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Soil Organic Matter as Indicator of Changes in the Environment. Anthropogenic Influences: Tillage

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Introduction

Soils contain a major pool of the active organic carbon (OC) reserve on Earth. Besides having an influence on soil colour, structure, nutrient supply, biological activity, soil organic matter (SOM) – through its transformation – has an impact on the atmospheric CO_2 concentrations. Its comprehensive role makes its management a problem of key importance in sustainable agricultural systems (STEVENSON, 1994).

From an agricultural point of view the understanding of the reactivity, stability and finally the function of SOM is important. The relatively stable SOM pool consists of humic substances (HS). This is the fraction of SOM that is able to resist microbial decomposition to the greatest extent, and hence, helps maintaining the organic matter level of soils, while at the same time it posesses a considerable chemical reactivity through which it contributes to the productivity of soils. It is believed, that the answer to the functionality of HS is hidden in their structural composition (HAYES et al, 1989). Therefore, investigating changes in the molecular composition of HS imposed by agricultural activity may provide substantial information on the effect of agricultural practices on their function in the soil.

One of the most radical agricultural interventions into the natural state of soils is tillage. Apart from influencing the physical, chemical and biological properties of soils, it also has an impact – partly through the above-mentioned properties – on the level and chemistry of SOM (e. g. GANTZER & BLAKE, 1978; MANN, 1986; HAVLIN et al., 1990; DALAL et al., 1991; CARTER & RENNIE, 1982).

In the literature little information is provided about the effect of tillage systems on the composition of the relatively stable SOM fraction, the HS. Results from ongoing examinations carried out by researchers in different parts of the world (STEARMAN et al., 1989a; ARSHAD et al., 1990; MACHADO & GERZABEK, 1993) seem to support one another. STEARMAN et al. (1989b) cautiously stated that tillage slightly influences HA composition and reactivity based on results from a 7-year experiment.

Infrared spectroscopy is frequently used in SOM characterization (GERASIMOWICZ & BYLER, 1984; NIEMEYER et al., 1992; BAES & BLOOM, 1989; WANDER & TRAINA, 1996; JOHNSTON et al., 1994). Under proper conditions ^{13}C CPMAS NMR proved to be a promising tool for both qualitative and quantitative analysis of humic materials (BALDOCK et al., 1990; KINCHESH et al., 1995). STEARMAN et al. (1989a) and SCHULTEN et al. (1990) successfully characterized cultivation effects on SOM with ^{13}C NMR and made conclusions for structural changes in HS.

The objective of this study was to measure the effects of tillage on SOM composition and consequently on its function by comparing the humic acid (HA) fraction of SOM extracted from no-till (NT) and conventionally tilled (plowed) (PL) soils from a 21- and 10-year long-term tillage experiment at the time of sampling.

Materials and Methods

Origin of soil samples

Soil samples were collected from the 0-15 and 15-30 cm layers of two long-term tillage experiments.

The Griffith plots at the Agronomy Research Center (ARC) of Purdue University (West Lafayette, Indiana, USA) were set up in 1975 on a Chalmers silty clay loam (fine-silty, mixed, mesic Typic Haplaquoll) (SOIL SURVEY STAFF, 1992). Detailed description of the experiment has been provided by WEST et al. (1996).

The long-term tillage experiment at the Throckmorton Purdue Agronomy Center (PAC) was set up in 1986 on a Toronto-Millbrook silt loam (fine-silty, mixed, mesic Udollic Ochraqualf) (SOIL SURVEY STAFF, 1992).

Plots that have been being continuously under PL and NT were examined. The cover crop on the examined plots was continuous corn in case of both tillage systems and treatments.

Except for tillage, the examined plots have been treated uniformly in each experiment.

Sampling, sample preparation

Although the examined long-term tillage experiments are small plot arrangements, single samples have been collected from 5 places and sub-composite samples (each containing 4 single samples) from 4 places of each plot. The PL and NT treatments were set up in four replicates in the ARC experiment and there were no replications of the treatments in the PAC experiment.

Humic substances were extracted and fractionated from composite samples in 6 replicates using 0.5 *M* sodium hydroxide (NaOH) as in the standard procedure described by STEVENSON (1994). The HA fractions were dialized against 0.1 *M* HCl, then against deionized water and finally were freeze dried and studied in solid state.

Methods of examination

Organic carbon (OC) content of soil samples were determined by rapid dichromate oxidation using the Walkly & Black protocol (SSSA, 1982).

The cation exchange capacity (CEC or T-value, as referred to in the Hungarian literature (STEFANOVITS, 1992)) of the soil samples has been determined by the modified Mehlich method (BUZÁS, 1988).

The total C and N content of the HA samples was determined by high temperature dry combustion using a Perkin Elmer NA 1500 Series 2 C/N Analyzer.

^{13}C CPMAS NMR measurements have been done using a Bruker 250 MHz spectrometer at 62.9 MHz. The aquisition 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. Tetramethylsilene was applied as internal standard.

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 the KBr 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.

Results and Discussion

Organic carbon content, CEC of soils and C/N of humic acids (HA) of the conventional tillage (PL) and no-till (NT) treatments

As expected, the OC content of soils under NT has significantly increased compared to soils under PL. There was no significant difference found between the OC content of the 15-30 cm layers of PL and NT, and the values for this layer were always lower than those of the 0-15 cm layers (Table 1).

The OC content of the soil samples has been measured for background information about the spatial variability of OC distribution. Since the OC content of soils is one of the most sensitive indicators of changes in management, it has been taken as a basic information to check the spatial variability of tillage effects on SOM between and within the PL and NT plots. The spatial variability of OC content did not prove to be significant in the PL treatments and was on the edge of significance in case of NT treatments. Therefore, composite soil samples have been used for further investigation.

The CEC values of soils and treatments resemble the SOM contents in case of the ARC experiment, where NT was applied for a sufficiently long time to increase SOM content to an extent that it also raises the CEC of the soil (Table 1). The higher the SOM content of the soil, the higher the CEC.

Table 1

OC content and CEC of soil samples and C/N ratio of extracted humic acids of the conventional tillage (PL) and no-till (NT) treatments

Sample	Treatment	Depth, cm	OC, %	CEC, cmol (+)/kg	C/N of HAs
ARC	PL	0-15	2.31	39.2	16.05
ARC	NT	0-15	2.82	41.5	15.31
PAC	PL	0-15	1.30	28.1	15.02
PAC	NT	0-15	1.50	27.7	14.54
ARC	PL	15-30	1.69	37.7	17.38
ARC	NT	15-30	1.70	37.3	15.40
PAC	PL	15-30	0.62	27.1	14.42
PAC	NT	15-30	0.87	27.1	14.36

Long-term tillage experiments: ARC: Agronomy Research Center of Purdue University, PAC: Throckmorton Purdue Agronomy Centre

From the total C and N data the C/N ratio of HAs could be calculated easily. The C/N ratio of HAs in the PL soils is higher than in the NT treatments. which indicates the enrichment of C or the depletion of N from the HA pool in PL soils (Table 1).

^{13}C CPMAS NMR of humic acids

^{13}C CPMAS NMR spectra of HA samples from ARC and PAC were analyzed by calculating areas of characteristic chemical shift regions corresponding to structural compounds of HAs. The areas of the characteristic regions are expressed in percentage of the total area of the corresponding NMR spectrum. Results of peak area calculations are presented in Table 2.

Difference could be detected in the molecular composition of HAs within the soil profile (between the tillage affected and the reference layers) and between the examined tillage systems.

Table 2

Relative distribution of the characteristic chemical shift regions of ^{13}C CPMAS NMR spectra of extracted humic acids, in percentage of total area

A. Purdue Agronomy Research Center (ARC - 21 years)

	P015PL ±0.94%	P015NT ±0.87%	P1530PL ±1.24%	P1530NT ±1.20%
Carbonyl (190 - 220 ppm)	8.44	3.87	7.97	9.50
Carboxyl (165 - 190 ppm)	16.66	11.00	18.52	17.20
Phenolic OH (145 - 165 ppm)	1.63	2.62	1.05	5.07
Aromatic (105 - 145 ppm)	28.66	25.87	35.05	32.68
Acetals	6.37	4.52	2.83	0.81
Carbohydrates	10.11	10.78	8.97	4.44
Methoxy. alc.. ethers	7.79	11.38	8.90	14.24
Alkyl chains (0 - 48 ppm)	20.33	28.96	16.72	16.10
Total aliphatic (105 - 0 ppm)	44.6	55.64	37.42	35.59

B. Purdue Agronomy Center, Throckmorton Research Farm (PAC - 10 years)

	T015PL ±1.31%	T015NT ±1.51%	T1530PL ±0.90%	T1530NT ±0.96%
Carbonyl (190 - 220 ppm)	8.70	2.22	9.00	8.98
Carboxyl (165 - 190 ppm)	16.85	10.22	17.72	19.95
Phenolic OH (145 - 165 ppm)	0.70	9.34	4.66	3.43
Aromatic (105 - 145 ppm)	28.91	20.32	26.55	29.44
Acetals	3.51	15.44	5.96	3.47
Carbohydrates	14.42	11.96	12.22	12.08
Methoxy. alc.. ethers	8.09	11.41	10.19	12.08
Alkyl chains (0 - 48 ppm)	18.83	19.09	13.71	10.56
Total aliphatic (105 - 0 ppm)	44.85	57.9	42.08	38.19

Carboxylic groups. – The overall carboxylic group content of HAs in the ARC experiment decreases with decreasing depth. In the 0-15 cm layer the PL treatment contains a relatively higher amount of carboxylic groups than NT.

In the PAC experiment the relative amount of carboxylic groups within the soil profile also decreases with decreasing depth, and the relative amount of the carboxylic groups is also higher in the PL sample as compared to the NT in the 0-15 cm layer. The difference in the carboxylic group content between the two treatments in the 15-30 cm layer is less pronounced than in the 0-15 cm layer.

The aromatic core. – The relative amount of the aromatic components of HAs is significantly lower in the upper, tillage affected layers than in the underlying ones, but the difference between the effect of the tillage systems cannot be considered significant. This is true for both experimental sites, with the difference that in the „younger" PAC experiment the lower aromatic content of the upper layer is less pronounced.

The aliphatic parts. – The aliphatic compounds constitute the major part of HAs, and they are represented in higher relative amount in the HA molecules in the upper layers than in the lower ones in both treatments.

A tendency in the change of the relative amount of the aliphatic parts in the samples from both experiments could also be observed. which is the decrease of the relative amount of the aliphatic parts in PL as compared to NT in the 0-15 cm layer. In the PAC experiment the difference between the two treatments is larger in the lower layer, that theoretically has not been influenced by tillage.

Although the analysis of the „pure" relative distribution of the structural components of HAs within and among the samples and treatments provides useful information about the structural composition of HAs, the examination of ratios of the relative occurrence of the structural components within each sample is more informative for the investigation of the change in the function of the HAs in the soil. The following ratios have been calculated for each sample: the aromatic/aliphatic ratio:

$$\textit{aromatic compounds, \% / total aliphatic compounds, \%}$$

and Hatcher's aromaticity (HATCHER et al., 1981):

$$\frac{\textit{aromatic compounds, \% + phenolic-OH, \%}}{\textit{aromatic compounds, \% + phenolic-OH, \% + total aliphatic, \%}}$$

to describe the ratio of the stable and labile structural elements, and the aromatic/carboxylic ratio:

$$\textit{aromatic compounds, \% / carboxylic groups, \%}$$

for the description of the ratio of the stable and reactive parts of HAs. The % amounts of structural components in the HAs are expressed in percentage as shown in Table 2. Ratios calculated for HA samples as measures of functionality of HAs are presented in Table 3.

The simple aromatic/aliphatic ratios show that the HAs in the NT treatments are less aromatic in nature in the 0-15 cm layer at both experimental sites, whereas the aromaticity of the 15-30 cm layer of the PL and NT treatments show only slight differences, which is not even significant in the samples from the ARC experiment.

The Hatcher aromaticity values show similar trends as compared to the simple aromaticity numbers (aromatic/aliphatic ratio). These also show an in-

Table 3

The aromatic/carboxyl and the aliphatic/aromatic ratios of extracted humic acids from conventional tillage (PL) and no-till (NT) treatments from the experimental areas

A. Purdue Agronomy Research Center (ARC - 21 years)

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
Aromatic/Aliphatic	0.6 ± 0.03	0.5 ± 0.04	0.9 ± 0.03	0.9 ± 0.03
Hatcher's aromaticity	51.86 % ± 0.94 %	41.39 % ± 0.87 %	58.49 % ± 1.24 %	55.44 % ± 1.2 %

B. Purdue Agronomy Center. Throckmorton Research Farm (PAC - 10 years)

Ratio	0-15 cm PL	0-15 cm NT	15-30 cm PL	15-30 cm NT
Aromatic/Carboxylic	1.72 ± 0.045	1.99 ± 0.060	1.50 ± 0.027	1.48 ± 0.028
Aromatic/Aliphatic	0.6 ± 0.04	0.3 ± 0.07	0.6 ± 0.03	0.8 ± 0.03
Hatcher's aromaticity	52.38 % ± 1.31 %	49.30 % ± 1.51 %	56.63 % ± 0.90 %	59.21 % ± 0.96 %

crease in the aromaticity of the PL samples in the 0-15 cm layers as compared to the NT ones in both experiments.

Both the simple and the Hatcher's aromaticity numbers indicate that, within the upper 30 cm of the soil profile, the aromaticity of the HA fraction of the soil decreases with decreasing depth. A tendency of decreasing aromaticity in the NT treatment by time of continuous NT can also be observed.

There is also a significant difference between the aromatic/carboxylic ratio of the PL and NT samples of the 0-15 cm layers in both ACR and PAC. However, according to statistics, the difference between the same ratios of the PL and NT samples from the 15-30 cm layers of the PAC experiment is also significant, this difference between the treatments in the lower layers of the ARC experiment is not. According to the aromatic/carboxylic ratios, PL treatments of each experimental site contain relatively more acidic functional (carboxylic) groups as compared to NT. This latter observation is supported by the redox titration data of whole soils and extracted HAs from the same PL and NT treatments, which indicated that PL HAs in the 0-15 cm layers are more oxidative in nature than HAs in NT (MADARI et al.. 1997). A correlation between the length of the long-term experiment in time and the evolved difference in the reactivity of HAs between PL and NT also appears.

The calculation of the aromatic/carboxylic ratio of humic substances is not usual in the literature. HATCHER et al. (1981) deal with the carboxylic group content with caution. Calculating aromaticity of HS, he excludes the carboxylic group content in the calculation, because in his opinion the distribution of carboxylic C between the aromatic and aliphatic parts is not known. We calculated the aromatic/carboxylic content, because in our opinion, in addition to providing information on the reactivity of the HA fraction in the soil, comparing this number with the measures of aromaticity may give information about the distribution of the carboxylic functional groups between the aromatic and aliphatic components of the humic molecules. However, further research is necessary to find real evidence for the latter.

FT-IR spectroscopy of humic acids

To support the results obtained by Carbon-13 NMR spectroscopy, FT-IR spectra of the HA fractions have been collected. In this study two characteristic and well identifiable absorption bands were selected in the spectra of HAs to compare tillage induced differences: between 1725 and 1720 cm^{-1} the absorption maximum of the C=O stretching of -COOH groups and ketones can be found, the 1620–1600 cm^{-1} region contains the aromatic C=C stretching (and/or asymmetric -COO stretching).

The aliphatic C-H stretching can be found in the 2940–2900 cm^{-1} absorption band region. The more well defined the absorbance bands of this region are, the more homogeneous and/or less decomposed the aliphatic components are in the HA fraction.

Similar results have been obtained in case of both the ARC (21-year) and PAC (10-year) experiment. The absorption maximum of the C=O stretching band at 1716 cm^{-1} in the humic acid sample extracted from the 0-15 cm layer of the PL treatments is relatively higher than the absorption maximum of the aromatic C=C stretching band at 1619 cm^{-1}, than the absorption maximum of the corresponding absorption band at 1716 cm^{-1} in the sample from the NT treatment as compared to the absorption maximum of the aromatic C=C stretching band at 1619 cm^{-1} (Figure 1).

In the 2940–2900 cm^{-1} absorption region the aliphatic C-H stretching peaks of the absorption bands are more well defined in the HA fraction extracted from the NT treatment of the 0-15 cm layer, than in the HAs from the PL treatment.

The aliphatic C-H stretching peaks in the 2940–2900 cm^{-1} absorption region are more pronounced in both treatments in the 15-30 cm layers than the aliphatic C-H stretching peaks in the PL treatment of the 0-15 cm layer (Figure 1).

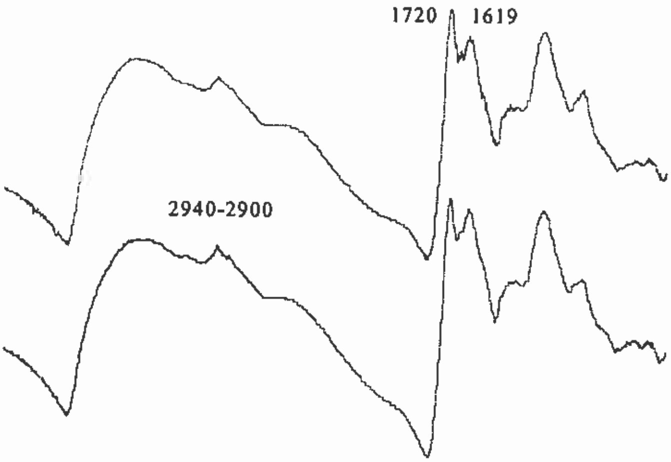

Figure 1
FT-IR spectra of extracted HAs of conventional tillage (PL – upper diagram) and no-till (NT – lower diagram) treatments in the long-term ARC and PAC experiments

Similarly to NMR, data obtained by FT-IR spectroscopy also indicate that HAs in PL soils in the tillage affected layer contain relatively less aliphatic compounds and more oxygen containing reactive acidic functional groups.

Summary and Conclusions

Analyzing data of the OC content of soil samples and the structural composition of the extracted HAs by ^{13}C CPMAS NMR and FT-IR spectroscopy led to the general observation that SOM content and the composition of HAs change both within the soil profile – between the tillage affected upper layer and the physically undisturbed layer – and between the PL and NT treatments in the tillage affected layer. The observed differences show a tendency to be more well defined as the duration of the long-term experiments increases.

Our results of ^{13}C NMR and FTIR investigations on HAs extracted from soils from 21- and 10-year experiments suggest that tillage influences the composition, hence the nature and function of humic substances in the soil.

Although a larger amount of SOM increases the overall CEC of soils, the change in the functionality of HAs is influenced by tillage by other means. HAs in the tillage affected layer of PL treatments contain less aliphatic components than the HAs of NT treatments. At the same time there are relatively more reactive oxygen containing functional groups (carboxylic groups as the most important ones) in HAs of PL treatments than in HAs of NT soils.

The higher relative aromaticity of PL HAs does not necessarily mean that the HA fraction in the PL treatments become more stable than the HA fraction in NT. The increase in the aromaticity is probably the result of the oxidative conditions in PL soils due to continuous mechanical disturbance and consequent aeration of the soil by plowing and the relatively higher aromaticity is the result of the faster decomposition rate of SOM, including the aliphatic parts of HAs.

The existence of more oxidative conditions in PL than NT soils seems to be evident anyway, but our own experimental results of redox titration of the examined soils also proved it in PL soils as compared to the NT treated ones (MADARI et al., 1997).

A further hint is the C/N ratios of HAs. The C/N ratios suggested that HAs contain relatively less N in PL soils in comparison to those in NT ones. This can be in association with the mineralization process, and consequently, the nutrient supply of soils. It seems that the higher decomposition rate of HAs in PL soils leads to the faster mineralization of aliphatic N, as compared to HAs in NT soils, where this process tends to be slower, and consequently more balanced.

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