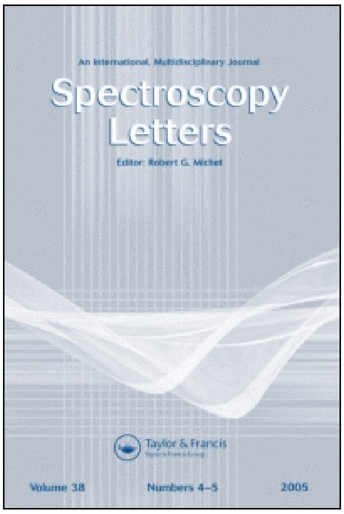

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Characterization of Soil Organic Carbon in Long-Term Amendment Trials

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Abstract: In the current study, total organic carbon (TOC) analysis and spectroscopic characterization (IRMS, DRIFT, and SERS) are applied to investigate the molecular changes in humic acids (HA) extracted from soil samples amended for more than 30 years with cattle manure (CM), cow slurries (CS), and crop residues (CR). All samples are compared to the unamended (T) sample. A variation in soil TOC percentage occurs in all samples, but a significant ($P < 0.05$) accumulation of C is observed only in CM. The TOC $\delta\ ^{13}C$ values appeared progressively less negative in all samples with respect to T. The DRIFT and SERS analyses of HA revealed a change in oxygenate groups (such as COO^- and OH) in the amendment with CS and CR. These results suggest how amendment might affect both C accumulation and the HA structure.

Keywords: $\delta\ ^{13}C$ isotopic ratio-mass spectrometry, DRIFT, humic acids, SERS, soil organic matter

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INTRODUCTION

Carbon flow through the soil organic matter (SOM) cycle is considered a major prospect for using the soil as a sink for carbon.[1] The accumulation of SOM depends on several variables such as pedo-climatic conditions, vegetation, and agricultural practices. It is widely accepted that the addition of organic amendments and crop rotation are two traditional farming practices employed to increase soil organic carbon (C) content.[2,3] Chemical and radioactive isotopes analyses have been performed to evaluate the dynamics of C and its residence time in soil.[1] However, this approch gives a partial picture of the pathways of changes in SOM. To further broaden our understanding of SOM, a study of its molecular structure is required. The chemical composition of SOM is poorly understood because of its chemical complexity. To obtain a dynamic picture of SOM, information is needed on the molecular structure of its humate fraction. This fraction is relatively resistant to the degradation process and, for this reason, is considered a main C sink of soil.

Spectroscopic techniques are powerful tools in distinguishing individual molecular structural differences in a heterogeneous organic mixture. In particular, diffuse reflectance infrared Fourier transform (DRIFT) spectroscopy is considered one of the most sensitive IR techniques for analysis of SOM and humic substances from different origins.[4–8] Additional information was obtained using surface-enhanced Raman spectroscopy (SERS).[7–9] The Raman study on roughened metal surface offers an additional advantage: the enhancement of those vibrational modes that are placed closer to the surface; it affords very valuable information about the molecules adsorbed on metal surface as well as their conformation.[9,10] Moreover, the use of stable isotope ratio, $\delta\ ^{13}C$, has allowed examination of the biogeochemical transformation occurring in soil.[11] The basis of this approach is that each individual organic compound in organic matter inherits a specific isotopic ratio resulting from its natural origins and its postdepositional transformation pathway.[11]

The main goal of this work is to use vibrational spectroscopy (DRIFT and SERS) and $\delta\ ^{13}C$ isotopic ratio-mass spectrometry (IRMS) to evaluate the changes in molecular structure of humic acids extracted from soil treated for more than 30 years with cattle manure (CM), cow slurries (CS), and crop residues (CR).

MATERIALS AND METHODS

The soil samples were taken from plots of a field experiment that had been in progress for more than 30 years at the University of Bologna, Agricultural Faculty's farm. The experimental design included plots amended during a 30-year period with cattle manure (CM), cow slurries (CS), and crop residues (CR) constituted of wheat straw or cornstalks (each biomass followed in succession), and unamended soil as the control (T).[12] Analyses were carried out on soil samples taken from (i) unamended soil in 1972 (T_0), (ii) after 30 years (T_{30}), and (iii) after a 30-year period of amendment with CM, CS, and CR. Humic acids were extracted by soil samples using 0.5 M sodium hydroxide as described in a previous paper.[8] Humic acids were freeze-dried before analysis.

Total Organic C and $\delta\,^{13}C$

Total organic C in soils and HA fractions were measured in an elemental analyzer (Thermo Finnigan model EA 1110, Bremen, Germany). The percentage of C in the sample was calculated by using acetanilide as a certified standard containing 71.09% of carbon. The HA samples were weighed in tin capsules and directly analyzed, whereas the soil samples were pretreated with 6 M HCl in order to eliminate the carbonate-bound C. About 10 mg of dry soil finely ground was weighed in silver capsules placed on a cast iron plate. A few drops of acid solution were added directly to the capsules, and the plate was heated at 80°C until the soil was dry. The samples were measured with continuous flow-isotope ratio mass spectrometry (CF-IRMS) by entering the CO_2 from elemental analyzer into an isotope ratio mass spectrometer (IRMS) model (Delta Plus Thermo Electron, Bremen, Germany). The C isotope values were expressed in delta notation (δ), where: δ (‰) $= [(R_{sample}/R_{standard}) - 1 \times 1000]$, and R_{sample} and $R_{standard}$ are $^{13}C/^{12}C$ of the sample and standard, respectively. Delta ^{13}C is relative to the Vienna pee dee belemnite (VPDB) standard.[13] All measurements were conducted in triplicate for each sample. The reproducibility of the delta ^{13}C values of the samples were, in 90% of the cases, better than 0.1‰. The coefficient variation was $<0.1\%$. Duncan's multiple comparison procedure was performed to discriminate statistically significant differences among the means.

DRIFT Spectroscopy

The spectra were recorded with a Nicolet Impact 400 FT-IR Spectrophotometer (Madison, WI, USA) equipped with an apparatus for diffuse reflectance (Spectra-Tech. Inc., Stamford, CT, USA).[8]

SERS Spectroscopy

Colloidal silver nanoparticles were prepared by using hydroxylamine hydrochloride as reducing agent.[14] The procedure for sample preparation has been described by Sanchez-Cortes et al.[9] The SERS spectra were recorded using a Renishaw Raman Microscope System RM2000, Gloucestershire, England, using the macro configuration equipped with an electrically refrigerated CCD camera. The 514.5-nm line of an Ar^+ laser was used. The laser power in the sample was 2.0 mW. Spectral resolution was 2 cm^{-1}.

RESULTS AND DISCUSSION

The total organic C (TOC), $\delta\,^{13}C$ of TOC, and $\delta\,^{13}C$ of HA are reported in Table 1. The total organic C variation (Δ%) was evaluated by comparing the TOC of soil samples after 30 years of treatment with the TOC measured at the beginning of the experiment in the unamended plot (T_0). The plot that did not receive organic treatment (T_{30}) and those amended with CR_{30} and CS_{30} showed a strong decrease in TOC (Table 1), whereas a significant increase appeared in CM with respect to T_0 (+3.7%) and T_{30} (+35%). Throughout the 30 years of amendment, the $\delta\,^{13}C$ of total organic C became progressively less negative (Table 1) in comparison to T_0. This increase of about 2 δ units was mainly due to the presence of corn in the farming rotation, as this C4 plant has a $\delta\,^{13}C$ value of −12‰. Unlike the TOC, the HA fraction was characterized by more constant $\delta\,^{13}C$ values, only CR_{30} and CS_{30} actually showed an increase in $\delta\,^{13}C$ values, which was, however, less pronounced than that of the TOC. This behavior would suggest that the corn-derived C, responsible for the shift of $\delta\,^{13}C$ of soil organic C toward less negative values, was a recent C source mainly present in the not-humic organic fraction.

Table 1. Effect of soil amended with crop residues (CR_{30}), cow slurries (CS_{30}), and cattle manure (CM_{30}) on total organic carbon (TOC), $\delta\,^{13}C$ of TOC and $\delta\,^{13}C$ of humic acids with respect to the control (T_0) at the start of the experiment and after 30 years of trials

Treatments	Soil TOC (%)	Soil Δ (%)[a]	Soil $\delta\,^{13}C$ (‰)	Humic acid $\delta\,^{13}C$ (‰)
T_0	0.82 ± 0.01	—	−24.1 ± 0.2	−24.6 ± 0.1^a
T_{30}	0.63 ± 0.005	(−23.1)	−22.0 ± 0.3^a	−24.5 ± 0.07^a
CR_{30}	0.74 ± 0.005^a	(−9.8)	−21.8 ± 0.1^a	−23.4 ± 0.3^b
CS_{30}	0.74 ± 0.001^a	(−9.8)	−21.5 ± $0.2^{a,b}$	−23.1 ± 0.1^b
CM_{30}	0.85 ± 0.003	(+3.7)	−22.4 ± $0.2^{a,b}$	−24.3 ± 0.1^a

Means followed in the same column by the same letter are not statistically different ($P < 0.05$) according to Duncan's test.

[a] Δ (%) percentage change = $[(TOC_{1972} - TOC_{2002})/TOC_{1972}] \times 100 \pm$ standard error.

Additional information on the effects of amendment can be gained from studies using DRIFT and SERS of HA (Figs. 1 and 2, respectively). The main regions of DRIFT spectra were attributed in accordance with previous papers.[4,5,7,8] Specifically, all spectra are characterized by intense bands at about 2930 and 2850 cm^{-1}, assigned to ν_{as} (CH_2) and ν_s (CH_2, CH_3) stretching vibrations in aliphatic structures, respectively. A strong band at about 1600 cm^{-1} is assigned to ν_{as} (COO^-) and ν(C=C) in aromatic ring vibrations.[4,5] The band intensity at 1660 cm^{-1} due to νs (C=C) in conjugate systems and νs (C=O) in ketonic groups increased in the T_{30}, CR_{30}, and CS_{30}

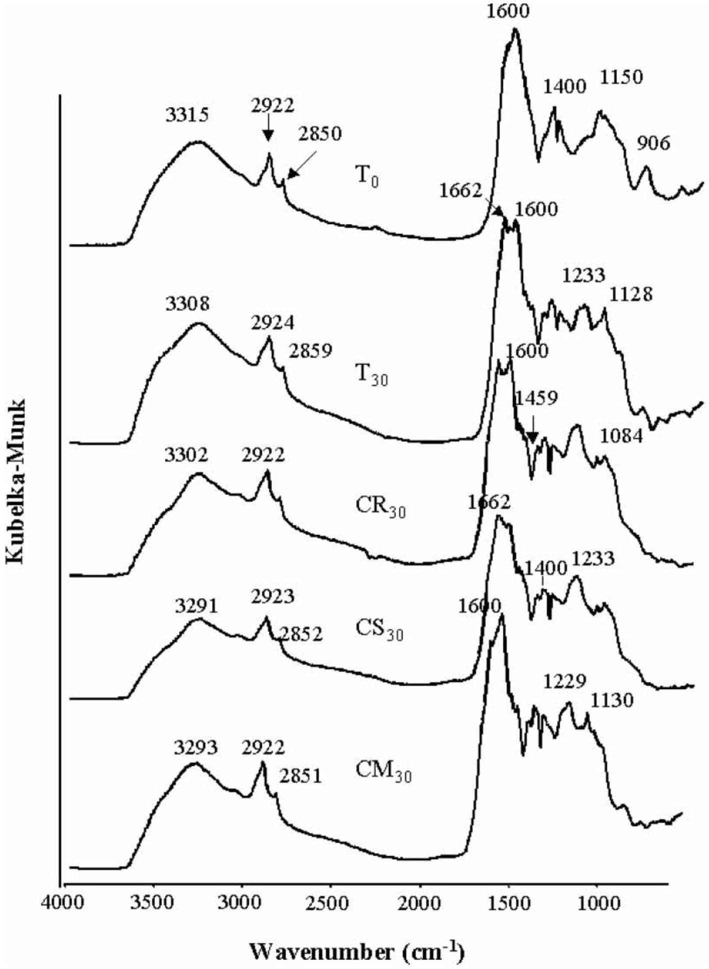

Figure 1. DRIFT spectra of HA extracted from unamended plots at the start of the experiment (T_0) and after 30 years (T_{30}), and amended with crop residues (CR_{30}), cow slurries (CS_{30}), and cattle manure (CM_{30}).

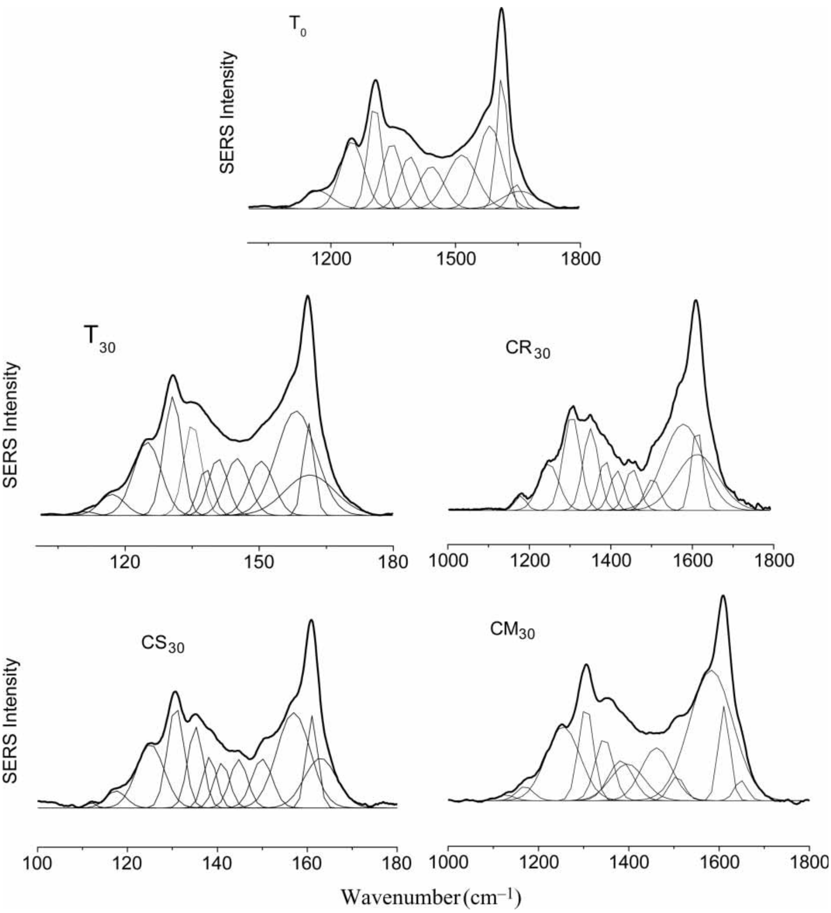

Figure 2. HA SERS curve fit spectra at the start of the experiment (T_0) and after 30 years (T_{30}) and amended for 30 years with crop residues (CR_{30}), cow slurries (CS_{30}), and cattle manure (CM_{30}).

spectra, while it appeared as a shoulder in the CM_{30} spectrum. We suggest that the presence of polar substituents might be the main cause of the changes in intensity and frequency of this band in the CM_{30} spectrum. In the 1460–1300 cm^{-1} region two bands appeared, one above 1460 cm^{-1}, the other at 1400 cm^{-1}: the first band corresponds to the δ(CH) motion of aliphatic groups and the second band can be assigned to $\delta(CH_2)$ and $\nu_s(COO^-)$ symmetric stretching motion.[4,5,8] The relative intensity of these bands is similar but slightly weaker in the CR_{30} and CS_{30} spectra compared to the CM. Furthermore, the band near 1370 cm^{-1}, assigned to the $\delta(CH_3)$ of alcohols and also to $\nu_s(COO^-)$, decreased in the CR_{30} and CS_{30} spectra. The broad band, centered at 1230 cm^{-1}, can be assigned to the ν(C–O) stretching motion of phenolic groups[7,8] and acid dimers with electron-withdrawing groups. This band increased in the CR_{30} and CS_{30} as compared to the T_0 and CM_{30} spectra. The bands between 1120 and 1084 cm^{-1} are due to ν_s(C–OH) and C–O–C in sugar and ether group vibrations, respectively.

The SERS spectra of these HA were recorded in the 1800–1000 cm^{-1} region at alkaline pH. This pH was used in order to help reveal the HA structure and to study, in greater detail, the different functional groups existing in the HA.[7–9] There are clear differences that involve both the overall SERS intensity as well as the relative intensity of the different bands. For instance, a higher absolute SERS intensity was observed for T_{30} compared to HA from amended plots. We suggest that different molecular structures are formed during amendment, as a more compact structure appeared in the CM spectrum due to the establishment of a large amount of H-bonds, while in CR, the SERS intensity was particularly weak. In order to analyze the SERS spectra of the different HA samples in further detail, the curve-fit spectra are shown in Fig. 2. These spectra showed bands of similar frequency, which correspond to the different functional groups that exist in HA. The main bands selected to carry out the comparison between samples correspond to the following regions: (a) 1650–1500 cm^{-1}, mainly attributed to C=C and C=O stretching vibrations in aromatic moieties; (b) 1460–1440 cm^{-1}, which can be attributed to (CH_2) and (CH_3) bending; (c) 1420–1400 cm^{-1}, due to both aromatic ring vibrations and to symmetric stretching vibrations of carboxylate groups; (d) 1390–1380 cm^{-1}, attributed to $-COO^-$ groups in aromatic rings and to (CH_3) bending; (e) 1350–1250 cm^{-1}, due to aromatic C–C stretching vibrations, together with C–H bending, coupled to C–O stretching vibrations (i.e., in phenols, ether, and aromatic ester moieties); and (f) 1200–1000 cm^{-1}, due to C–C and C–O stretching vibrations in aliphatic chains.[8,9] The analysis of the fitted spectra corresponding to T_0 and T_{30} shows only small differences between them, mainly due to a different relative composition of aliphatic and aromatic groups. This result agrees with studies carried out by the same authors on the effect that time has on the structure and composition of SOM.[10] The main difference between these two samples lies in the composition of the carboxylates of the 1420–1380 cm^{-1} region, where a relative decrease of aromatic carboxylates is seen, progressing from T_0 to T_{30}. In addition, the decrease in intensity of bands in the 1350–1100 cm^{-1} region could be related to a decrease in OH groups in the T_{30} sample. Among all the samples, CR_{30} exhibits the largest differences due to a greater quantity of aromatic groups and a corresponding reduction in the aliphatic component. In addition, a relative decrease in the bands below 1420 cm^{-1} is observed for this sample. This latter result is probably related to a reduction in oxygenated functional groups and aromatic C–H bonds and an increase of the substituents in the aromatic rings. The CS_{30} spectrum only shows slight differences compared to T_{30}, although a small increase in the aromatic component and a subsequent decrease in the aliphatic fraction is observed, together with an increase in oxygenated functional groups. In contrast, CM showed a greater loss of aromatic rings and a remarkable increase of aliphatic rings with an increase in the content of both aromatic and aliphatic carboxylate groups.

CONCLUSIONS

The application of total organic carbon and spectroscopic (IRMS, DRIFT, and SERS) analyses has provided a coherent picture of the molecular changes taking place in humic substances induced by amendment with CR, CS, and CM in a long-term experiment. After 30 years, amendment with CM produced a higher proportion of TOC than amendment with CS or CR. The presence of corn in the farming rotation caused greater changes in TOC $\delta\,^{13}C$ values than in HA suggesting that the corn-derived C was probably a recent C source mainly present in the nonhumi fraction. We suggest that the structural modifications of the humic fraction are related to the type of amendment with CR and CS causing a decrease in oxygenated functional groups and aromatic C–H in contrast to CM amendment, which produced an increase in aliphatic and carboxylate groups of both aromatic and aliphatic structures.

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