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Application of DSC–TG and NMR to study the soil organic matter

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Abstract The environmental concern on soil exploitation, linked to global warming by the Kyoto protocol, is responsible for increasing interest in the understanding of the role of the composition and structure of the soil organic matter (SOM) on soil carbon, C, dynamics. Thermal analysis and nuclear magnetic resonance (NMR) are applied to study the thermal properties, the structure and composition of the SOM of six samples with different C contents in order to improve the interpretation of results given by thermal analysis. Results showed that the direct integral of the combustion peaks obtained by DSC and the percentage of SOM given by TG were both directly related to the quantity of total soil C. Thus, soils with higher C content showed higher energy content too. The combustion temperatures of the curves given by DSC are those reported for labile OM. NMR results indicated the presence of aliphatic C, carbohydrates, and a weak signal in the aromatic C band in all the samples that was not detected in the DSC curves. Only two samples showed carboxyl/carbonyl C which was not detected by DSC also.

Keywords Thermal analysis · NMR · Soil organic matter

Introduction

The emission of CO2 from the soil surface through the degradation of the soil organic matter (SOM) is considered as one of the largest fluxes in the global carbon cycle [1]. Its contribution to global warming and climate change is recognized by the Kyoto protocol that suggests carbon sequestration as a technique for long-term storage of CO2 to mitigate global warming [2]. This feature is responsible for increasing interest in the understanding of the role of the composition and structure of SOM on soil carbon dynamics [3], and for increasing demands for rapid and quantitative assessments on SOM quality. Up to now, no single analytical method can adequately describe the complete variety of materials that comprise SOM. One approach has been the development of a large number of physical, chemical, and biological fractionation schemes to separate SOM into more homogeneous pools for characterization. [4, 5].

Thermal analysis is an attractive option for characterization of SOM because it rapidly provides the distribution of the labile and stable fractions and requires little sample preparation. These studies are usually focused on the identification of the typical patterns in the thermal curves that describe the degree of organic matter stability in relation with its thermal stability [6, 7]. However, trying to go beyond a qualitative interpretation of combustion temperatures is a complicate task that requires matching the combustion peaks obtained by thermal analysis with the properties theoretically expected on the basis of the exact chemical structures and composition of the SOM determined by other analytical methods. Despite their difficulties, such type of quantitative analysis would greatly benefit the understanding of the soil thermal properties utterly related with the SOM degradability. Then, the

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temperature limits defining SOM stability for the different exothermic peaks obtained in soils by Differential Scanning Calorimetry (DSC) has not been rigorously defined and varies significantly [7]. For these reasons, the information about the labile/recalcitrant SOM nature cannot be obtained by the thermal analysis alone and requires the application of alternative methods to complete the information given by DSC, and to match the given temperatures with a certain compound. These features underline the idea that a better quantitative interpretation of the soil thermal properties is possible by bringing together relevant thermodynamic and chemical composition data obtained with the appropriate analytical techniques.

In this article, we present the application of DSC, Thermogravimetry Analysis (TG), and Nuclear Magnetic Resonance (NMR) to study the SOM properties of soil samples with different C contents. The main goal is the improvement in the interpretation of the exothermic peaks of the DSC in an attempt to associate them with the chemical composition of the SOM given by the NMR results. The approach should permit to compare the combustion temperatures of different SOM components providing a better understanding of the heats of combustion calculated for different soils by integration of the DSC curves. Finally, it would permit application of DSC in a more quantitative way that would benefit soil research well known thermodynamic models that connect thermal properties with substrates degradability [8].

Material and methods

Soil samples

Soils were collected in the north of Spain (Asturias) in places with different vegetation. Data dealing with the location and vegetation of the sampling sites is given in Table 1. For collection of these samples, 100 m^2 of each land area was divided into six randomly chosen 1 m^2 sites. Before collecting samples, the plant litter on each site was removed. Soil was collected at a depth of 5–10 cm in each of the six sites to make a unique sample of about 500 g. Then, each sample was sieved with a mesh size of 2 × 2 mm and stored in polyethylene bags until measurements at 4 °C.

Soil elemental analysis

Carbon, C, and Nitrogen, N, percentages were determined with a Perkin-Elmer 2400B elemental analyzer. Soil pH was determined by means of a Crison pH-meter. The measurements were performed by introducing the electrode in a supernatant solution prepared with 10 g of soil and 25 mL of water.

Table 1 Soil samples chemical properties (pH and Nitrogen percentage, N) and sampling data

Samples	Location	Vegetation	N/%	pH
Caror	43°21,177′	Oak forest		
	5° 07,500′		0.17	4.62
Carocc	42°56,293′	Oak forest		
	6°30,875′		0.60	3.96
Hayor	43°06,357′	Beech forest		
	5°14,338′		0.37	4.58
Pinocc	43°31,552′	Pinus forest		
	6°36,957′		0.33	4.32
Tojor	43°15,559′	Ulex		
	5°04,156′	Europaeus	1.06	4.05
Vi-Pom	43°29,622′	Apple arable		
	5°25,049′	Land	0.51	7.47

Soil thermal analysis

Thermal analysis was performed using a differential scanning calorimeter, DSC, and by thermogravimetric analysis (TG) (Mettler Toledo TGA/SDTA851, DSC 822). Soil was dried and gently grounded in an agate mortar. These experiments were conducted with a heating rate of 10 °C/min under a flux of dry air and nitrogen (20 cm^3/s) from 20 to 600 °C, as previously described [9, 10]. The direct integral of the exothermic DSC curves under the flux of dried air, using as reference the curve obtained under the flux of nitrogen, gives the heat of combustion of the soil in Kilojoules per gram. The percentage of combusted SOM was calculated by the TG traces expressed as total weight loss associated with thermal decomposition between aprox. 150 and 600 °C. All measurements were done on dry weight basis.

NMR spectroscopy

The following six soil samples were studied by solid and liquid NMR spectroscopy: *carocc*, *hayor*, *tojor*, *caror*, *vi-pom*, and *pinocc.*

Solid NMR 1D CPMAS (Cross Polarization Magic Angle Spinning) experiments were performed for each soil sample at room temperature in a 17.6 T Varian Inova-750 spectrometer operating at 750 MHz proton frequency equipped with a T3 Varian solid probe. Each soil sample was prepared in a 3.2 mm rotor with an effective sample capacity of 22 μL which corresponds to approximately 30 mg of the powder sample. Carbon chemical shifts were referred to the carbon methylene signal of solid adamantane at 28.92 ppm. This sample was also used for the calibration of the 1D CPMAS experiments. 1D CPMAS experiments were acquired with the following conditions: the MAS rate was 15 kHz, a ramped cross polarization period of 1 ms was applied, the inter-scan relaxation delay was 2.5 s, and the number of scans was 20000. The total acquisition time of each spectrum was ~14 h. Heteronuclear decoupling during acquisition of the FID was performed with Spinal-64 with a proton field strength of 70 kHz. During cross polarization, the field strength of the proton pulse was set constant to 75 kHz and that of the 13C pulse was linearly ramped with a 20 kHz ramp near the matching sideband.

Solution NMR experiments were acquired for chloroform extracts of each soil sample. The samples were prepared by dissolving independently 400 mg of each sample in 1 mL $CDCl_3$ during 6 h at room temperature. A volume of 0.5 mL of the liquid supernatant of each sample was transferred to an NMR tube. Liquid NMR spectra were acquired on a 11.7 T Bruker DRX-500 spectrometer. The following experiments were acquired for each extract: ^1H, 2D HMQC ^1H-^{13}C, 2D HMBC ^1H-^{13}C and 2D TOCSY ^1H-^1H.

Solid and liquid NMR spectra were processed and analyzed with Mestre-C software (Mestrelab Research Inc).

Results and discussion

DSC and solid NMR experiments

In this study, six samples of soils collected in the north of Spain (Asturias) in places with different vegetation are studied (Table 1). Figure 1 shows the DSC curves of the soil samples. Some of them show clear differences either in the combustion profiles and/or in the intensity, possibly reflecting the different nature of the OM and different C content among the samples. The combustion peaks maximum height and so does the total area of the curves increases with the total C percentage in the soil. Figure 2 shows that there is a positive and approximate linear correlation between the integral of the DSC curve, given in Joules per gram of soil, and the moles of C per gram of soil. The integral of the curves given in Joules per gram of soil also positively correlates to the percentage of OM measured by TG as shown in Fig. 3. The slope in the plot of Fig. 3 is 25 kJ/g OM. Thus, soils with higher C and OM contents have higher values of heat, and it could be expressed either as Joules per gram of soil, Joules per gram of OM, or Joules per mol of total soil C.

Table 2 shows the results obtained when the integral of the DSC curve is related to the quantity of OM and C in each soil sample. For comparison, the heat of combustion for glucose is 15.55 kJ/g or expressed in kilojoules per mole of C: 467 kJ/mol C.

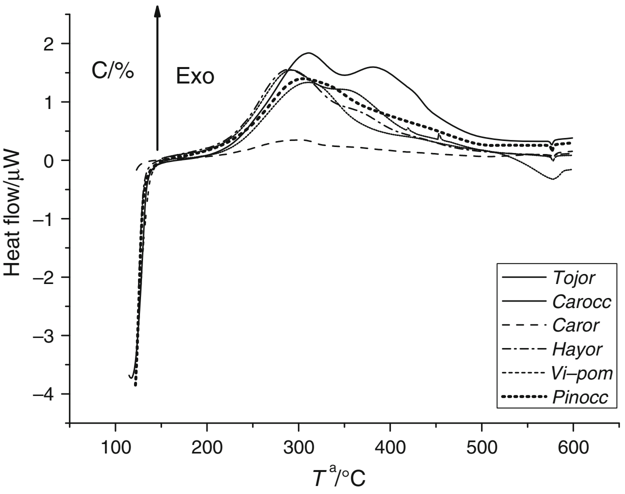

Fig. 1 DSC curves of the six soil samples studied. The *arrow* indicates increased C percentage and the sign of the exothermic reaction

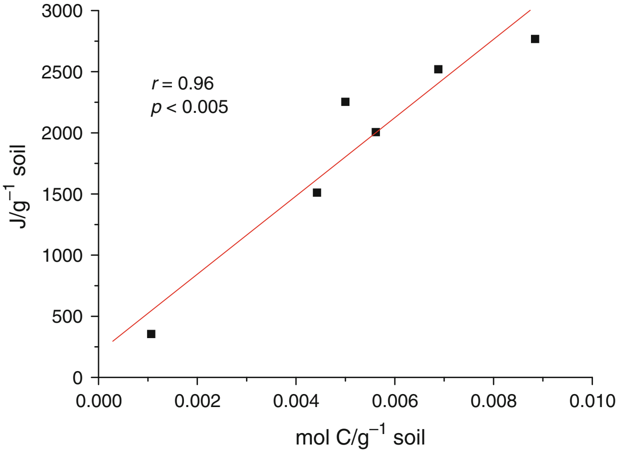

Fig. 2 Correlation between the values calculated by the direct integral of the DSC curves, in Joules per gram of soil, and the total C of the samples given in mol per gram of soil

In order to complement the information of the DSC curves and the quantitative values obtained from them, the carbon composition of the samples was studied by 1D CPMAS ^{13}C spectra. Figure 4 shows the spectra obtained for the samples *Carocc*, *Hayor*, and *Tojor*. From these spectra it is clear that the total amount of C follows the order *Tojor* > *Hayor* ~ *Carocc*. The sample *Tojor* has the highest C content; it shows three intense signals in the NMR spectrum of Fig. 4c that can be attributed to aliphatic carbons (0–40 ppm), carbohydrates (60–110 ppm), and carbons in carbonyl/carboxyl groups (160–240 ppm).

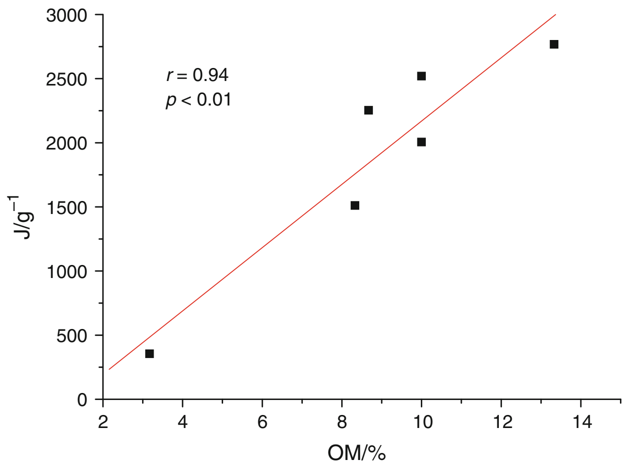

Fig. 3 Correlation between the direct integral of the DSC curves and the percentage of organic matter given by the TG analysis

Table 2 Data of total C percentage, C, Aliphatic C percentage, Calif, percentages of organic matter, OM, and heats of combustion in kilojoules per gram of organic matter (kJ/g OM) and in kilojoules per mol of C (kJ/mol C)

Sample	C/%	kJ/g OM	kJ/mol C	Calif/%	OM/%
Carocc	8.26	25	515	2.30	10.00
Caror	1.28	11	309	0.12	3.17
Hayor	6.00	26	451	1.86	8.67
Pinocc	6.74	20	356	1.08	10.00
Vi-pom	5.31	18	339	0.69	8.33
Tojor	10.61	21	316	3.18	13.33

Signals correspondingly to aromatic carbons (110–140 ppm) are detected with relatively much lower intensity. The *Hayor* sample spectrum (Fig. 4b) shows that the most relevant signals are those of aliphatic carbons followed by carbonylic/carboxylic carbons. A broad signal covers the carbohydrate and aromatic regions from 60 to 140 ppm though with relative lower intensity. The NMR spectrum of *Carocc* given in Fig. 4a only shows a relative intense signal that corresponds to aliphatic carbons. The presence of other type of carbons is very modest in this sample.

The DSC curves of the samples *Carocc*, *Hayor*, and *Tojor* are shown in Fig. 1 and also presented with more detail in Fig. 5. In each curve, two combustion exothermic peaks can be distinguished, one occurring in the range 289–310 °C and the second one in the range 356–385 °C. *Tojor* has the highest intensity for both peaks indicating a much higher amount of C content and a more complex OM nature, in concordance with the 1D CPMAS spectra of Fig. 4.

The first peak maximum in the DSC curve of Fig. 5 was assigned with the assistance of the 1D CPMAS spectra of Fig. 4. It was attributed essentially to the content in carbohydrates with very modest or no contribution of the content in carbonyl/carboxyl groups. Such statement is based on the following observations: (i) the relative intensity of the first peak in the DSC curves follows the order *Tojor* $\gg$ *Carocc* $\sim$ *Hayor*, which is the same order as the relative content in carbohydrates based on the NMR spectra of Fig. 4 and (ii) while the content of carbonyls/carboxyls is higher in *Hayor* than in *Carocc* in the 1D CPMAS spectra, the intensity of the first combustion peak in the DSC curve is slightly higher for *Carocc* than for *Hayor*, which denotes the little influence, if any, of the carbonyl/carboxyl groups to this peak of the curve.

The second peak maximum in the DSC curve of Fig. 5 was attributed solely to the content in aliphatic C with the assistance of the 1D CPMAS spectra of Fig. 4. Such assignment was based on the following observations: (i) the three samples *Carocc*, *Hayor*, and *Tojor* have an important contribution of aliphatic carbons in the NMR spectra of Fig. 4 and (ii) the possibility of this second peak to be assigned to aromatic carbons was ruled out because the range of temperatures is much lower than that described in the literature for aromatic compounds in soil, between 400 and 500 °C [11, 12].

The 1D CPMAS spectra of the samples *Caror*, *Vi-Pom*, and *Pinocc* are given in Fig. 6. In contrast with samples *Carocc*, *Hayor*, and *Tojor*, samples *Caror*, Vi-*Pom*, and *Pinocc* do not show relevant peaks either in the aliphatic and/or in the carbonylic/carboxylic regions. The only visible peak in these spectra is a very broad signal with very low intensity covering the whole region 50–150 ppm that is typically assigned to carbohydrate and aromatics carbons. Given the similarity in the three spectra of Fig. 6 it should be expected that these samples have approximately the same SOM pattern independently on the exact C quantity. The small intensity of the unique signal in the spectra of Fig. 6 is in concordance with the low content of C of these samples determined by elemental analysis and shown in Table 2.

The DSC curves of samples *Caror*, *Vi-Pom*, and *Pinocc* are given in Fig. 7. For these samples, the only combustion peak maximum occurs in the range 301–312 °C. This range of temperatures is typical of carboxyl and carbohydrates, it is named in the literature as EXO1 and it is considered as labile SOM [9, 13] but the temperatures for EXO 1 in these samples are higher than those of the first combustion peak in samples *Hayor* and *Carocc* indicating, assuming the interpretation of these temperatures, a more stable labile SOM in spite of having lower or similar total C than samples *Hayor* and *Carocc*. The peak maximum in the DSC curves of Fig. 7 is attributed solely to the content of carbohydrates with the assistance of the 1D CPMAS spectra of Fig. 6. Such assignment is based on the

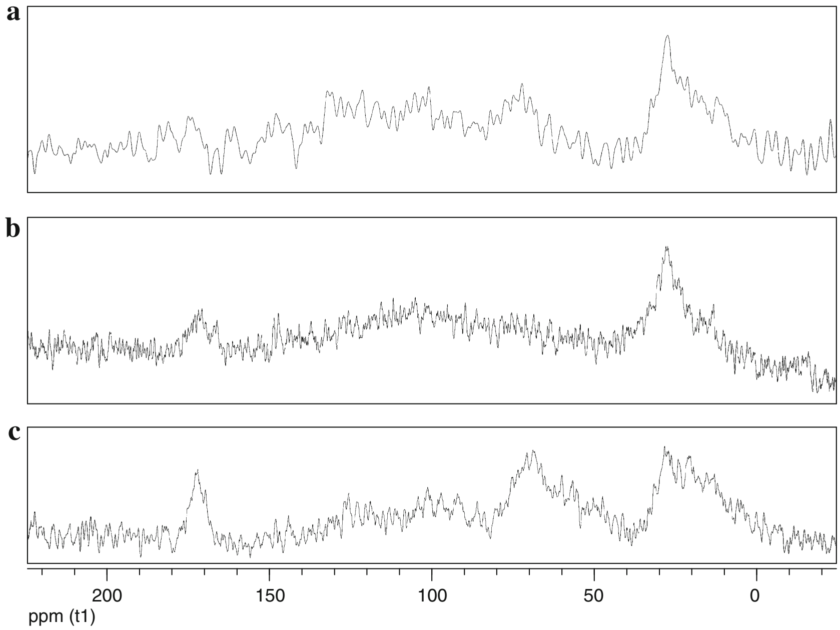

Fig. 4 1D ^{13}CPMAS spectrum of samples **a** *Carocc*, **b** *Hayor*, and **c** *Tojor*

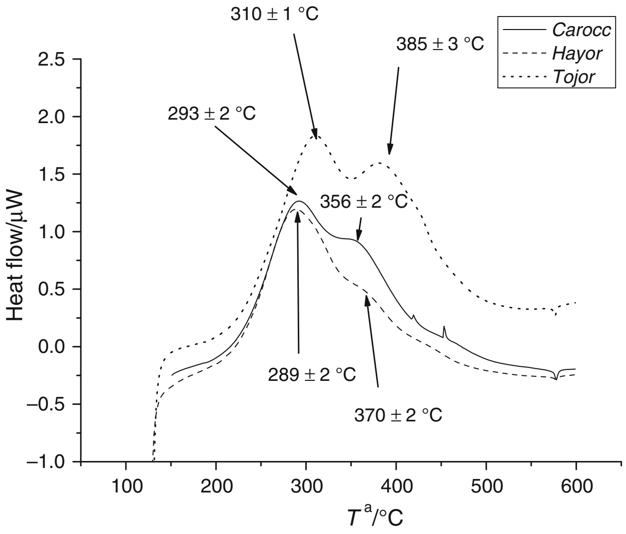

Fig. 5 DSC curves of samples *Carocc*, *Hayor*, and Tojor

following observations: (i) The only relevant peak in the spectra of Fig. 6 denotes the presence of carbohydrates and (ii) The possible contribution of aromatic compounds to the peak that is seen in the DSC curves is ruled out since aromatic compounds typically appear in the range 400–500 °C [11, 12].

Liquid NMR experiment of extracts of the soils

NMR experiments were performed for liquid extracts of the soils upon mixing with chloroform (see Experimental part). A 2D HMQC spectrum was acquired for each soil sample. This experiment provides a ^{1}H-^{13}C two dimensional correlation between a hydrogen and the carbon that is directly attached in the molecular structure. The peaks in the HMQC spectrum are a fingerprint of the protonated carbons. They are related with the chemical structures that are present in the organic soluble part of the soil. The 2D HMQC spectra of the extracts of the six samples studied herein are represented in Fig. 8. Leaving apart the 2D HMQC spectrum of *Tojor* whose signals in the spectrum of Fig. 8c do not have enough intensity, the 2D HMQC spectra of the other samples in Fig. 8 show basically the same peak patterns. Each spectrum contains a number of strong peaks of aliphatic carbons in the region ^{1}H 0–2.5 ppm/^{13}C 0–50 ppm. The peaks assigned to carbohydrates appear in the region ^{1}H 3–5 ppm/^{13}C 60–100 ppm with a lesser intensity. Besides, the signals of aromatics appear in the region ^{1}H 6.5–7.5 ppm/^{13}C 100–140 with even lower intensity.

The quantitative analysis of the whole set of peaks in each HMQC spectra follows the order:

$$Pinocc \sim Vi-Pom \sim Caror > Carocc \sim Hayor \gg Tojor$$

Despite the fact that the extracts were prepared similarly and the 2D HMQC spectra were obtained under the same experimental conditions, such order of intensities are in contrast and opposite to that determined in the intact solid samples of the soils such as the carbon content given in Table 2 and the 1D CPMAS spectra of Figs. 4 and 6. Such unexpected result indicates that the soil samples with lower C content are those that present a more abundant organic soluble fraction, possibly because they contain a larger fraction of organic polymers with lower molecular weight. The heat of combustion values are lower in these samples

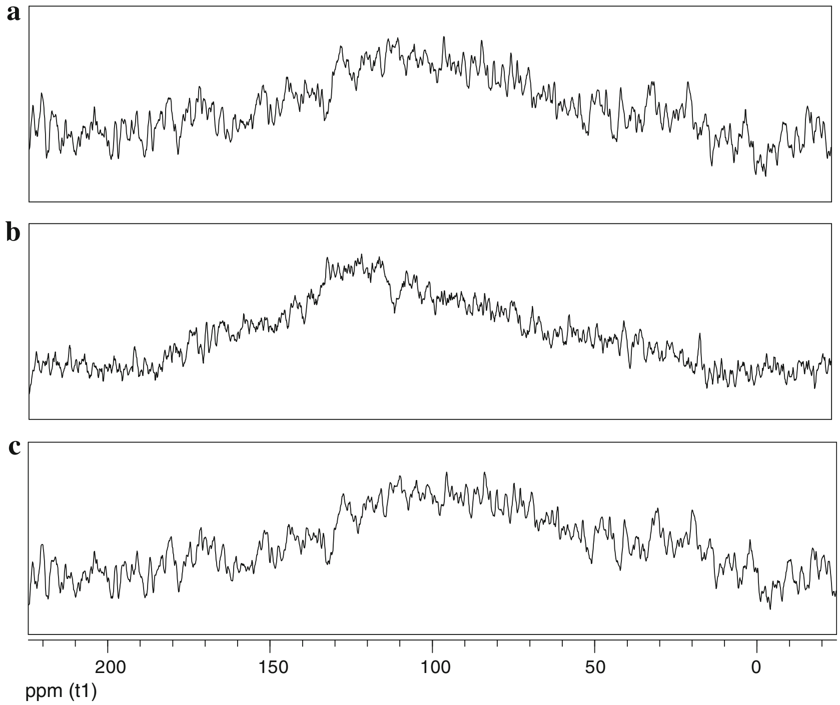

Fig. 6 1D ^{13}CPMAS spectrum of samples **a** *Caror*, **b** *Vi-Pom*, and **c** *Pinocc*

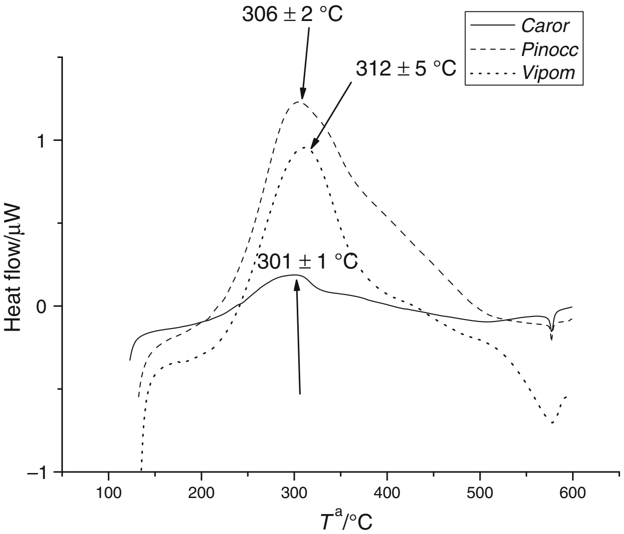

Fig. 7 DSC curves of samples *Caror*, *Pinocc*, and *Vi-Pom*

with more extractable OM than *Carocc* and *Hayor*. *Tojor* appears to follow a completely different behavior.

In previous studies using NMR to analyze SOM, it was found that the structure and composition of the SOM are quite stable and do not show big changes among soils under different environmental conditions [14]. All the samples in this article show a similar pattern of carbohydrates, aliphatic C, and low aromatic C. The presence/absence of carboxyl/carbonyl groups appear to follow a different pattern since they are detected by the NMR analysis in some of the soils and not detected in others with similar C values in this study. NMR gives aliphatic C and carbohydrates in all the samples while this is not reflected in the DSC curves. The only conclusion based on the thermal analysis data exclusively and on the temperature combustion peaks would be a "labile nature" of SOM in these soils according to literature, while NMR shows a more complex OM in the samples than that observed by the DSC curves exclusively.

Integration of the aliphatic bands showed positive correlation of aliphatic C with the total C and OM content. Results of the aliphatic C percentages obtained by integration of the spectra are shown in Table 2. The obtained values ranged from 0.01 to 3.37 mg per gram of soil. Literature gives values from 0.1 to 6.5 mg in humic acids and relates the quantity of aliphatic C with degree of soil humification, that decreases as humification increases [15]. In this article, it is found that aliphatic C increases with total C enhancement but we can not confirm if the reason for lower C quantities in our samples is caused by high humification degree of the soil or by higher degradation rates of the OM as found in other studies [16, 17].

These results improve the interpretation of the DSC curves representing the combustion of SOM. Most of the article using thermal analysis to study SOM report the existence of two combustion peaks that have been commonly attributed to labile/recalcitrant nature of the OM [5] but the existence of four different exothermic peaks was reported by TGA analysis for different soils, and the second exothermic peak at the temperature registered in this study in Fig. 5, is assigned to fulvic acids in previous

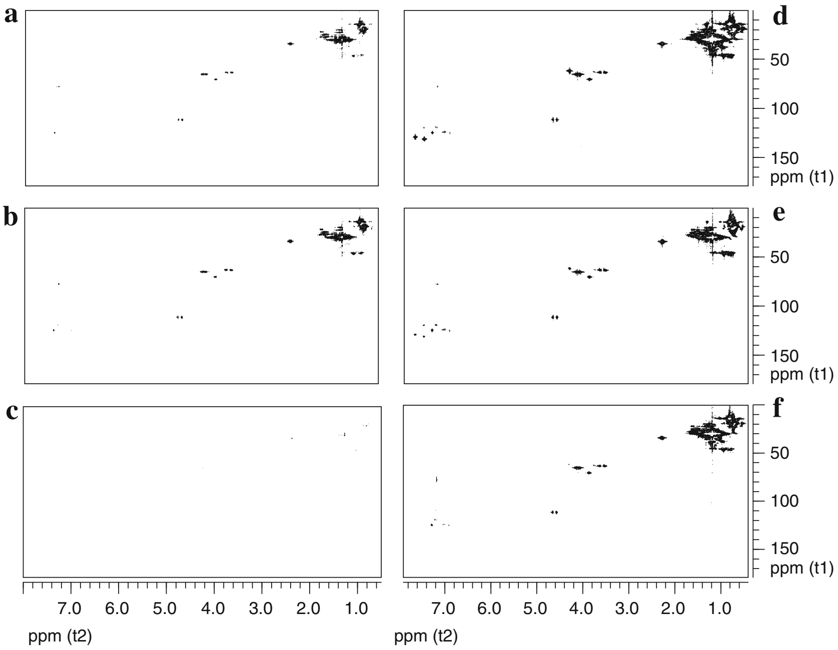

Fig. 8 2D HMQC spectra of samples **a** *Carocc*, **b** *Hayor*, **c** *Tojor*, **d** *Caror*, **e** *Vi-Pom*, and **f** *Pinocc*. The spectra are processed identically and represented at the same contour level, therefore, the peak intensities can be considered as semi-quantitative

article [13]. Here, samples with aliphatic, carbohydrates and aromatic C in their composition given by the NMR results do not show a peak at the temperature expected for aromatic C [11, 12], while samples with clear peaks in the aliphatic band of the CPMAS ^{13}C spectrum show a second peak in the DSC curves at temperatures considered as labile OM by the literature, although aliphatic C takes part of the composition of recalcitrant biomacromolecules [18]. The height of the second combustion peak decreased with decay of total C and aliphatic C content. Thus, although it is truth that different SOM constituents have different thermal stabilities and, therefore, the formation of a peak in a certain temperature range can be related to the decomposition of specific SOM structures, the quantity of total C, and aliphatic C, affects the DSC curve too and that quantity of C in soil can be responsible for the lack of combustion peaks if the quantity of that compound is too low in soil to be detected by the DSC. In that sense, application of TOCSY and HMQC has been very useful to show the existence of those structures in the soil in spite of not being detected by CPMAS ^{13}C NMR and by DSC. This indicates that thermal analysis by itself can lead to erroneous interpretations about the labile/recalcitrant nature of the OM.

Another important point in this study is the role of the integral of the DSC curves and its relation with the heat of combustion of the soil. The obtained values normalized to total C content and to the OM content could be considered as a thermal property of the soil reflecting a combination of the C quantity and the nature of the SOM that perhaps could be related to the capacity of the soil to keep C if the Thorton′s rule is considered [19], and if it is assumed that the value of that integral must be close to the heat of combustion. It relates the heat of combustion of a substrate with its oxidation degree. It is well established that substrates more reduced than biomass are less available to microbial attack [20] and thus, more recalcitrant. That means that SOM with heat of combustion values higher than those given for carbohydrates would be more difficult to degrade by the soil biomass and thus C would tend to be kept in the soil system. Since that point of view, sample *Carocc* would be the one with the most reduced OM. That is in agreement with the 1D CPMAS ^{13}C spectrum which shows in this sample the most intense peak in the aliphatic band in relation with the intensity of the peaks in the carbohydrates region. Aliphatic C is more reduced than carbohydrates [21]. A higher ratio aliphatic/carbohydrates could be responsible for higher heat combustion values in this case, but the last is still something to study in depth.

Conclusions

The maximum combustion peaks in the DSC curves depend on the quantity and nature of the soil C. The total C quantity in soil is a factor that strongly affects to the existence/lack of a combustion peak at a given interval of temperature. The predominance of a given C compound in relation to the others C components in soil also determines the existence or not of a combustion peak at a given temperature in the DSC curves.

The integral of the DSC curves related to the OM and C content of the soil could inform about the nature of the OM too, while that integral related to the soil mass is a function of the C and OM quantity in soil.

NMR is a helpful alternative to thermal analysis that improves the interpretation of the DSC curves.

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