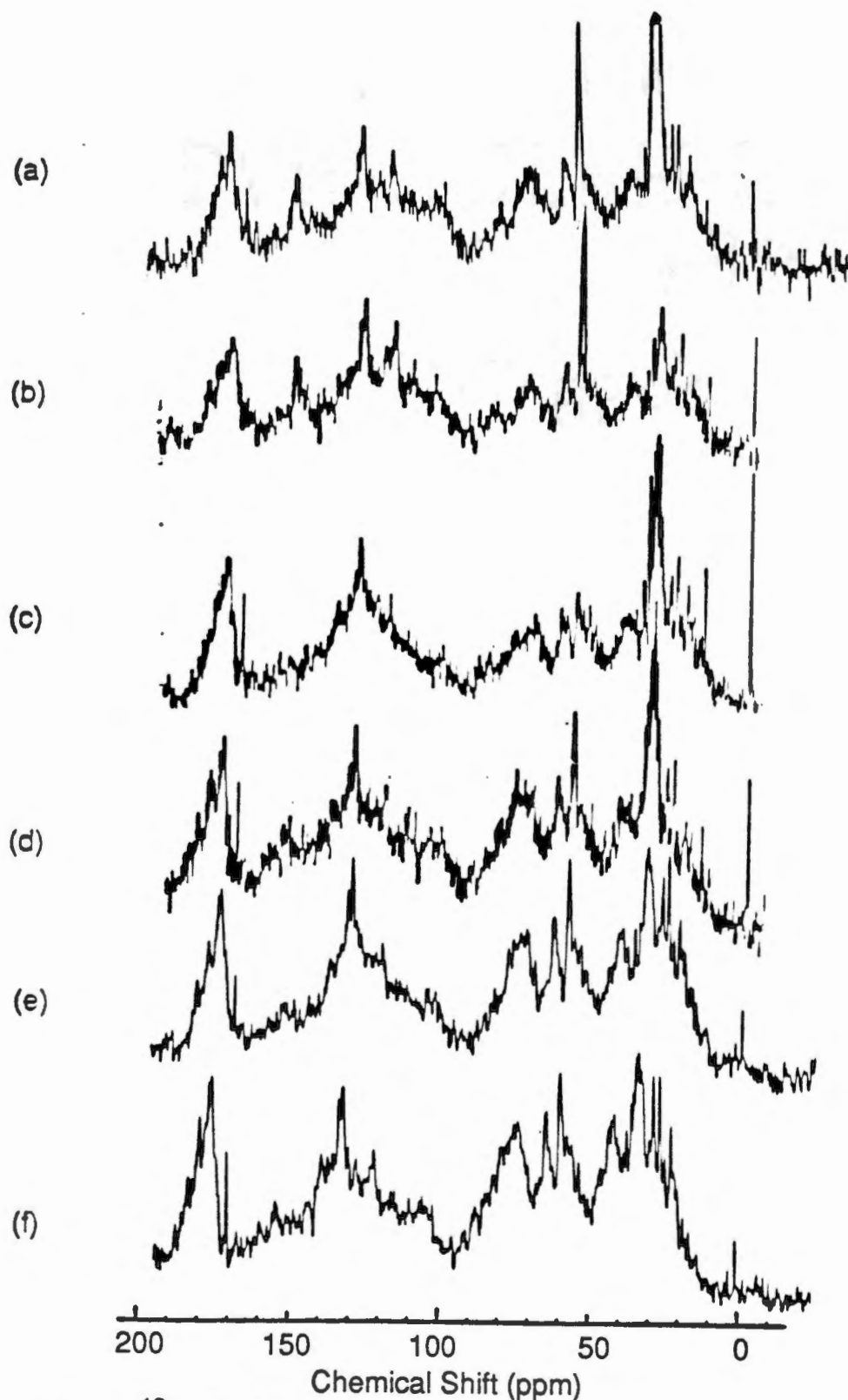


Fig. 1.  $^{13}\text{C}$  NMR spectra of no-tilled and tilled corn, soybeans and cotton humic acids (in NaOD) from 0-2 cm depth, (a), corn, NT, (b), corn, T, (c), soybean, T, (d), soybean, NT, (e), cotton, T, and (f), cotton, NT.



Table 4. Interpretation of  $^{13}\text{C}$ -NMR spectra of humic acid from cotton, corn, and soybean plots, 0-2 cm depth.

Chemical Shift	Corn		Soybean		Cotton	
	NI	I	NI	I	NI	I
<u>PPM</u>	<u>wt/wt%</u>					
0-40	27	19	19	25	22	24
40-62	16	15	13	14	17	16
62-105	16	17	22	17	21	20
105-150	26	31	27	30	24	27
150-170	6	7	7	4	5	4
170-190	9	11	12	11	11	10
Aliphatic	61	52	54	56	60	59
Aromatic	32	38	34	34	30	31
Ratio Al/Ar	1.9	1.4	1.6	1.6	2.0	1.9
Aromaticity	36	42	39	38	34	34





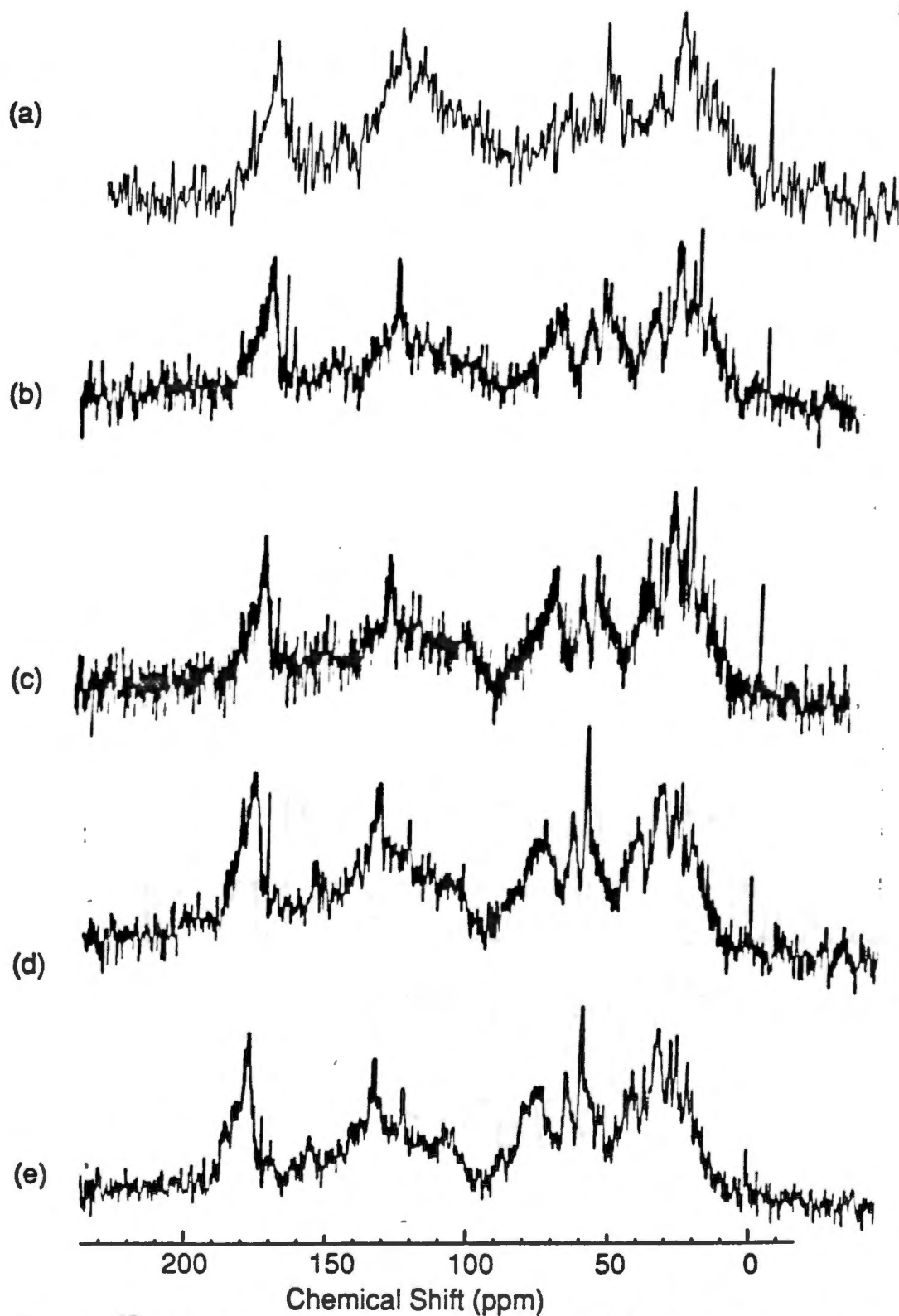


Fig. 2.  $^{13}\text{C}$  NMR spectra of humic acids (in NaOD) extracted from cotton plots with vetch and wheat cover crops, (a), vetch, 7.5-15.0 cm, (b), vetch, 1-2 cm, (c), vetch, 0-1 cm, (d), wheat, 1-2 cm, and (e), wheat, 0-1 cm.



Table 5. Interpretation of  $^{13}\text{C}$ -NMR spectra of humic acid from cotton plots.

Chemical Shift	Cotton,NT,vetch cover			Cotton,NT,wheat cover	
	0-1cm	1-2cm	7.5-15cm	0-1cm	1-2cm
<u>PPM</u>	<u>wt/wt%</u>				
0-40	28	29	18	30	21
40-62	15	17	14	17	15
62-105	19	18	17	18	19
105-150	22	22	32	22	27
150-170	5	4	8	3	7
170-190	10	11	12	10	12
Aliphatic	63	64	48	65	55
Aromatic	28	25	40	25	33
Ratio Al/Ar	2.3	2.5	1.2	2.6	1.7
Aromaticity	31	29	46	28	38



that as organic matter decomposes it becomes more aromatic in structure. This was attributed to the process of decomposition, where the more easily decomposed carbohydrates and aliphatic structures degrade leaving more resistant lignin derived aromatic structures. The aliphatic-aromatic C ratio for HA from the 7.5 to 15.0 cm treatment was close to one (Table 5), while the ratio was above two for the shallow samples where total C was larger and in a less decomposed state. There was a significant increase in peak area of the 105 to 170 ppm region in the deep sample. Therefore, as soil HA decomposed it had a larger percentage of the more resistant aromatic components. This observation is illustrated in Fig. 3, where the ratio of aliphatic to aromatic C components increased with C. Therefore, the no-tilled surface treatments, consisting of relatively large amounts of C, had larger aliphatic to aromatic ratios, because the HA from the no-tilled treatments contained less decomposed organic matter, of which cellulose and carbohydrates were constituents.

The three spectra of HA from the soybean plots are presented in Fig. 4. The spectra are quantified based on peak areas in Table 6. The surface no-tilled and tilled HA spectra were similar both quantitatively and qualitatively in peak area, while soil HA from the 7.5 to 15.0 cm depth was more aromatic and the aliphatic to aromatic ratio was close to one, as was the case with the deeper HA from the cotton treatment. Also, the HA from the deeper sample had more carboxyl groups, again similar to the deep cotton sample.

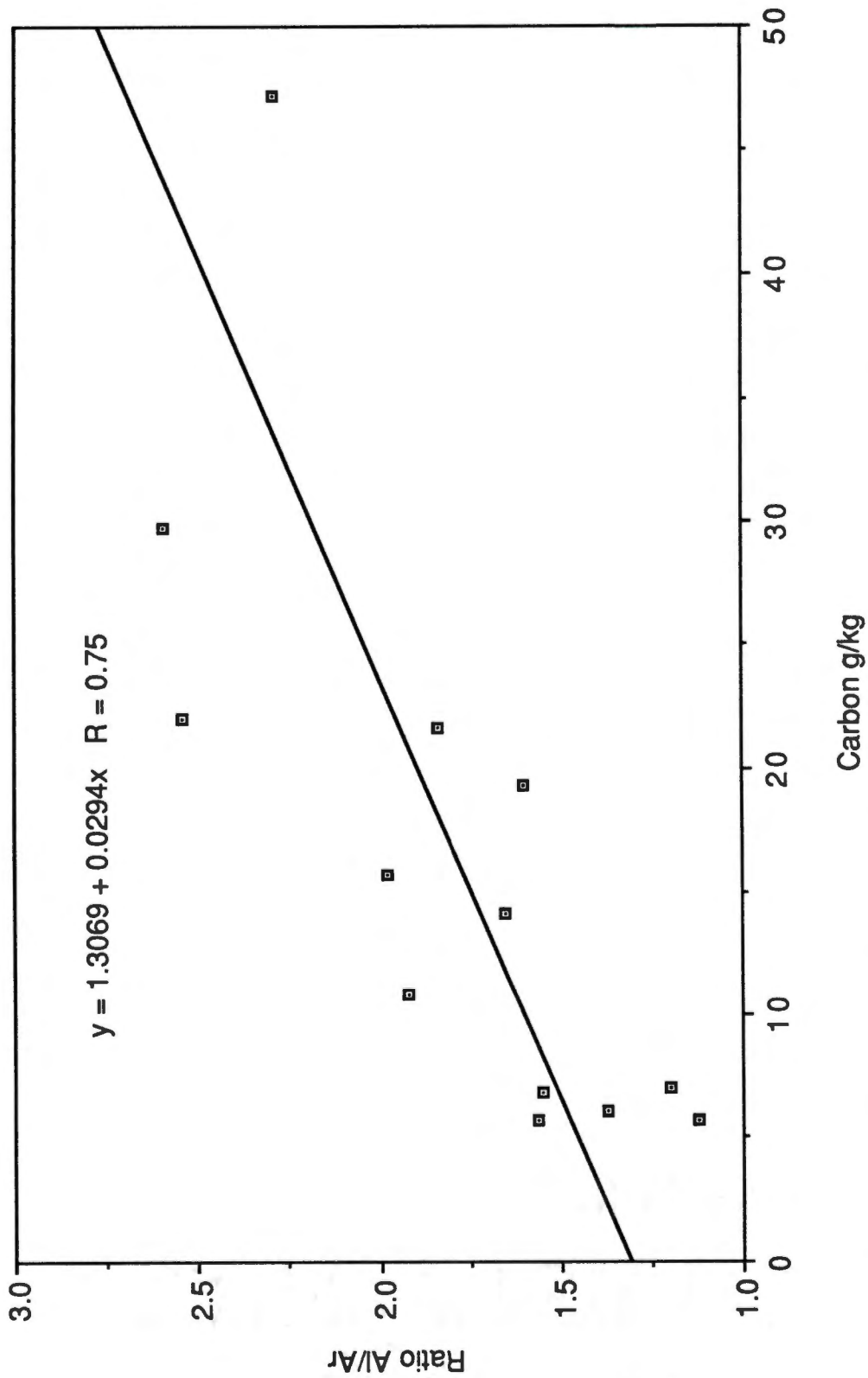


Fig. 3 Aliphatic to aromatic carbon ratio of humic acid as a function of carbon in soils.



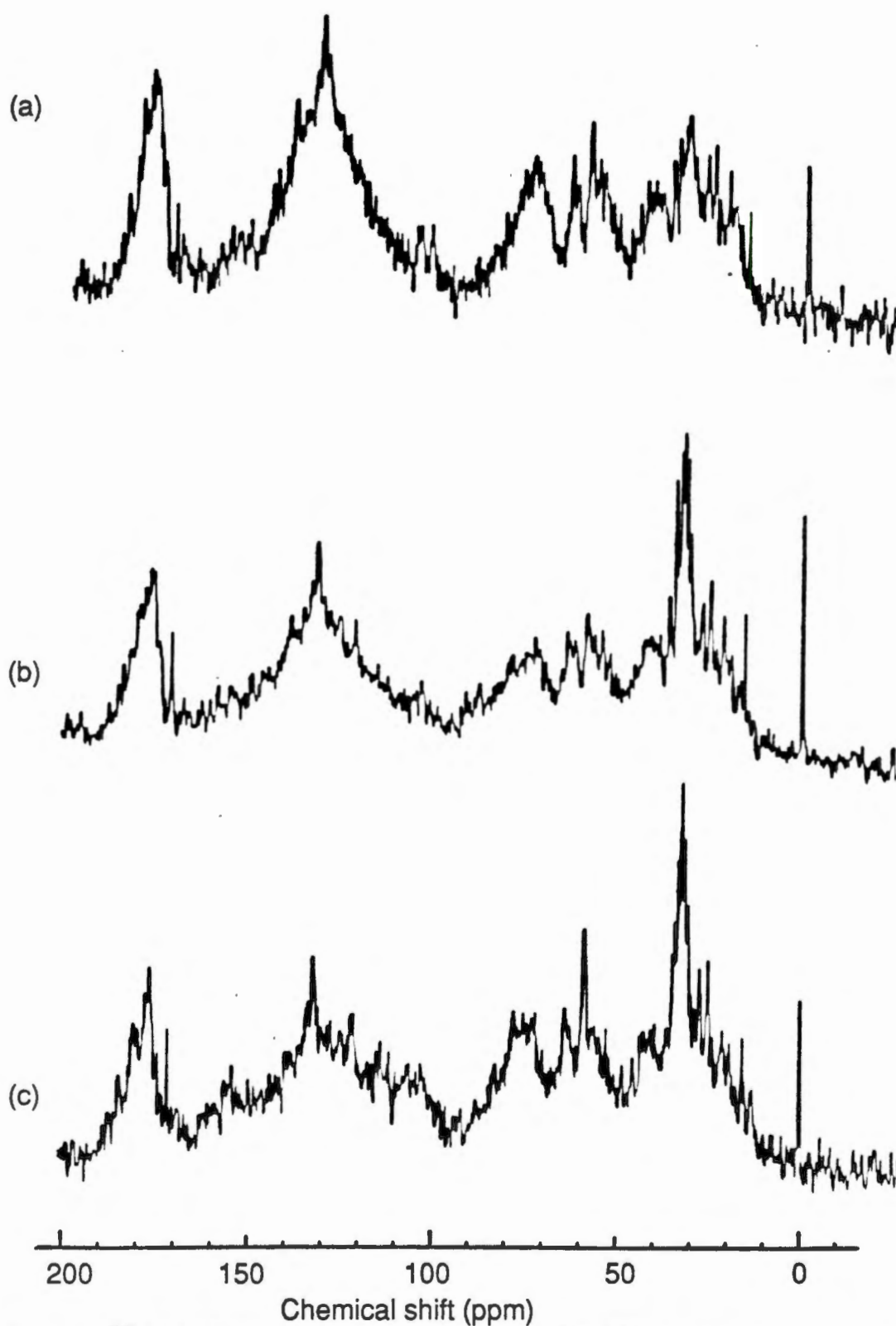


Fig. 4.  $^{13}\text{C}$  NMR spectra of humic acids (in NaOD) extracted from soybean plots, (a), no-tilled, 7.5-15.0 cm, (b), tilled, 0-2 cm, (c), no-tilled, 0-2 cm.



Table 6. Interpretation of  $^{13}\text{C}$ -NMR spectra of humic acid from soybean plots.

<u>Chemical Group</u>	<u>No-Tilled 0-2 cm</u>	<u>Tilled 0-2 cm</u>	<u>No-Tilled 7.5-15.0 cm</u>
<u>ppm</u>	<u>wt/wt%</u>		
Aliphatic C 0-105	54	56	48
Aromatic C 105-170	34	34	40
COOH C 170-190	12	11	13
Ratio Al/Ar	1.6	1.6	1.1
Aromaticity	39	38	46



The distribution of C in HA grouped by crop, tillage and depth is summarized in Table 7. The corn, cotton, and soybean averages were based on the 0 to 2 cm no-till and till treatments and the 7.5 to 15 cm treatments. Most obvious differences between HAs were by depth, where the aliphatic to aromatic ratio decreased with depth. Soybean plot HAs had more carboxyl groups and contained more aromatic structures than did the cotton or corn plot HAs. Differences were slight between tillage systems, although no-tilled treatments were more aliphatic and had less carboxyl groups than tilled treatments.

#### E. CONCLUSIONS

Of thirteen humic acids characterized, carboxyl group determination by  $^{13}\text{C}$ -NMR agreed more closely with total acidity than carboxyl group determination by chemical titrations. Fulvic acid increased with depth of sample. The larger amounts of organic C in surface no-tilled plots were correlated with greater amounts of aliphatic groups in HAs extracted from these plots. The HAs derived from the deeper samples had an aliphatic to aromatic C ratio close to one. Humic acids extracted from soybean plots had the largest values for carboxyl groups and slightly more aromatic groups than HA extracted from corn and cotton plots. Humic acid from corn and cotton crops were similar both quantitatively and qualitatively in peak areas. Differences in HA composition were



Table 7. Summary of mean C distribution of soil humic acid from  $^{13}\text{C}$ -NMR spectra.

Plots	Aliphatic Carbon	Aromatic Carbon	Ratio Al/Ar	Aliphatic + Aromatic	CO <sub>2</sub> H	Aromatic + CO <sub>2</sub> H	Aroma- ticity
Tilled 0-2 cm	55	34	1.6	89	11	45	38
No-Tilled 0-2 cm	58	32	1.8	90	10	42	36
7.5-15.0 cm	50	39	1.3	89	11	50	44
Cotton	56	34	1.7	89	11	45	38
Corn	56	35	1.6	90	10	45	39
Soybean	52	36	1.5	88	12	48	41
Cotton, vetch cover	63	26	2.4	89	11	37	30
Cotton, wheat cover	60	29	2.1	89	11	40	33



more pronounced with depth than with tillage differences. No-tilled and tilled treatments were similar in HA structural properties.





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#### IV. SOIL ORGANIC MATTER FRACTIONS OF NO-TILLED AND TILLED COTTON PLOTS AND THEIR REACTIVITY WITH HERBICIDES

##### A. ABSTRACT

Soil humus fractions and characteristics were determined on surface soil samples of no-tilled and tilled cotton field plots in West Tennessee. Soil humic and fulvic acid were extracted by standard methods. Total carbon ranged from 9.9 g kg<sup>-1</sup> in the tilled soil to 13.5, 16.6, and 23.5 g kg<sup>-1</sup> in the no-tilled soils, with rye, vetch, and crimson clover cover crops, respectively. The tilled soil had the largest fraction of extractable C and fulvic acid, relative to total soil C. Humic acid was characterized by <sup>13</sup>C-NMR, titration of functional groups, infrared and elemental analysis. Carboxyl groups determined by <sup>13</sup>C-NMR ranged from 6.1 to 7.7 mol kg<sup>-1</sup> humic acid and compared more closely with total acidity than with carboxyl group determinations by titration. All infrared spectra were similar. A greenhouse herbicide bioassay was conducted and sorghum growth was measured to indicate soil organic matter effects on phytotoxicity of herbicides, metribuzin and oxyfluorfen. Herbicide activity was inversely related to soil C, extractable C, carboxyl groups of humic acid, and fulvic acid C of soils. Fulvic acid C best predicted herbicide activity. Results indicated that C was more reactive in tilled than in no-tilled soils.

## B. INTRODUCTION

Phytotoxicity of many herbicides is inversely correlated with organic matter in soils (Harrison et al., 1976; Upchurch and Mason 1962; Upchurch et al. 1966; Peter and Weber, 1985a,b,c). Soil organic matter increases in long term no-tilled plots compared to tilled plots (Blevins et al., 1977; 1983). Tyler et al. (1983) reported significant increases in surface (0 to 5 cm) no-tilled soil organic matter levels after two years compared to tilled soils. Giddens (1957) demonstrated that plowing increased rates of organic matter decomposition in soils. Important components of soil organic matter include humic acid (HA), fulvic acid (FA), and humin, defined by their solubility in acid or base. These reactive components of soil organic matter are able to adsorb herbicides by several mechanisms, including ion exchange, hydrophobic bonding, hydrogen bonding, charge transfer, Van der Waals attraction, and ligand exchange. Some herbicides must be applied to soil at up to 20 times the normal rate to compensate for their adsorption by organic soils and to achieve adequate weed control (Vaughan and Malcolm, 1985).

Upchurch et al. (1966) examined 5 herbicides at 17 locations in North Carolina and found organic matter to account for 66% of total variation in herbicide activity. Weber et al. (1987) reported soil organic matter, as determined by chromic acid oxidation, to be highly correlated ( $r=0.89$ ) with soil humic matter content as determined by NaOH/DTPA/alcohol extraction of 201 soils. In field



and greenhouse studies at various locations in North Carolina, soil organic matter, clay, and surface area were inversely correlated with activity of five herbicides (Peter and Weber, 1985a,b,c). Slack et al. (1978) studied tillage and pH effects on herbicide activity in field and greenhouse experiments and reported less persistence of simazine under no-tilled corn than conventionally tilled corn. This was explained by greater organic matter content of no-tilled soil, adsorption by organic residue on the surface, and increased moisture in surface soil of no-tilled plots. Bioassay techniques have been used to measure herbicide phytotoxicity. A distinct advantage of the bioassay is the assurance that the phytotoxic portion of the herbicide is determined (Lavy and Santelman, 1986; Appleby, 1985).

Stevenson (1972) observed that in herbicide reactions, not only is the amount of organic matter important, but also the nature of organic matter. Hayes, 1970, pointed out that mechanisms of herbicide-organic matter interactions will remain obscure until more is known about the chemistry of humic substances, particularly HA.

Extraction, fractionation, and purification of soil organic matter (humic acid and fulvic acid), have been conducted using various extractants. Recently, attempts have been made by the International Humic Substances Society to standardize the methods of extraction and purification (R. L. Malcolm, 1985, personal communication). A standardized method of soil organic matter





extraction has yet to be published because of continual refinement to extraction and purification procedures.

Nuclear magnetic resonance spectroscopy has become a powerful tool in characterizing a complex material such as HA. Liquid state  $^{13}\text{C}$ -NMR spectroscopy has enabled researchers to compute aromatic, aliphatic, and carboxyl groups (Preston and Schnitzer, 1984; Schnitzer and Preston, 1986; 1987; Hatcher et al., 1981; Wilson, 1981; Wilson et al., 1986). Schnitzer and Preston (1986) calculated carboxyl groups from  $^{13}\text{C}$ -NMR spectra of HA and found them to be more closely associated with total acidity titrations than with carboxyl group titrations (Schnitzer and Gupta, 1965). Therefore, a substantial portion of HA reactive groups may be present as carboxyl groups.

The objectives of this study were (1) to compare soil organic matter fractions from tilled and no-tilled cotton plots with different cover crops, (2) to characterize HA using  $^{13}\text{C}$ -NMR spectroscopy, elemental analysis, titration of functional groups, and infrared spectroscopy, and (3) to relate soil organic matter fractions from no-tilled and tilled cotton plots to herbicide phytotoxicity using greenhouse bioassay techniques.

## C. MATERIALS AND METHODS

### 1. Experimental Soils

Soil samples were collected from the 0 to 3.75 cm depth of adjacent cotton plots continuously cropped for seven-years in an

experiment designed to study tillage effects. This experiment was located at the West Tennessee Agricultural Experiment Station in Jackson, Tennessee. The soil was a Lexington silt loam (fine, silty, mixed, thermic Typic Paleudalfs). Cotton plots consisted of three no-till treatments with vetch, rye, and crimson clover cover crops, and one tilled treatment with no cover. All plots received 90 kg ha<sup>-1</sup> of N and uniform amounts of P and K. Lime was uniformly applied at the second growing season. At sampling, soil contained seven percent water ( $\pm 1\%$ ). All soil samples were ground to pass a 2.0 mm sieve.

Soil analyses conducted to characterize the soils included soil pH with a glass electrode pH meter and 1:1 soil to water ratio by volume (McLean, 1982, p.208-209), C by dry combustion (Nelson and Sommers, 1982, p.549-50), cation exchange capacity by summation of cations using ammonium acetate (Thomas, 1982, p.160-161; Yuan, 1959) with Ca, Mg, and K determined by atomic absorption, clay analysis by the pipet method (Gee and Bauder, 1986), and water content at -33kPa, using the pressure plate method (Richards, 1965, p.134-135).

## 2. Soil Organic Matter Fractions and Characterization of Humic Acid

Humic and fulvic acids were extracted based on procedures recommended by the International Humic Substances Society (R. L. Malcolm, 1985, personal communication). Briefly, 90 g of air dry soil was shaken for 30 minutes after adding 50 mL of 1.0 mol L<sup>-1</sup>



HCl and 850 mL of 0.1 mol L<sup>-1</sup> HCl, allowed to settle for two hours and centrifuged (1800 x *g*) for 10 minutes. An aliquot of the supernatant was saved for analysis of soluble fulvic acid C. The supernatant was decanted and 70 mL of 1.0 mol L<sup>-1</sup> NaOH was added to the soil followed by the addition of 830 mL of 0.1 mol L<sup>-1</sup> NaOH. Nitrogen gas was bubbled into the bottles for two minutes and the soil shaken for eight hours and allowed to settle overnight. The soil was centrifuged for ten minutes at 1800 x *g*, and the supernatant decanted into another 1 L centrifuge bottle and acidified to pH 1 with 6.0 mol L<sup>-1</sup> HCl. A sample of extracted soil was saved and acidified with 0.1 mol L<sup>-1</sup> HCl, then dried in an oven at 105<sup>o</sup> C and analyzed for nonextracted carbon. The extracted HA and FA was centrifuged and an aliquot of the supernatant saved for carbon (FA) analysis. Suspended clays were removed by dissolving HA in a minimum volume of 0.1 mol L<sup>-1</sup> KOH under N<sub>2</sub> and adding KCl to make the system 0.3 mol L<sup>-1</sup> with respect to K. Nitrogen gas was bubbled into the containers and they were allowed to stand for 4 h and suspended solids removed by centrifugation. The clear HA solution was acidified to pH 1 and the HA allowed to coagulate. The latter was separated by centrifugation and shaken repeatedly for 24 h at room temperature with 0.1 mol L<sup>-1</sup> HCl-0.3 mol L<sup>-1</sup> HF solution. The HA was washed with small aliquots of deionized water to remove chloride ions, freeze dried, and stored in a desiccator over P<sub>2</sub>O<sub>5</sub> at room temperature.

### 3. Analytical Methods

Carbon in the soils and HA was determined with a Leco Carbon Determinator CR12 by dry combustion. The amount of fulvic acid C was measured using a Beckman Total Organic Carbon Analyzer, Model 915-B. Humic acid (HA) was purified based on the previously cited methods and characterized by elemental composition,  $^{13}\text{C}$ -NMR and infrared spectroscopy, and titrations for total acidity and carboxyl groups. Humic acid N and H were determined by dry combustion with a Perkin Elmer 240 elemental analyzer (Galbraith Laboratories, Inc., Knoxville, Tennessee). Oxygen was calculated by difference. Moisture content of HA was determined by heating at  $105^{\circ}\text{C}$  for 24 h and ash by ignition at  $750^{\circ}\text{C}$  for 4 h. Total acidity and carboxyl groups were determined by methods described by Schnitzer and Gupta (1965). Briefly, total acidity was determined by titration with  $0.5\text{ mol L}^{-1}$  HCl after treatment with  $0.1\text{ mol L}^{-1}$   $\text{Ba}(\text{OH})_2$  solution. Carboxyl groups were determined by titration with  $0.1\text{ mol L}^{-1}$  NaOH after reaction with  $0.5\text{ mol L}^{-1}$  calcium acetate. All titrations were replicated two to four times. Infrared (IR) spectra were recorded on KBr pellets (5.0 mg of HA and 200 mg of dry KBr) from  $4000$  to  $700\text{ cm}^{-1}$  on a Beckman IR 4260 spectrophotometer.

The  $^{13}\text{C}$ -NMR solution spectra for HA's were determined on a JEOL FX 90Q spectrometer. The HA sample (100mg) was dissolved in 2 ml  $0.5\text{ mol L}^{-1}$  NaOD, vortexed 3 minutes, and filtered through a coarse sintered filter. Chemical shifts were measured relative to sodium 3-(trimethylsilyl)propionic acid (TSP). Solution

$^{13}\text{C}$ -NMR spectra were obtained at 22.5 MHz using double precision 16k data points with a 15015 Hz spectral width. A  $45^\circ$  pulse width with a 0.9 sec pulse delay was used to give an acquisition time of 0.534 sec. Optimum conditions were chosen to maximize signal to noise ratio. Between 95,000 and 110,000 scans were collected in the FT mode requiring 36 to 45 h total acquisition time. The spectra were divided into six areas 0 to 40, 40 to 62, 62 to 105, 105 to 150, 150 to 170, and 170 to 190 ppm, and broadly divided from 0 to 105 ppm, aliphatic carbons; 105 to 170 ppm, aromatic carbons; and 170 to 190 ppm carboxyl carbons. The same scale for all NMR spectra was used. Areas were computed by razor cutting the spectra and determining total weight of each group. Areas were also measured with a VT area meter using a video screen. Area measurements agreed  $\pm 5\%$  between the two methods employed and results were reported by averaging the numbers.

#### 4. Greenhouse Experiments

Greenhouse studies were conducted with the four differently treated soil samples to evaluate the reactivity of C, extractable C, fulvic acid, and carboxyl groups in humic acid with herbicides. The tests were conducted using 0.25 L styrofoam pots containing 200 g of soil at 7% moisture. Soils were limed to approximately the pH of the tilled soil (6.2) by adding  $\text{CaCO}_3$ . Nitrogen was added as soluble  $\text{NH}_4\text{NO}_3$  at rates of  $150 \text{ kg ha}^{-1}$ . The soil was moistened to field capacity and allowed to stand overnight before seeding. Twelve pregerminated sorghum (*Sorghum bicolor* (L.) Moench.) seeds

of approximately the same size were planted 1.0 cm deep in each pot. Metribuzin (4-amino-6-*tert*-butyl-3-(methylthio)-1,2,4-triazin-5(4H)one) or oxyfluorfen (2-chloro-1-(3-ethoxy-4-nitrophenoxy)-4-(trifluoromethyl)benzene) were applied to the surface of the soils using a calibrated plant mister at 70 and 140 g ha<sup>-1</sup>. Pots were arranged in a completely random design with four replications and rotated in the greenhouse every 24 h. Plants were harvested at 25 days and plant wet and dry weight recorded. Water use was recorded the last five days of growth. The replications were averaged, divided by the control and linearly regressed against C, extractable C, FA, and COOH of HA.

#### D. RESULTS AND DISCUSSION

##### 1. Soil Properties and Humic Acid Characterization

The four soil samples had similar properties, as expected, since they were from the same soil, (Table 1). However, pH and C varied between soils, with the tilled soil having the highest pH and lowest C values. The largest fraction of total C extracted was from the conventionally tilled soil sample, perhaps indicating that the organic matter in the conventionally tilled soil was more reactive (Table 2). The sample with the next largest amount of extractable C was the no-tilled vetch soil. The largest fraction of FA extracted was from the tilled soil. Humic acid extracted was greatest from the no-tilled vetch soil, followed by the tilled soil. Therefore, the C in these soils was more easily extracted and



Table 1. Chemical and physical characteristics of soils.

<u>Soil Treatment</u> <u>Tillage &amp;</u> <u>Cover</u>	<u>pH</u> <u>1:1</u> <u>(soil/</u> <u>water)</u>	<u>CEC</u> <u>cmol(+)</u> <u>kg<sup>-1</sup></u>	<u>Clay</u> <u>g kg<sup>-1</sup></u>	<u>Water</u> <u>Content</u> <u>at -33kPa</u> <u>%</u>	<u>Total</u> <u>Carbon</u> <u>g kg<sup>-1</sup></u>
NT, Vetch	4.8	8.5	231	21.8	16.6
NT, Rye	5.2	11.1	237	22.2	13.5
NT, Crimson Clover	5.4	11.0	167	23.1	23.5
Till, No Cover	6.2	10.2	194	22.4	9.9

Table 2. Extractable carbon, humic acid, and fulvic acid of soils.

<u>Soil Treatment</u>	<u>Total Carbon</u> <u>g kg<sup>-1</sup></u>	<u>% of Total C</u> <u>Extracted</u>	<u>% of Total C</u>	
			<u>FA</u>	<u>HA</u>
NT, Vetch	16.6	27.7	11.1	8.5
NT, Rye	13.5	25.7	9.9	6.9
NT, Crimson Clover	23.5	22.1	9.5	7.1
Till, No Cover	9.9	33.0	15.7	8.9



perhaps more reactive than C in the no-tilled rye and crimson clover soil treatments. This observation was also apparent from Fig. 1, where it was obvious that C from the tilled soil ( $9.9 \text{ g kg}^{-1}$ ) was able to decrease herbicide activity more than an equal amount of C from the no-tilled soil. It is interesting to note that there was a somewhat linear relationship between the three C values associated with the no-tilled soils. Other studies, using soil organic matter or humic matter to predict herbicide activity, have demonstrated similar results, Weber et al. (1987). However, the fact that C from no-tilled soils was not as reactive as C from tilled soils with these herbicides is important in herbicide applications where rates are often based on soil organic matter content.

Elemental composition reported on a moisture and ash free basis (Table 3) indicated that HA was similar in H composition and except for the no-tilled rye cover treatment was similar in N content. Since the rye cover soil was a non-legume it was expected to have a lower N content. The values for the H/C, O/C, and N/C ratios were in the range reported by Steelink (1985) although the high N to C ratio may be due to N fertilizer added to the soils, as well as the N contained in the legume cover crops. The N from these sources becomes incorporated into soil organic matter fractions.

Infrared spectra of all humic acids resembled each other. No quantitative determinations were made due to broad, overlapping peaks.

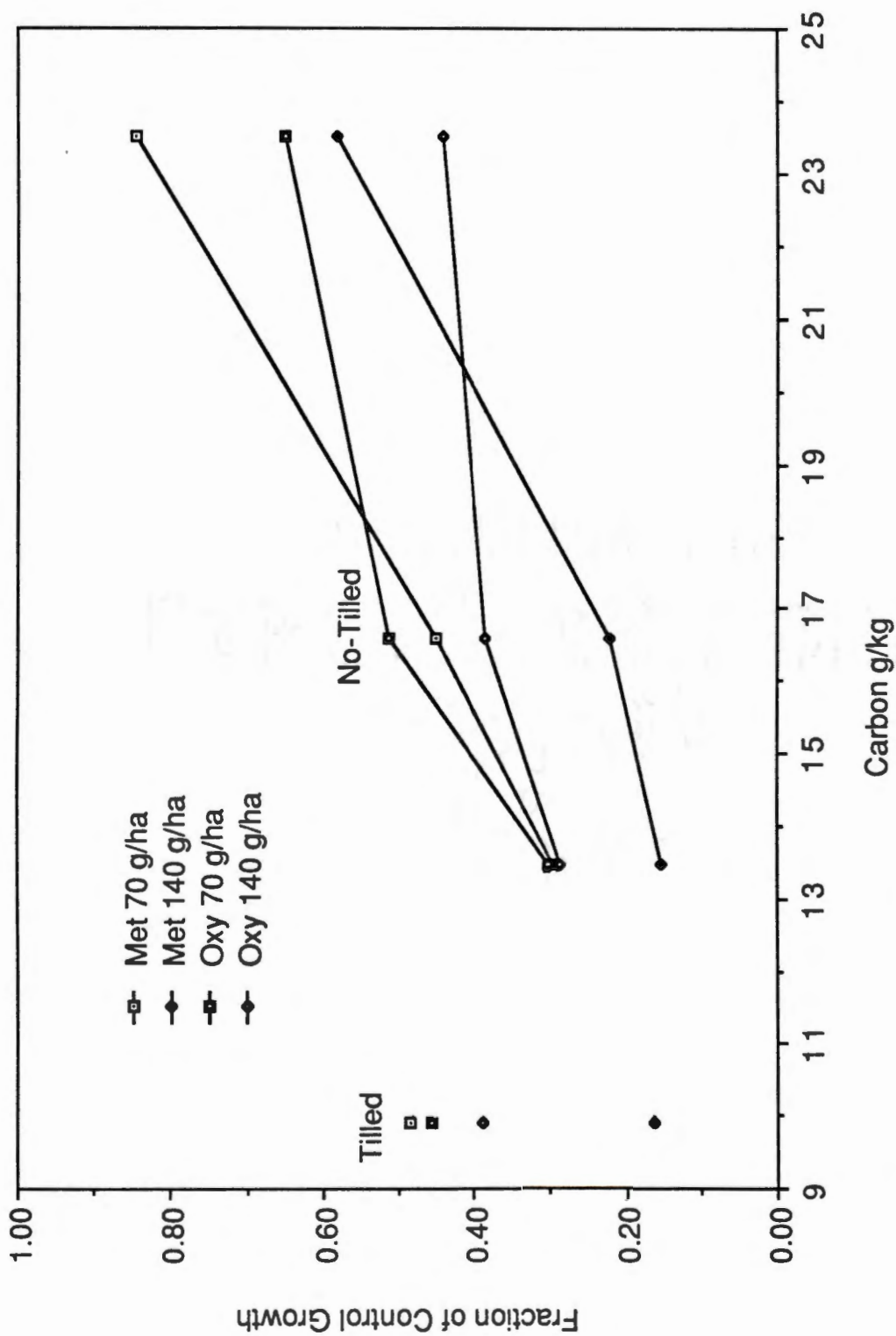


Fig. 1. Relative fresh plant weight as a function of carbon in soil.



Table 3. Elemental analyses and atomic ratios of purified humic acids.

<u>Treatment</u>	<u>(wt/wt%)</u>				<u>Atomic ratios</u>		
	C	H	N	O	H/C	O/C	N/C
NT, Vetch	56.5	5.7	5.2	32.6	1.21	.43	.08
NT, Rye	57.0	5.4	4.1	33.5	1.13	.44	.06
NT, C.Clover	50.6	5.5	5.0	38.9	1.30	.58	.08
Till, No Cover	50.2	5.3	5.3	39.2	1.27	.59	.09





Comparisons of total acidity and carboxyl group determinations of HA by titration, and solution state  $^{13}\text{C}$ -NMR determination of carboxyl groups showed that the total acidity numbers compared more closely with carboxyl groups as determined by NMR (Table 4). Schnitzer and Preston (1986) also, found this to be the case. The values for carboxyl groups by titration were close to that found in their study, while the values reported for total acidity were slightly smaller than those listed by Schnitzer and Preston (1986). Their results for carboxyl groups as determined by NMR were similar in range to those reported here.

The NMR spectra of the HAs are shown in Fig. 2. The integrated peak areas demonstrated the overall similarity between HAs, except the no-tilled vetch soil treatment, which had a larger aliphatic to aromatic ratio (Table 5). The ratio of aliphatic to aromatic groups was close between the other HAs. Of particular interest was the peak area of 170 to 190 ppm where carboxyl groups were located. The carboxyl groups of HA were similar except for the smaller amounts in the no-tilled rye plot. This soil treatment also had the lowest sorghum yield, relative to the control, indicating it was not able to reduce herbicide activity as significantly as other soil treatments.

## 2. Greenhouse Results

Data from the bioassays is depicted in Figures 1,3,4,5 where yield (plant fresh weight) is reported as growth divided by the control growth (fraction of control growth). Plant fresh weight,



Table 4. Concentration of humic acid COOH groups by solution-state  $^{13}\text{C}$ -NMR and titration, and measurements of total acidity.

<u>Soil</u> <u>Humic acid</u>	<u>COOH groups</u>		
	<u><math>^{13}\text{C}</math>-NMR</u> <u>Solution-state</u>	<u>Titration</u>	<u>Total acidity</u>
	<u>mol kg<sup>-1</sup></u>		
NT, Vetch	7.7	3.4	6.9
NT, Rye	6.1	3.9	7.7
NT, Crimson Clover	7.0	4.6	6.9
Till, No Cover	7.3	3.7	7.8



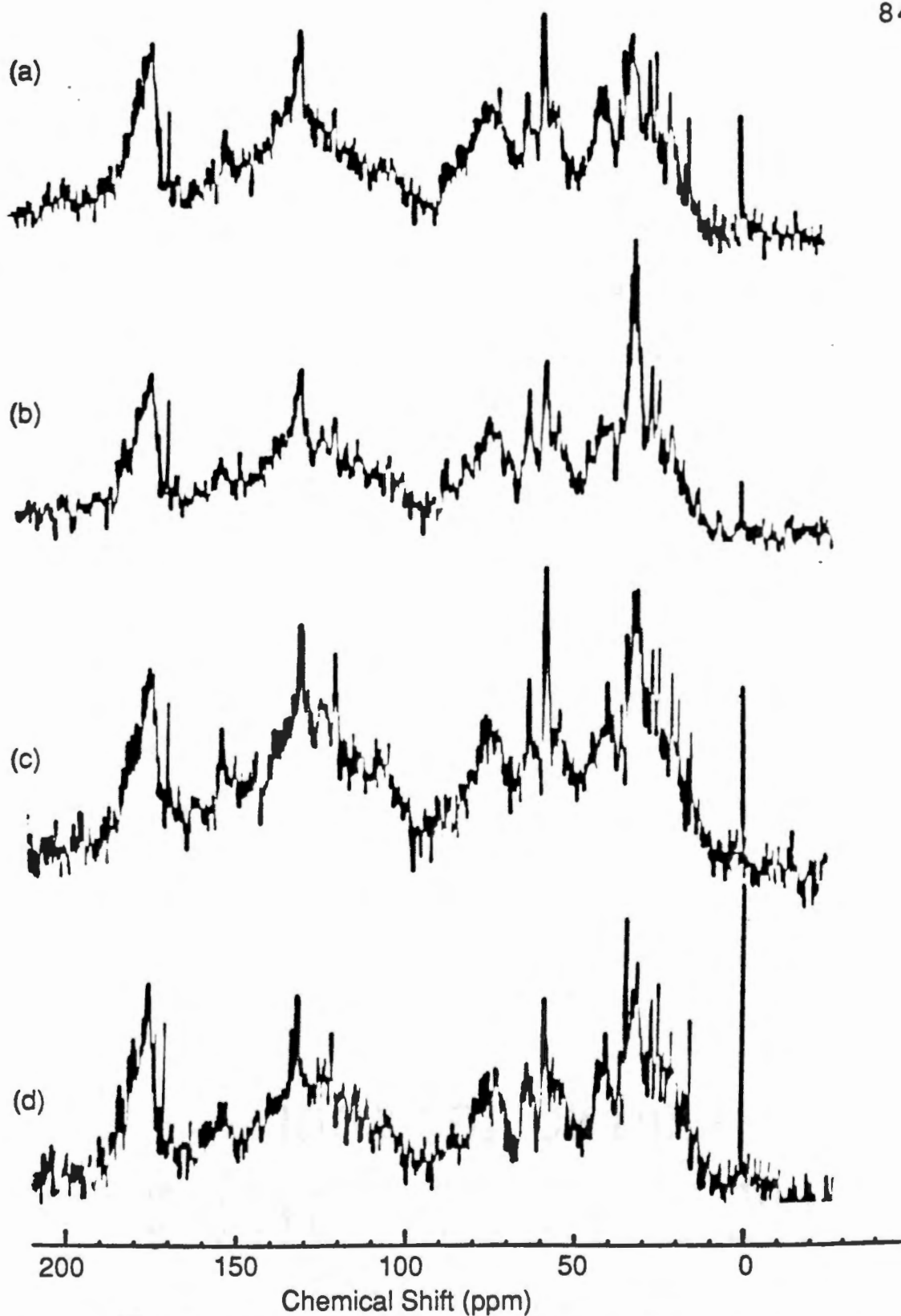


Fig. 2.  $^{13}\text{C}$  NMR spectra of humic acids extracted from cotton plots at 0-3.75 cm depth, (a), tilled, no cover, (b), no-tilled, crimson clover cover, (c), no-tilled, rye cover, and (d), no-tilled, vetch cover.



Table 5. Interpretation of  $^{13}\text{C}$ -NMR spectra of humic acid from cotton plots, 0-3.75 cm depth.

Chemical Shift ppm	<u>NT.Vetch</u>	<u>NT.Rye</u>	<u>NT.C.Clover</u>	<u>Tilled</u>
	<u>wt/wt%</u>			
0-40	24	26	23	19
40-62	17	17	15	16
62-105	19	16	19	20
105-150	25	27	26	28
150-170	4	5	6	6
170-190	11	9	12	12
Aliphatic C	60	58	57	54
Aromatic C	29	33	32	33
Ratio Al/Ar	2.1	1.8	1.8	1.6
Aromaticity	33	36	36	38





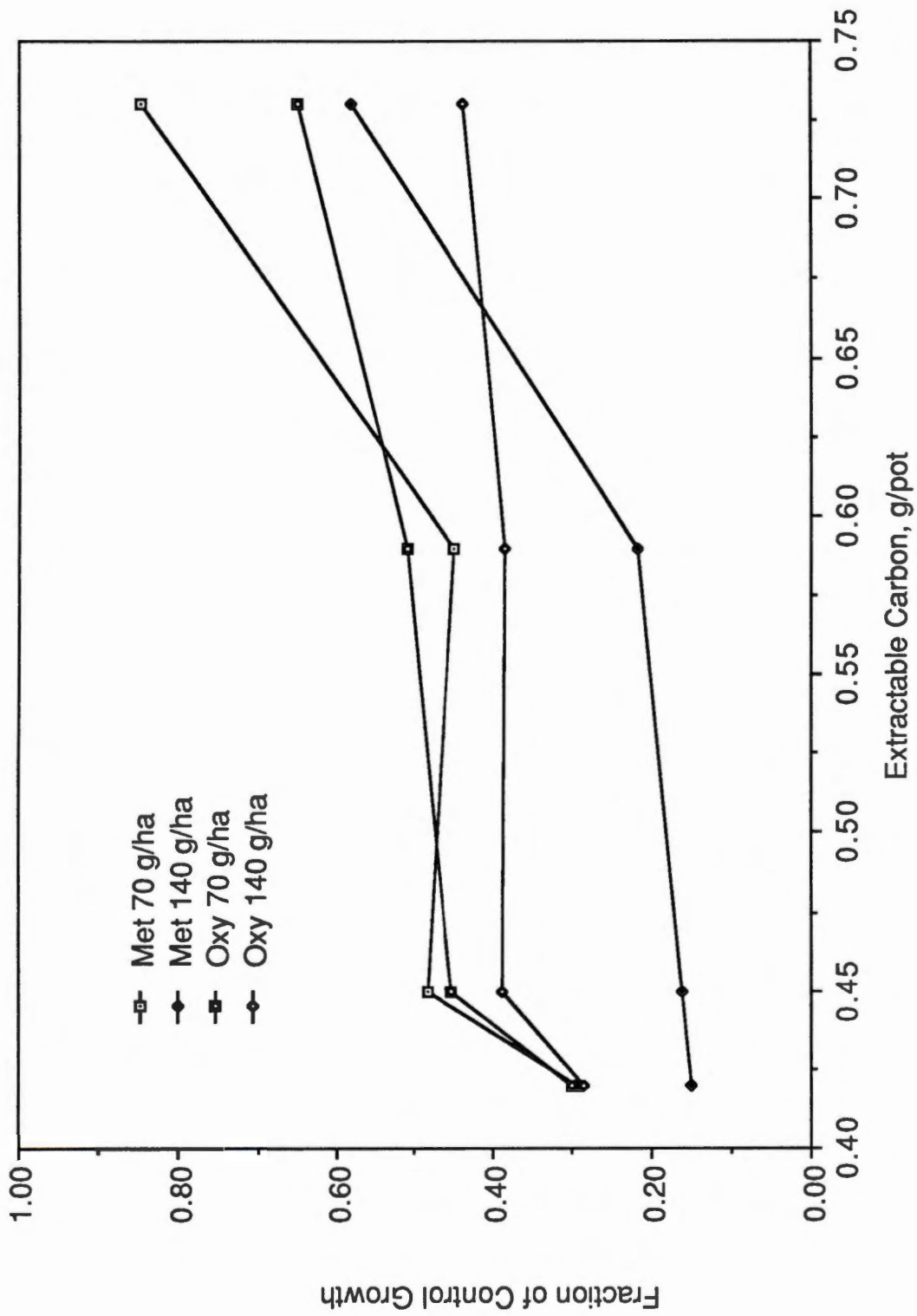


Fig. 3. Relative fresh plant weight as a function of extractable carbon in soil.



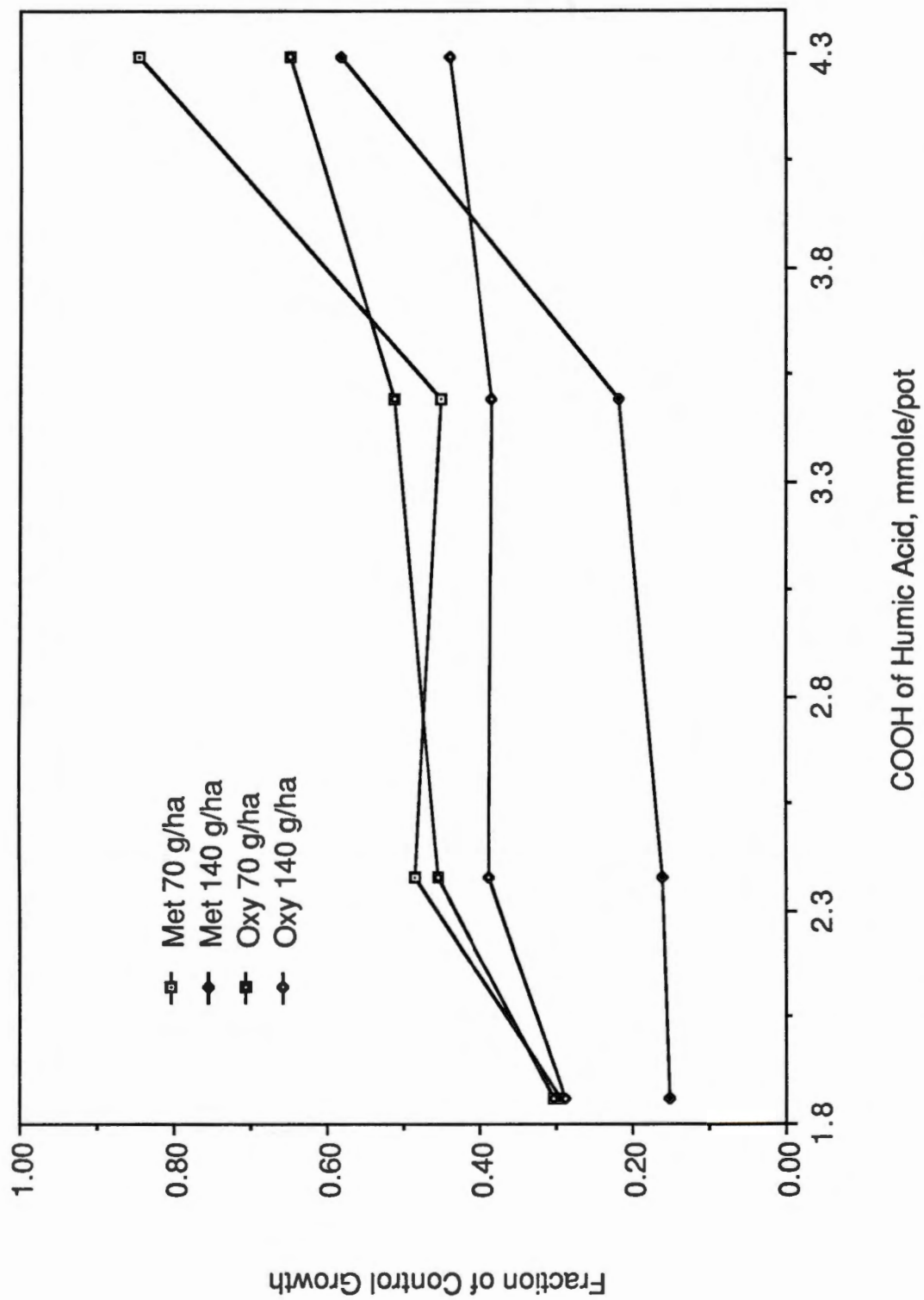


Fig. 4 Relative fresh plant weight as a function of humic acid carboxyl groups determined by  $^{13}\text{C}$ -NMR.



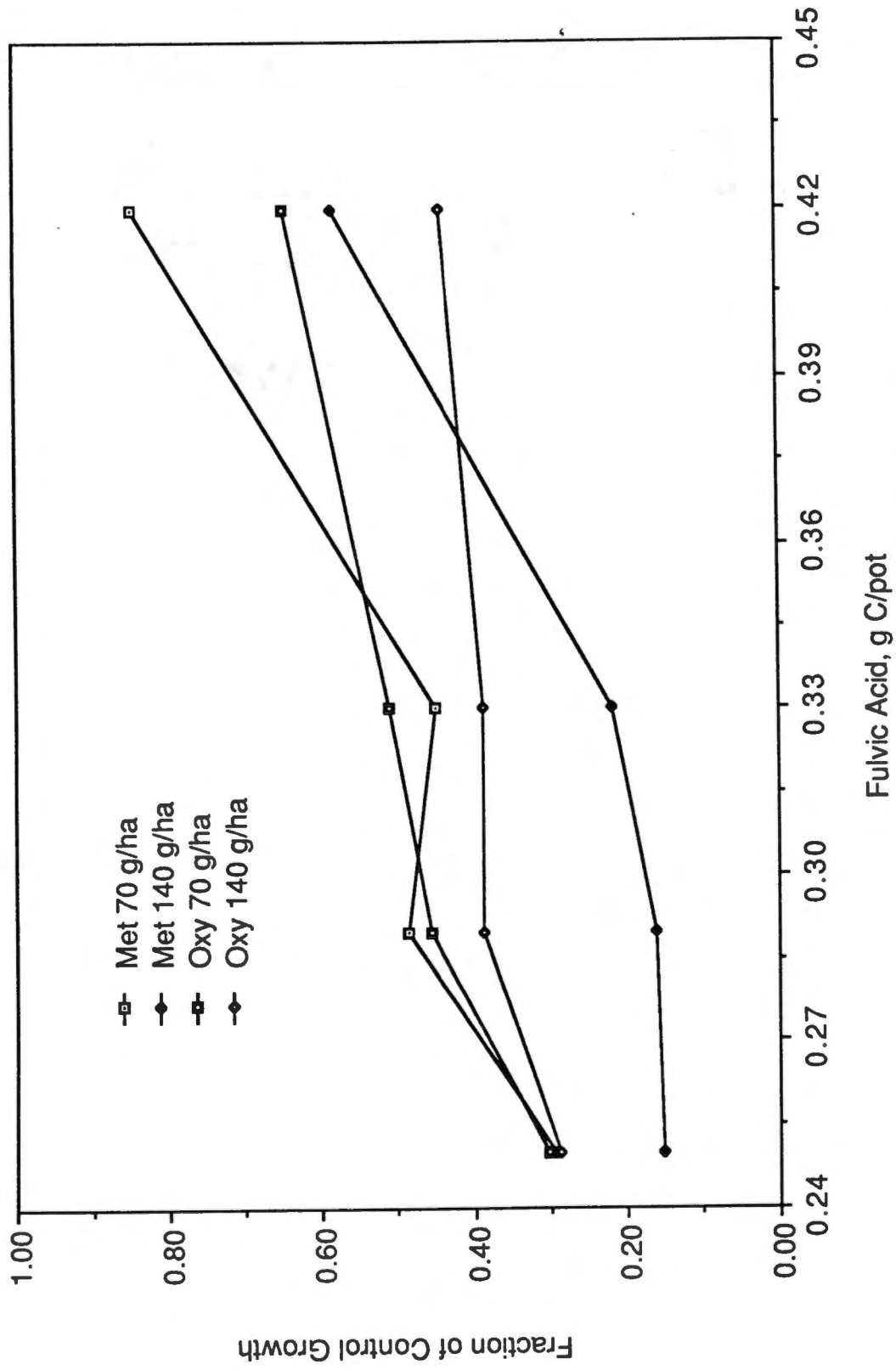


Fig. 5. Relative fresh plant weight as a function of fulvic acid carbon in soil.



dry weight, and water use were highly correlated, and only plant fresh weight was used to portray results. Yield as a function of soil C is reported in Fig. 1. A deviation from linearity was apparent between the first three points, indicating all C was not equally reactive in adsorption of herbicides. The tilled soil treatment was separated from the no-tilled soil treatments, which were connected linearly. The coefficients of determination ( $r^2$ ) indicated that when total C using all four points was linearly regressed against yield the lowest  $r^2$  resulted except with metribuzin at 140 g ha<sup>-1</sup> (Table 6). The tilled soil (C=9.9 g kg<sup>-1</sup>, Table 1) decreased herbicide activity more at a given rate compared to no-tilled soils. However, at the 140 g ha<sup>-1</sup> rate of metribuzin, the tilled soil responded similarly to the no-tilled soils. At the higher rate, sorghum growth was reduced to less than 20% of the control, and the adsorption capacity of the tilled soil was thought to be exceeded. This accounted for low growth with the first three C values, followed by the significant growth increase (59% of control) with the 23.5 g kg<sup>-1</sup> C soil. With increased use of no-tillage the probability that C is less reactive in no-tilled soil compared to tilled soil becomes more important and perhaps less herbicide per total C can be applied to no-tilled soil to achieve the same weed control as in tilled soil. Increased microbial activity in no-tilled soils causing degradation of herbicides might negate the differences in C reactivity.

Yield versus extractable C (Fig. 3) and yield versus carboxyl groups of HA (Fig. 4) demonstrated the similar response of





Table 6. Coefficients of determination ( $r^2$ ) of sorghum fractional growth on NT and T soils receiving herbicide as expressed by various expressions of soil C content.

<u>Regressor</u>	<u>Metribuzin</u>		<u>Oxyfluorfen</u>	
	<u>70 g ha<sup>-1</sup></u>	<u>140 g ha<sup>-1</sup></u>	<u>70 g ha<sup>-1</sup></u>	<u>140 g ha<sup>-1</sup></u>
Total C	.62	.85**	.57	.33
Extractable C	.79	.85*	.86	.87
COOH of HA	.76	.75	.92	.75
Fulvic acid C	.92	.89**	.94+	.79

\*\*Significant at .01 level

\*Significant at .05 level

+Significant at .10 level

increased yield with increasing C fractions. The increased yield with the largest carboxyl value in Fig. 4 was noteworthy; perhaps the herbicides at these rates were inactivated by carboxyl groups at this particular concentration, and growth approached that of the control. When FA was regressed against plant growth the best fit of data resulted (Fig. 5 and Table 6). Perhaps, because of FA content and solubility, it was the most influential in controlling herbicides applied in solution. The improved correlation coefficients (Table 6) compared to other C fractions, indicated that FA content was the best predictor of herbicide phytotoxicity and was an important factor in reducing herbicide activity. Although FA was not characterized in this study, it is considered to be the most reactive portion of organic matter. Fulvic acid has more oxygen functional groups and a larger number of reactive sites than does HA.

#### E. CONCLUSIONS

Based on results from the plant bioassay, C from the tilled soil was more reactive than C from the no-tilled soils. Fulvic acid was important in herbicide availability and can be used to predict herbicide phytotoxicity. However, more study, with increased sample size, is necessary to substantiate these conclusions, which were based on small sample size. Soil HAs were similar in aliphatic to aromatic ratios, with the exception of the no-tilled vetch HA which had more aliphatic components. Yield increased with larger amounts of HA carboxyl groups, extractable C, and FA in

the soil treatments. These C groups were thought to be largely responsible for reduced herbicide activity. Although the tilled soil had the lowest amount of C, it had the greatest fraction of extractable C and FA, and the most reactive C based on decrease in herbicide activity. Both the amount of C in soils and the type of C were important in predicting activity of the herbicides used here.



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## V. SUMMARY

No-tillage has become a popular system of farming in Tennessee and the United States. No-tilled agricultural soils have increased surface organic matter compared to tilled soils under otherwise similar management. The presence of a humus layer and different chemical, physical, and microbiological properties in the surface layers of long term no-tilled soils contrast with the more uniform tilled soil characteristics. Since soil organic matter is a reactive component of soils, it influences herbicide activity and is especially important in the no-till system where herbicides control weed growth. This study was conducted to quantify soil organic matter fractions and to characterize humic acid from various tillage, crop, and depth treatments. A second objective was to correlate tilled and no-tilled soil organic matter fractions with herbicide activity.

Soil from continuous (seven-year) soybean and cotton tillage plots at the West Tennessee Agriculture Experiment Station and corn plots from Ames Plantation were sampled at various surface depths and 7.5 to 15.0 cm depth to compare organic matter fractions. A sorghum greenhouse bioassay was conducted to determine effects of soil organic matter fractions on metribuzin and oxyfluorfen herbicide biological activity using a no-tilled and tilled cotton soil, sampled at 0 to 3.75 cm.

Soil organic matter composed of humic and fulvic acid, was extracted from the soil samples. Carbon, extractable C, FA, and carboxyl groups in HA were compared between soil treatments and correlated with the activity of metribuzin and oxyfluorfen. Fresh sorghum weight was inversely correlated with herbicide activity. Purified HA from soil organic matter was characterized by  $^{13}\text{C}$  liquid state nuclear magnetic resonance spectroscopy, titration of functional groups, and infrared and elemental analyses, and compared based on tillage, depth, crop, and cover crop.

Results demonstrated that the tilled soil was more reactive and reduced herbicide activity to a greater extent per C molecule, than did the no-tilled soils. There was a nearly linear relationship between the no-tilled soils' C and plant growth, indicating that C reacted in a similar manner in these no-tilled plots. In the tilled system, increased plant growth and therefore, reduced herbicide activity, as a function of soil C, indicated the more reactive nature of the tilled soil organic matter. The tilled soil treatment also, had more extractable C and FA than did the no-tilled treatments. Of the soil organic matter fractions, FA best predicted herbicide activity with coefficients of determination ( $r^2$ ) of 0.92 and 0.94 for metribuzin and oxyfluorfen at the  $70 \text{ g ha}^{-1}$  rate.

Soil organic matter and extracted FA increased with sample depth. In the no-tilled surface samples, less decomposed soil

organic matter consisted of less reactive, plant identifiable compounds. This was substantiated by  $^{13}\text{C}$ -NMR solution state characterization of HA where the tilled treatments had larger amounts of aromatic C than did the no-tilled treatments. The aliphatic to aromatic C ratio increased with larger amounts of soil C, which were associated with no-tilled surface soils. With increasing depth and lower soil organic matter a more aromatic HA resulted, indicative of a more decomposed state. As organic matter decomposed the more easily weathered carbohydrates and other aliphatic C were the first to oxidize, and the more resistant aromatic structures remained.

Humic acid from all treatments had relatively large amounts of N (4.8%), and H (5.6%), indicating an aliphatic C structure. Carbon (52.7%), and O (36.9%) of humic acid were similar in range to values reported by other studies. The vetch cover no-tilled soil sample and soybean soil samples had, as expected, slightly larger amounts of HA nitrogen, compared to non-legume crops and cover crops.

Humic acid from cotton and corn plots was quantitatively and qualitatively similar in their NMR spectra, while that from soybean plots had slightly more aromatic and carboxyl compounds. Tilled and no-tilled HAs were slightly different, with the tilled being more aromatic and carboxyl in nature. Carboxyl groups as determined by NMR compared more closely with total acidity than carboxyl group determination by titration.

The most pronounced differences between HAs occurred with depth as surface samples had more aliphatic C, while subsurface 7.5-15.0 cm samples had more aromatic C. Small differences were observed between tillage systems, although in terms of reactivity in bioassay studies, the tilled soil was able to reduce herbicide activity to a greater degree than the no-tilled soil on a per C basis.

Nuclear magnetic resonance spectroscopy characterization of HAs, revealed structural differences between samples based on depth, crop, and tillage. Perhaps in the near future, soil organic matter will be more specifically characterized by solid-state  $^{13}\text{C}$ -NMR without using a strong, alkaline extracting solution, which may create artifacts, as well as mask differences between samples.

100X200mm PAPER  
1. MICROFILM FORM I  
- 2014-2015

**APPENDIX**

Sorghum Bioassay Raw Data

<u>Plant fresh weight g</u>	<u>Plant dry weight g</u>	<u>Water use ml</u>	<u>Carbon g kg<sup>-1</sup></u>	<u>FA g pot<sup>-1</sup></u>	<u>Ext. C</u>	<u>COOH of HA mmole pot<sup>-1</sup></u>
<u>Control</u>						
2.05	0.26	35.3	16.6	0.33	0.59	3.49
2.36	0.31	35.8	16.6	0.33	0.59	3.49
2.76	0.36	46.5	16.6	0.33	0.59	3.49
2.13	0.28	38.2	16.6	0.33	0.59	3.49
2.17	0.24	36.0	13.5	0.25	0.42	1.86
3.00	0.33	46.8	13.5	0.25	0.42	1.86
2.49	0.29	38.9	13.5	0.25	0.42	1.86
2.93	0.33	50.8	13.5	0.25	0.42	1.86
1.58	0.21	31.7	23.5	0.42	0.73	4.29
1.88	0.23	37.4	23.5	0.42	0.73	4.29
1.74	0.19	35.5	23.5	0.42	0.73	4.29
2.07	0.25	37.2	23.5	0.42	0.73	4.29
2.70	0.30	48.1	9.9	0.29	0.45	2.38
2.92	0.35	46.4	9.9	0.29	0.45	2.38
3.25	0.37	57.4	9.9	0.29	0.45	2.38
3.10	0.35	50.5	9.9	0.29	0.45	2.38
<u>Metribuzin 70 g ha<sup>-1</sup></u>						
1.22	0.15	27.0	16.6	0.33	0.59	3.49
0.90	0.13	21.5	16.6	0.33	0.59	3.49
1.41	0.19	30.3	16.6	0.33	0.59	3.49
0.68	0.11	23.9	16.6	0.33	0.59	3.49
0.64	0.12	18.3	13.5	0.25	0.42	1.86
0.44	0.08	17.4	13.5	0.25	0.42	1.86
0.82	0.14	20.6	13.5	0.25	0.42	1.86
1.22	0.14	30.8	13.5	0.25	0.42	1.86
1.72	0.21	29.8	23.5	0.42	0.73	4.29
1.49	0.19	34.3	23.5	0.42	0.73	4.29
1.86	0.23	32.3	23.5	0.42	0.73	4.29
1.09	0.15	29.0	23.5	0.42	0.73	4.29
2.03	0.23	35.6	9.9	0.29	0.45	2.38
1.34	0.17	28.8	9.9	0.29	0.45	2.38
1.18	0.17	27.3	9.9	0.29	0.45	2.38
1.24	0.15	30.9	9.9	0.29	0.45	2.38

Sorghum Bioassay Raw Data

<u>Plant fresh</u> <u>weight g</u>	<u>Plant dry</u> <u>weight g</u>	<u>Water use</u> <u>ml</u>	<u>Carbon</u> <u>g kg<sup>-1</sup></u>	<u>FA</u> <u>g pot<sup>-1</sup></u>	<u>Ext. C</u>	<u>COOH of HA</u> <u>mmole pot<sup>-1</sup></u>
<u>Metribuzin 140 g ha<sup>-1</sup></u>						
0.53	0.10	22.1	16.6	0.33	0.59	3.49
0.33	0.11	20.7	16.6	0.33	0.59	3.49
0.75	0.11	25.4	16.6	0.33	0.59	3.49
0.41	0.07	22.1	16.6	0.33	0.59	3.49
0.26	0.08	19.5	13.5	0.25	0.42	1.86
0.25	0.10	19.8	13.5	0.25	0.42	1.86
0.40	0.08	17.9	13.5	0.25	0.42	1.86
0.69	0.12	21.1	13.5	0.25	0.42	1.86
1.29	0.17	31.1	23.5	0.42	0.73	4.29
1.04	0.15	24.1	23.5	0.42	0.73	4.29
0.75	0.12	27.5	23.5	0.42	0.73	4.29
1.16	0.15	26.7	23.5	0.42	0.73	4.29
0.24	0.11	19.2	9.9	0.29	0.45	2.38
0.41	0.14	25.6	9.9	0.29	0.45	2.38
0.67	0.13	25.7	9.9	0.29	0.45	2.38
0.58	0.10	20.9	9.9	0.29	0.45	2.38
<u>Oxyfluorfen 70 g ha<sup>-1</sup></u>						
0.93	0.12	25.7	16.6	0.33	0.59	3.49
1.48	0.19	32.8	16.6	0.33	0.59	3.49
1.31	0.17	26.8	16.6	0.33	0.59	3.49
1.05	0.14	29.6	16.6	0.33	0.59	3.49
0.85	0.11	27.4	13.5	0.25	0.42	1.86
1.13	0.13	30.2	13.5	0.25	0.42	1.86
0.39	0.05	20.5	13.5	0.25	0.42	1.86
0.82	0.10	26.5	13.5	0.25	0.42	1.86
1.29	0.16	30.9	23.5	0.42	0.73	4.29
1.14	0.15	31.9	23.5	0.42	0.73	4.29
1.05	0.12	30.7	23.5	0.42	0.73	4.29
1.25	0.16	29.8	23.5	0.42	0.73	4.29
1.24	0.15	28.4	9.9	0.29	0.45	2.38
1.49	0.18	30.1	9.9	0.29	0.45	2.38
1.06	0.12	27.2	9.9	0.29	0.45	2.38
1.66	0.21	33.8	9.9	0.29	0.45	2.38

Sorghum Bioassay Raw Data

<u>Plant fresh</u> <u>weight g</u>	<u>Plant dry</u> <u>weight g</u>	<u>Water use</u> <u>ml</u>	<u>Carbon</u> <u>g kg<sup>-1</sup></u>	<u>FA</u> <u>g pot<sup>-1</sup></u>	<u>Ext. C</u> <u>g pot<sup>-1</sup></u>	<u>COOH of HA</u> <u>mmole pot<sup>-1</sup></u>
		<u>Oxyfluorfen 140 g ha<sup>-1</sup></u>				
0.79	0.11	26.8	16.6	0.33	0.59	3.49
0.84	0.12	25.2	16.6	0.33	0.59	3.49
0.56	0.08	25.8	16.6	0.33	0.59	3.49
1.41	0.19	32.0	16.6	0.33	0.59	3.49
0.48	0.07	23.0	13.5	0.25	0.42	1.86
1.03	0.12	31.1	13.5	0.25	0.42	1.86
0.73	0.09	22.1	13.5	0.25	0.42	1.86
0.80	0.11	21.9	13.5	0.25	0.42	1.86
0.35	0.05	21.8	23.5	0.42	0.73	4.29
0.89	0.11	32.0	23.5	0.42	0.73	4.29
1.14	0.15	25.0	23.5	0.42	0.73	4.29
0.80	0.11	25.1	23.5	0.42	0.73	4.29
0.59	0.08	23.4	9.9	0.29	0.45	2.38
1.30	0.16	30.9	9.9	0.29	0.45	2.38
2.35	0.27	43.1	9.9	0.29	0.45	2.38
0.41	0.05	21.6	9.9	0.29	0.45	2.38



## VITA

Gordon Kim Stearman was born in Phoenix, Arizona on September 12, 1951. He attended elementary schools in that city, and upon graduation from Central High School in June 1969, attended Amherst College in Massachusetts for two years. After working in construction for three years in Arizona and Florida he enrolled at the University of Arizona and graduated, May, 1976, with a B.S. degree in Agricultural Chemistry and Soils. After working for Asgrow Seed Co. for six months he joined the Peace Corps where he was an agronomist for a basic grains cooperative in Jalapa, Nicaragua for two years. In August, 1981, he completed his M.S. degree in Agronomy (Soil Chemistry), from the University of Missouri where he studied phosphate solubility and equilibria in soils. He then worked for the Cooperative Extension Service at the College of the Virgin Islands, setting up the first soil, plant, and water testing laboratory in the area and teaching courses in agronomy. He enrolled at the University of Tennessee in September, 1984, and received the Doctor of Philosophy degree with a major in Plant and Soil Science in December, 1987.

The author is a member of Sigma Xi and Gamma Sigma Delta, scientific and agricultural honor societies, respectively. He is married to the former Gail Ann Wehrmann, and has a son, Bo. The birth of his son, Taylor was celebrated as he completed his dissertation.