

Long-term Effects of Tillage on the Composition of Soil Organic Matter: Spectroscopic Characterization

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Soil organic matter (SOM) is a significant component of soils and is a fundamental contributor to the overall soil quality. It is also a sensitive indicator of changes in agricultural management.

Humic acids (HA) are conventionally defined as the alkali soluble and acid insoluble fraction of SOM (STEVENSON, 1994). Due to their relatively large molecular size, they belong to the colloidal fraction of soils. This fraction is functionally stable and yet is still chemically reactive in the soil. From an agricultural point of view both the stability and reactivity characteristics of humic substances are important, as these characteristics play a significant role in determining SOM levels in the soil, and in the contribution of SOM to soil productivity.

Spectroscopic methods are important in the investigation of the structure and composition of humic substances. Infrared spectroscopy is frequently used in SOM characterization (GERASIMOWICZ & BYLER, 1984; NIEMEYER et al., 1992). Most studies were done by transmission (FTIR) (KBr pressed-pellet method) and diffuse reflectance (DRIFT) Fourier transform infrared spectroscopy (BAES & BLOOM, 1989). WANDER & TRAINA (1996) made semi-quantitative examinations with DRIFT on SOM searching for management induced differences in SOM chemistry. They determined the chemical reactivity of humic substances by comparing the reactive to recalcitrant functional group peak intensity ratios.

¹³C nuclear magnetic resonance (NMR) spectroscopy, both liquid and solid phase, is a relatively new but informative technique for SOM characterization (PRESTON, 1996). A number of studies have been addressed to examine and establish conditions for the reliable determination of the composition of extracted humic substances and in whole soil samples (e.g. PRESTON & SCHNITZER, 1987; HATCHER et al., 1981, KINCHESH et al., 1995). ¹³C cross-

polarization magic angle spinning (CPMAS) NMR has been successfully applied for both qualitative and quantitative analysis of humic materials (BALDOCK et al., 1990). SCHULTEN et al. (1990) characterized cultivation effects on soil organic matter with ^{13}C NMR and made conclusions for the change in the aromaticity of humic substances.

Tillage is one of the most aggressive interferences in the natural conditions of soils. The effect of tillage systems on the physical, chemical and biological properties of soils, including the quantity and distribution of SOM, has long been of interest in agricultural research (e.g. MANN, 1986; DALAL et al., 1991). On the other hand the effect of tillage on the composition of SOM has not been thoroughly studied. The objective of this study was to examine the effects of two tillage systems - conventional tillage (mouldboard plough) (CT) and no-till (NT) - on the function of soil organic matter (SOM), with special attention to the humic acid (HA) fraction. ^{13}C CPMAS NMR and FTIR spectroscopy supplemented with redox titration have been applied in the investigation.

Materials and Methods

Soil samples have been collected from a 21 year long-term tillage experiment at the Agronomy Research Farm, Purdue University, Indiana, USA. Treatments consisted of two tillage systems: conventional tillage (mouldboard plough) (CT) and no-till (NT). The cover crop on the examined plots was continuous corn. Detailed description of the experimental sites has been provided by WEST et al. (1996).

The soil is a fine-silty, mixed, mesic Aquic Haplaquoll (Soil Survey Staff, 1992). Composite samples were collected from the 0-15 cm (tillage affected) and the underlying 15-30 cm (reference or undisturbed) layers. The SOM content of the treatments was 2.31 and 2.82 percent in the 0-15 cm layer and 1.69 and 1.70 percent in the 15-30 cm layer in CT and NT, respectively.

Humic substances were extracted with sodium hydroxide (NaOH) and fractionated from each sample in 6 replicates by the standard procedure (STEVENSON, 1994).

^{13}C CPMAS NMR and transmission FTIR spectroscopy have been applied to investigate characteristics of HAs in solid state and redox titration has been used to examine their properties in (colloid) solution.

FTIR data have been obtained by a Perkin-Elmer 1800 Fourier Transform Infrared Spectrophotometer and analyzed by the GRAMS/386 Version 2.01 software. Transmission FTIR spectra of KBr pellets of HAs were obtained. To prepare pellets 1.25 mg humic acid was mixed with 250 mg KBr and 50 tons pressure was applied to the dies for 10 minutes under vacuum.

The ^{13}C CPMAS NMR measurements have been done using a Bruker 250 MHz spectrometer at 62.9 MHz. The acquisition time was 0.051 ms. The spectra were smoothed with 50 Hz line broadening function. A 5.5 μsec pulse

width and a contact time of 3 msec was applied. A delay of 3 sec was used between each pulse sequence. Tetramethylsilane was applied as internal standard.

Redox titration has been carried out with NaOCl on whole soil samples and on the HA fractions by a titrator assembled by Imre Czinkota.

Results and Discussion

^{13}C CPMAS NMR

The HAs gave well defined ^{13}C NMR spectra in which all the major peaks of humic substances can be identified: carbonyl (190-220 ppm), carboxyl (165-190 ppm), phenolic-OH (145-165 ppm), aromatic (105-165 ppm), substituted alkyls (48-105 ppm), alkyls (0-48 ppm) (PICCOLO et al., 1990; HATCHER et al., 1981).

The spectra of HAs extracted from the tillage affected layer and the underlying reference layer of the NT and CT soils are shown in Figure 1.

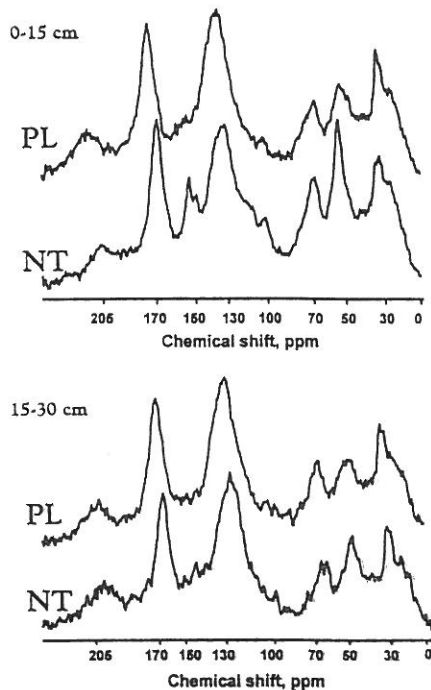


Figure 1

^{13}C CPMAS NMR spectra of HAs in the tillage affected (0-15 cm) and reference (15-30 cm) layers of CT (upper spectrum) and NT (lower spectrum) soils

To specify the differences between the spectra of NT and CT soils, the percentage contribution of each peak to the total area of the NMR spectra has been calculated, and subsequently the following ratios, as measures of functionality of HAs, have been obtained for each sample:

The *aromatic/carboxylic* ratio is aimed to describe the proportion of the stable and reactive compounds in the HA fractions.

The *aromatic/aliphatic* ratios describe the proportion of the stable and labile, readily decomposable parts.

Hatcher's aromaticity was first calculated by HATCHER et al. (1981): in this index the relative amount of the total aromatic part is compared to the whole area of the spectrum.

The values for the above defined ratios are shown in Table 1.

Table 1
Ratios of functionality of extracted HAs from CT and NT soils
in the tillage affected (0-15 cm) and reference (15-30 cm) layers

Ratio	0-15 cm PL	0-15 cm NT	15-30 cm PL	15-30 cm NT
Aromatic/ Carboxylic	1.72 ± 0.032	2.35 ± 0.041	1.89 ± 0.047	1.90 ± 0.046
Aliphatic/ Aromatic	1.56 ± 0.029	2.15 ± 0.037	1.07 ± 0.026	1.09 ± 0.026
Hatcher's aromaticity	51.86 % ± 0.94 %	41.39 % ± 0.87 %	58.49 % ± 1.24 %	55.44 % ± 1.2 %

The *aromatic/carboxylic* ratios of the tillage affected layers indicate that HAs in the CT soils contain relatively more carboxylic compounds than HAs under NT treatments, as this ratio is smaller for the CT samples.

Both the simple *aromatic/aliphatic* ratio and Hatcher's aromaticity indices suggest that HAs in CT soils are more aromatic in nature.

In the reference layer, the above examined characteristic ratios do not show to be significantly different.

FTIR

To support the results obtained by ¹³C NMR spectroscopy, FT-IR spectra of the HA fractions have been collected. Due to the heterogeneity of the HA fraction, and the constituent molecules themselves, there is a number of characteristic absorption bands in the IR spectrum. Most of the absorption bands are not characteristic for one type of molecule vibration only, thus in certain cases it may be difficult to identify them. In this study two characteristic and relatively well identifiable absorption bands were selected to compare tillage induced differences in the composition of HAs: at 1720 cm⁻¹ wavenumber the

(carbonyl) C=O stretching of carboxylic groups, and at 1619 cm^{-1} wavenumber the C=C stretching of basically aromatic compounds can be found. The intensity of these two peaks compared to each other can give qualitative information on the relative occurrence of carboxylic and aromatic compounds in HAs.

The stretching region between $2940\text{--}2900\text{ cm}^{-1}$ is characteristic of the C-H stretching in aliphatic compounds. The more well defined this area is, the more homogeneous, and/or less decomposed the aliphatic compounds are in the sample. Representative FTIR spectra of HAs in the tillage affected layer of CT and NT soils can be seen in Figure 2.

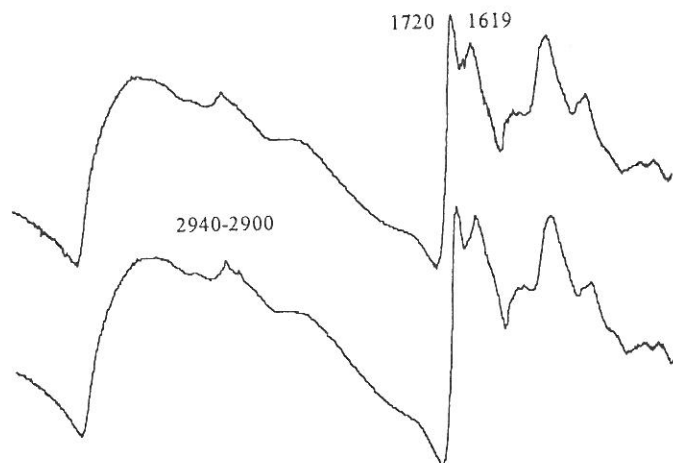


Figure 2

Representative FTIR spectra of HAs in the tillage affected (0-15 cm) layer of CT (upper spectrum) and NT (lower spectrum) (Absorbance in wavenumbers, cm^{-1})

In the tillage affected layer, the intensity of the (carbonyl) C=O stretching peak compared to the aromatic C=C stretching one is higher in the CT than in the NT treatment, which indicates relatively more carboxylic compounds in the CT treatment compared to NT. The aliphatic C-H stretching region of HAs in NT is better defined than that in CT, suggesting that the aliphatic components in the NT treatment have a more homogeneous character.

Redox titration

With redox titration the redox buffer capacity of soils and HAs was measured. NaOCl as a weak oxidizing agent cannot oxidize the C-C and C-H bonds (neither aromatic nor aliphatic), but can oxidize alcohols, aldehyds, occasionally phenols and terciar amines. The more NaOCl the material needs to reach the same redox potential change the less oxidized the material was before titration.

Redox titration curves indicate (Figure 3) that NT soils are in a less oxidized state and the same trend can be noticed in case of the extracted HAs. The HAs extracted from the tillage affected layer of the CT soil proved to be more oxidative, hence chemically more reactive than HAs in soils under NT.

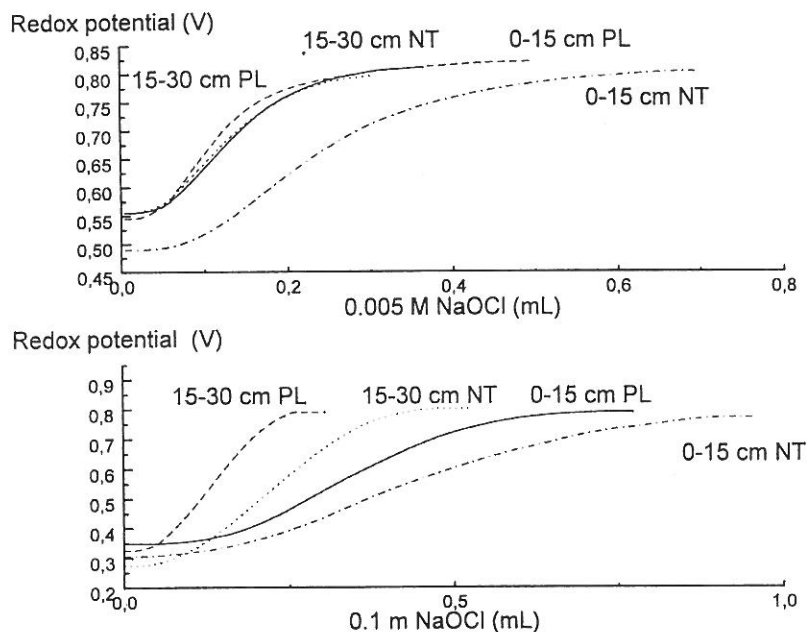


Figure 3
Redox titration curves of whole soils (lower diagram) and extracted HAs (upper diagram)

Conclusions

Data from ^{13}C CPMAS NMR and FTIR spectroscopic analysis of HAs indicate that HAs in the tillage affected layer of CT soils are more aromatic in nature than HAs under NT. At the same time HAs in soils under CT contain relatively more reactive oxygen containing functional (carboxylic) groups than those in soils under NT. The differences between HAs in the two tillage systems have been detected only in the tillage affected layer, while HAs in the reference layer of the CT and NT treatments showed no significant difference.

The differences in the tillage affected layers of the two examined tillage systems could be due to the aeration of the soil following mechanical disturbance. Aeration of the soil may contribute to a faster decomposition of SOM, including HAs. The observed higher aromaticity in HAs of CT soils can be the result

of this, so that the more readily decomposable aliphatic chains in the HA fraction break down faster under CT. Consequently, the higher relative aromaticity of HAs in CT soils does not necessarily combine with higher stability of SOM. The higher relative amount of oxygen containing reactive functional groups in HAs of CT soils may also be the result of intense aeration of the soil. This latter result has also been supported by redox titration of whole soils and extracted HAs of the same CT and NT treatments, suggesting that both whole soils and HA extracts from the CT treatment are more oxidative in nature than those under NT.

The importance of physical (spectroscopic) methods in the characterization of SOM pools combined with chemical analysis should also be emphasized. The relatively new powerful techniques, like NMR, provide the possibility for a more detailed investigation on the effect of agricultural management systems on SOM changes at the molecular level. Concerning that the properties and function of SOM, and mainly the humified fraction, is determined by its molecular composition (HAYES, 1994), being able to exploit this information for the prediction of management induced changes of SOM quality in sustainable agricultural systems is of utmost importance.

Summary

The effects of two tillage systems - conventional tillage (including mould-board plough) (CT) and no-till (NT) - on the function of soil organic matter (SOM), with special attention to the humic acid (HA) fraction were studied in the framework of the research project supported by the USA-HU S&T (F.J. No. 93-b379) and are presented in this paper. ^{13}C cross-polarization magic angle spinning nuclear magnetic resonance (^{13}C CPMAS NMR) and Fourier transform infrared (FTIR) spectroscopy and redox titration have been applied in the investigation. The data obtained from spectroscopic methods were supported by data from chemical analysis.

^{13}C CPMAS NMR and FTIR spectroscopic investigations proved to be adequately sensitive to examine slight tillage induced changes in the molecular composition of HAs and suggested that HAs in the tillage affected layer of soils under CT are relatively more aromatic and less aliphatic in nature than under NT. HAs under CT also contain relatively more oxygen containing acidic functional groups (carboxylic groups). This latter result was supported by redox titration suggesting an overall more oxidized state of CT soils and extracted HAs compared to NT.

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