

# Organic matter decomposition: bridging the gap between Rock–Eval pyrolysis and chemical characterization (CPMAS $^{13}\text{C}$ NMR)

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**Abstract** Organic matter (OM) is a key component of soils but information on its chemistry and behavior in soils is still incomplete. Numerous methods are commonly used to characterize and monitor OM dynamics, but only a few include the qualities required to become routine techniques *i.e.* simple, rapid, accurate and at low cost. Rock–Eval pyrolysis (RE pyrolysis) is a good candidate, as it provides an overview of OM properties by monitoring four components related to the main major classes of organic constituents (from A1 for the labile biological constituents to A4 for the mature refractory fraction). However, a question is still pending: do these four major classes used in the literature reflect a pertinent compositional chemical counterpart?  $^{13}\text{C}$  Nuclear Magnetic Resonance Spectroscopy in the solid state

( $^{13}\text{C}$  CPMAS NMR) has been used to answer this question by collecting information on structural and conformational characteristics of OM. Moreover, in order to avoid the blurring effect of pedogenesis on OM dynamics, a “less complex OM” source, *i.e.* compost samples, has been used. Results showed significant and high determination coefficients between classes, indices (of transformation of plant biopolymers, humification...) from RE pyrolysis, and the main classes of OM characterized by  $^{13}\text{C}$  NMR, *e.g.* A1 & A2 with labile/easily degradable components (alkyl C et O-alkyl C), A3 & A4 with humified OM (with aromatic C and phenolic C). The R index (contribution of biopolymers) is correlated with phenolic and aromatic C, whereas the I index (related to immature OM) refers to labile—easily degradable components (alkyl C et O-alkyl C). The results confirm the pertinence of RE pyrolysis to monitor OM dynamics.

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## Introduction

Soil organic matter (SOM) benefits of a positive perception since a long time. It is clearly associated with the concept of soil fertility and, more recently, of soil quality. Although a minor component of soil in quantitative terms, organic matter influences many soil properties, which in turn affects several soil functions: for example, it provides nutrient reservoirs, it is a substrate for microbial activity, it preserves the environment, and it is critical for sustaining and increasing agricultural productivity (Schnitzer 2005). However, information on its chemistry and behavior in soils is still incomplete. Numerous techniques have been used to characterize and monitor SOM dynamics (Kogel-Knabner 2000). Nonetheless, very few are used routinely, because of the common need for preliminary sample preparation (*e.g.* extraction or purification), or due to their complexity and cost (*e.g.* NMR techniques). Only a few techniques have the necessary qualities to become routine techniques, *i.e.* simple, rapid, accurate and at low cost, in order to be used on a very large sampling design. Rock–Eval pyrolysis (RE pyrolysis; Disnar et al. 2003; Sebag et al. 2006) or Near-Infrared Spectroscopy (NIRS; Albrecht et al. 2008a; 2009) constitute two potential candidates for routine techniques.

This study focuses on RE pyrolysis as a method to evaluate both the nature and behavior of natural organic matter (OM). Although initially designed for petroleum exploration (Lafargue et al. 1998), RE pyrolysis has been applied to a variety of other materials such as soils or recent sediments because of its simplicity.

RE method of pyrolysis consists of a programmed heating to quantitatively and selectively determine (1) free hydrocarbons contained in samples and (2) hydrocarbon- and oxygen-containing compounds ( $\text{CO}$ ,  $\text{CO}_2$ ) that are volatilized during the cracking of OE contained in the sample. Disnar et al. (2003) estimated the various pools of organic compounds by using their specific cracking temperatures. Several authors referred to this approach for various objectives, such as quantification of refractory OM,

assessment of humification processes, and also in order to study the outcome of fossil OM in modern environments, as well as diagenesis of sedimentary OM and quantification of carbon content in soils (Copard et al. 2006; Sebag et al. 2006; Marchand et al. 2008; Poot et al. 2009; Tambach et al. 2009; Graz et al. 2011; Saenger et al. 2013; Gillespie et al. 2014; Hare et al. 2014). The main advantages of this method are repeatability and rapidity in providing an overview of OM properties and content.

Nevertheless, there is still a question pending: do the major classes of OM defined by this approach accurately reflect and with pertinence OM chemical composition? To answer this question,  $^{13}\text{C}$  Nuclear Magnetic Resonance Spectroscopy in the solid state ( $^{13}\text{C}$  CPMAS NMR) has been used to collect direct information on structural and conformational characteristics of OM. NMR resonances were assigned to chemical structures, according to five main forms: alkyl C, O-alkyl C, aromatic C and phenolic C, and carbonyl–carboxyl C. The pertinence of RE pyrolysis is evaluated using a comparison between pyrolysis results and those of the  $^{13}\text{C}$  CPMAS NMR reference tool.

In this study, natural OM from compost samples is used for the following reasons: (1) contrary to soils, compost variability only comes from transformation of OM and type of inherited parent materials; (2) composting processes are well described and referenced in the literature (De Bertoldi et al. 1983; Epstein 1997); (3) the samples used have already been studied in previous works (Albrecht et al. 2008b; 2009) and are well constrained; (4) finally, this type of natural OM allows complex “interferences” due to pedogenic processes occurring in soils to be avoided.

## Materials and methods

### Experimental materials

Composts are obtained from local dewatered digested municipal sewage sludge, green wastes, and pine barks at a 1:1:1 v/v ratio (Company Biotechna, Ensues, Bouches du Rhône, France). Pine barks are incorporated into other biowastes to improve aeration during the process. The mixture was composted for 20 days in impervious boxes ( $100\text{ m}^3$ ) with forced aeration, and then stored in windrows (10 m long, 4 m high, and

5 m deep) on a composting platform for 6 months. The piles are mixed several times to promote humification of OM. Approximately 1 kg of homogenized compost was collected from each windrow at nine different stages of composting (4, 18, 40, 57, 67, 84, 101, 114 and 128 days) with four replicates for 36 samples in total. All samples were sieved (<20 mm mesh). Samples were freeze-dried and ground with a Cyclotec 1,093 mill (FOSS) to 1 mm.

#### Chemical and physical analyses of OM

RE pyrolysis has been performed with a ‘Turbo model Rock–Eval 6 pyrolyser’ (Vinci Technologies, Rueil-Malmaison, France). A homogenized sample of about 100 mg undergoes a pyrolysis step followed by a complete oxidation of the residual material. A FID detector measures hydrocarbons released during pyrolysis, while CO<sub>2</sub> and CO are detected by infrared absorbance during both steps. Pyrolysis starts isothermally at 200 °C for 3 min, after which, the sample is heated to 650 °C. The oxidation step starts isothermally at 400 °C (3 min) and then heats up to 850 °C. Deconvolution of signals results in four main peaks: the S1 peak (hydrocarbons released during the isothermal phase), the S2 peak (hydrocarbons produced between 200 °C and 650 °C), the S3 peak (CO<sub>2</sub> from pyrolysis of OM up to 400 °C), and the S4 peak (CO<sub>2</sub> released from residual OM below around 550 °C during the oxidation step). Mineral carbon decomposition is recorded by the S3’ peak (pyrolysis–CO<sub>2</sub> released above 400 °C), and the S5 peak (oxidation–CO<sub>2</sub> released above about 550 °C).

The present work focuses on hydrocarbon compounds released between 200 and 650 °C. The thermal maturity (TpS2—temperature peak of the S2 pyrogram in °C) was determined as the temperature corresponding to the maximum of the S2 curve. This study refers to the method developed by Disnar et al. (2003) to estimate the contribution of each pool of organic compounds defined by their specific cracking temperature. S2 curves are deconvoluted into a combination of elementary components. The residual method, which consists of subtracting the major Gaussian elementary distribution centered on the main mode, has been used first on the initial S2 signal, and then on successive residual distributions (Sebag et al. 2006; Hetényi et al. 2010). This empirical approach arbitrarily reduces the number of elementary

components. Five elementary Gaussian distributions as “components” were chosen according to the following ranges (from A1 to A5): 205–340, 340–400, 400–460, 460–550 or 550–600 °C.

Humic (HA) and fulvic acid (FA) fractions were analyzed using a precipitation method (Swift 1996). Compost samples were diluted with NaOH (0.1 M) in a conical flask and shaken for 4 h. Then, samples were centrifuged (Sorval Super T21), to recover the supernatant fraction containing the humic material. The supernatant was acidified to pH 1.0 using HCl (6 M), and then centrifuged to separate HA (precipitate) and FA (supernatant) fractions. Both fractions were dried (105 °C, 24 h) and weighed. HA and FA substances were expressed in mg kg<sup>-1</sup> DM.

All solid-state <sup>13</sup>C CPMAS NMR spectra were obtained on a Bruker Avance-400 MHz spectrometer (Bruker, Bremen, Germany) operating at a <sup>13</sup>C resonance frequency of 100.7 MHz. Samples were placed in a 7 mm zirconium rotor and spun at the magic-angle at 6 kHz. All measurements were made at room temperature. The <sup>13</sup>C chemical shifts were referenced to tetramethylsilane and calibrated with the glycine carbonyl signal, set at 176.5 ppm. Deconvolution of the NMR spectra was performed using the DmFit software (Massiot et al. 2002). This software adjusts the spectra to obtain the line-width and the peak positions (in ppm), allowing each peak to be integrated to get the percentage of each contribution. NMR resonances were assigned to chemical structures according to previous NMR studies on compost and SOM (Inbar et al. 1991; Vincelas-Akpa and Loquet 1997). The chemical shift range of <sup>13</sup>C NMR spectra for compost corresponds to the following dominant forms: alkyl C, e.g. from amino acids, lipids and waxes (0–45 ppm), O-alkyl C, e.g. from cellulose and hemicelluloses (45–110 ppm), aromatic C (110–145 ppm) and phenolic C, e.g. from lignin (145–165 ppm), and finally carbonyl–carboxyl C (165–210 ppm).

#### Statistical analysis

The relationships between major classes of organic constituents given by RE pyrolysis and <sup>13</sup>C CPMAS NMR were calculated using Pearson coefficients of determination ( $r^2$ ). Significant coefficients were retained for  $p$  value < 0.05. Principal component analysis (PCA) was performed to get dimension reduction in RE pyrolysis and <sup>13</sup>C NMR dataset. The

key idea of PCA is to represent the variation of a data matrix in a reduced number of dimensions to obtain the general structure of the variability. A circle of correlation expresses the influence of chemical compounds and RE pyrolysis main parameters on the PCA sample ordination. All statistical analyses were performed using XLSTAT 2012.

## Results

### Elementary components obtained with RE pyrolysis

RE pyrolysis results of compost samples are given in Table 1. S2 curves were deconvoluted into a combination of the elementary components A1–A5. Trends displayed by the four elementary components A1–A4 are particularly interesting. Two major and opposite trends are easily identified (they are partly co-linear as A1–A5 components constitute a closed dataset—their sum is equal to 1). The first group (biopolymers), which comprises A1 and A2 components, steadily decreases from 32 to 33 % (after 4 days) to a final value of 23 and 26 % after 128 days of composting, for A1 and A2 respectively. The other group (medium to high mature OM) comprises A3 and A4 components, and increases from 20 to 26 % and 14 to 23 %, for A3 and A4, respectively. TpS2 increases from 312.5 to 327.3 °C between samples of four and 128 days, respectively. <sup>13</sup>C NMR and chemical results

from previous studies on the same samples (Albrecht et al. 2008b; 2009) are given in Table 2.

### Statistical analysis of RE pyrolysis versus <sup>13</sup>C CPMAS NMR indices

Pearson's correlation coefficients calculated using RE pyrolysis and <sup>13</sup>C CPMAS NMR indices are given in Table 3. Significant figures are presented in bold. Main significant and interesting high  $r^2$  are the following:

- Alkyl C is significantly and positively correlated with A1 component ( $r = 0.478$ ) but significantly and negatively correlated with A4 component ( $r = -0.406$ );
- O-alkyl C is not correlated with RE pyrolysis components, but when O-alkyl C and alkyl C fractions are added together, this new “labile OM and plant biopolymers” component is significantly and positively correlated with A1 and A2 components ( $r = 0.621$  and  $0.709$ , respectively), but also significantly and negatively correlated with A3 and A4 components ( $r = -0.431$  and  $-0.756$ , respectively);
- Aromatic C is significantly and negatively correlated with A2 component ( $r = -0.508$ ) and significantly and positively correlated with A4 component ( $r = 0.450$ );
- Phenolic C is significantly and negatively correlated with A1 and A2 components ( $r = -0.606$  and  $-0.458$ , respectively) and significantly and

**Table 1** Rock-Eval pyrolysis results on compost samples

Age (d)	Tmax (°C)	A1 (%)	A2 (%)	A3 (%)	A4 (%)	A5 (%)	R-Index	I-Index
4	312.5 (2.1)	33.4 (1.3)	32.1 (2.3)	19.9 (1.3)	13.9 (1.6)	0.5 (0.6)	0.34 (0.01)	0.52 (0.02)
18	319.0 (4.2)	30.1 (2.6)	33.4 (0.8)	20.5 (1.1)	15.3 (2.1)	0.7 (0.5)	0.37 (0.04)	0.49 (0.05)
40	317.8 (2.2)	31.1 (1.6)	30.7 (2.9)	20.5 (0.4)	16.1 (1.9)	1.5 (1.1)	0.38 (0.03)	0.48 (0.03)
57	322.6 (4.0)	27.6 (0.2)	27.8 (0.5)	21.6 (0.2)	20.2 (0.6)	2.7 (0.1)	0.45 (0.01)	0.41 (0.01)
67	324.2 (1.6)	26.3 (0.1)	28.8 (0.1)	21.4 (0.0)	21.1 (0.0)	2.2 (0.0)	0.45 (0.02)	0.41 (0.02)
84	328.3 (1.1)	25.8 (0.3)	28.3 (1.9)	22.3 (0.5)	21.1 (1.2)	2.4 (1.1)	0.46 (0.02)	0.38 (0.01)
101	329.1 (1.4)	25.3 (0.3)	27.6 (0.3)	22.7 (0.9)	21.1 (0.6)	3.2 (0.2)	0.47 (0.01)	0.37 (0.02)
114	326.0 (1.7)	24.6 (0.6)	27.3 (0.9)	24.3 (0.3)	22.6 (0.5)	1.0 (0.3)	0.48 (0.01)	0.33 (0.01)
128	327.3 (3.3)	23.4 (0.8)	26.2 (0.7)	26.1 (0.7)	22.9 (1.3)	1.4 (1.3)	0.50 (0.02)	0.28 (0.01)

Values in parentheses are standard error ( $n = 4$ )

A1 (205–340 °C), A2 (340–400 °C), A3 (400–460 °C), A4 (460–550 °C) or F5 (550–600 °C), R-index = A3 + A4 + A5, I-index =  $\log[(A1 + A2)/A3]$

**Table 2**  $^{13}\text{C}$  NMR and chemical results from previous studies (Albrecht et al. 2008b; 2009)

Time/ day	carbonyl–carboxyl C 165–210 ppm	phenolic C 145–165 ppm	aromatic C 110–145 ppm	O-alkyl C 45–110 ppm	alkyl C 0–45 ppm	OM % DM	HA mg kg <sup>-1</sup> DM	FA mg kg <sup>-1</sup> DM
4	8.3 (1.4)	4.3 (0.4)	10.2 (1.1)	54.8 (1.7)	22.4 (1.2)	58.5 (3.0)	28.3 (7.5)	54.9 (6.6)
18	8.3 (1.1)	4.3 (0.3)	9.8 (0.7)	58.7 (2.0)	19.0 (1.0)	57.5 (3.6)	28.3 (1.9)	51.9 (9.1)
40	8.1 (0.9)	4.1 (0.2)	10.2 (0.7)	57.9 (1.0)	19.8 (1.0)	60.3 (3.0)	31.5 (5.4)	52.0 (6.3)
57	8.7 (0.5)	4.6 (0.3)	10.8 (0.4)	54.9 (0.7)	21.0 (1.1)	48.3 (2.1)	39.3 (1.8)	38.2 (4.5)
67	8.4 (1.8)	4.3 (0.7)	10.0 (1.1)	57.6 (1.8)	19.7 (1.8)	53.5 (5.9)	26.6 (8.0)	47.7 (6.7)
101	8.9 (0.7)	4.9 (0.3)	10.9 (0.5)	56.9 (2.6)	18.5 (1.5)	52.6 (2.7)	53.5 (4.7)	46.8 (5.1)
114	9.3 (1.9)	4.9 (0.6)	11.5 (1.5)	56.5 (3.6)	17.9 (0.5)	50.8 (4.1)	59.5 (7.7)	47.9 (4.4)
128	8.5 (1.3)	4.4 (0.3)	10.4 (0.6)	57.1 (2.5)	19.6 (1.5)	50.5 (4.3)	51.9 (4.6)	47.2 (4.2)

Values in parentheses are standard error ( $n = 4$ )

OM Organic matter content, HA humic, FA fulvic acids

**Table 3** Pearson's correlation coefficients between Rock–Eval pyrolysis and  $^{13}\text{C}$  CPMAS NMR indices

	A1 %	A2 %	A3 %	A4 %	A5 %	R-Index	I-Index
Alkyl C	<b>0.478</b>	0.287	-0.243	<b>-0.406</b>	-0.160	0.231	0.323
O-Alkyl C	-0.038	0.198	0.057	-0.101	-0.146	-0.240	0.000
Alkyl + O-Alkyl C	<b>0.621</b>	<b>0.709</b>	<b>-0.431</b>	<b>-0.756</b>	<b>-0.483</b>	0.154	<b>0.601</b>
Aromatic C	-0.221	<b>-0.508</b>	0.107	<b>0.450</b>	0.315	0.178	-0.248
Phenolic C	<b>-0.606</b>	<b>-0.458</b>	0.340	<b>0.623</b>	<b>0.422</b>	-0.372	<b>-0.488</b>
Carboxyl C	-0.176	-0.326	0.106	0.338	0.092	0.089	-0.185
Tmax	<b>-0.884</b>	<b>-0.719</b>	<b>0.643</b>	<b>0.869</b>	<b>0.572</b>	<b>0.886</b>	<b>-0.803</b>
C/N	0.295	0.318	-0.282	-0.306	-0.194	0.092	0.320
HA	<b>-0.796</b>	<b>-0.611</b>	<b>0.740</b>	<b>0.763</b>	0.193	<b>-0.523</b>	<b>-0.799</b>
HA/FA	<b>-0.728</b>	<b>-0.592</b>	<b>0.571</b>	<b>0.730</b>	0.352	<b>-0.444</b>	<b>-0.683</b>
OM	<b>0.499</b>	<b>0.471</b>	-0.388	<b>-0.519</b>	-0.346	0.235	<b>0.480</b>

Value in bold for p-value < 0.05

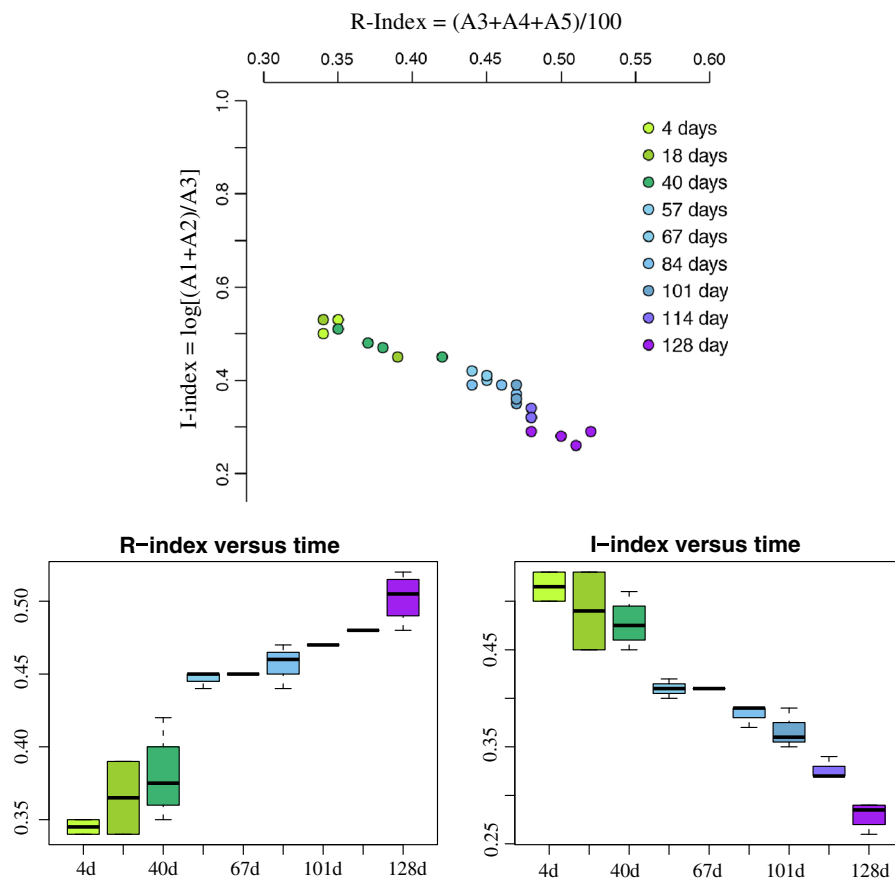
HA humic acid, FA fulvic acid, OM organic matter, R-index =  $A3 + A4 + A5$ , I-index =  $\log[(A1 + A2)/A3]$

- positively correlated with A3 and A4 components ( $r = 0.623$  and  $0.422$ , respectively);
- Tmax (TpS2) is significantly and negatively correlated with A1 and A2 components ( $r = -0.884$  and  $-0.719$ , respectively) and obviously significantly and positively correlated with A3 and A4 components ( $r = 0.643$  and  $0.869$ , respectively);
- HA (humic acids), as a HA/FA ratio (i.e. ratio between humic and fulvic acids) displays a highly significant and negative correlation with A1 and A2 components and is significantly and positively correlated with A3 and A4 components;
- OM (organic matter content) is significantly and positively correlated with A1 and A2 components

( $r = 0.499$  and  $0.471$ , respectively) and finally significantly and negatively correlated with A4 component ( $r = -0.519$ ).

#### Transformation of OM (R vs. I index map)

Sebag et al. (2006) proposed a novel Rock–Eval based-diagram, using two indices defined as  $I = \log(A1 + A2)/A3$  related to immature OM and  $R = (A3 + A4 + A5)/100$ , which represents the mature OM contribution to the S2 signal. Figure 1 shows a R versus I indices plot with two opposite trends throughout the time of composting. The R-index increases with values of 0.66 after 4 days and 0.75 after 128 days of composting. In contrast,



**Fig. 1** I-index versus R-index plot (*top*) and I-index and R-index box plots (*bottom*; *d* for day). Box plots display gaps in OM evolution with a threshold at 57/67 days for both the I- and the R-indices

I-index decreases from 0.27 to 0.24 (Table 1) during the same time span. Moreover, another presentation of the I- and R-indices versus time of composting using box plots brings up gaps in OM evolution with a threshold at 57/67 days for I and R indices (Fig. 1).

PCA was performed on RE pyrolysis and  $^{13}\text{C}$  NMR measured parameters to confirm correlation between thermal resistance and OM transformation. PCA made it possible to ordinate RE pyrolysis data according to the stage of composting on both first and second principal components PC1 and PC2 (Fig. 2). PC1 and PC2 explained 37.2 and 27.4 % of total variance in RE pyrolysis data, respectively. Three clusters are identified in the factorial projection. Young composts (4–57 days) are clearly separated from all others on PC1 and form a first cluster (left hand side of the diagram). Fifty-seven and 101 day-old composts form

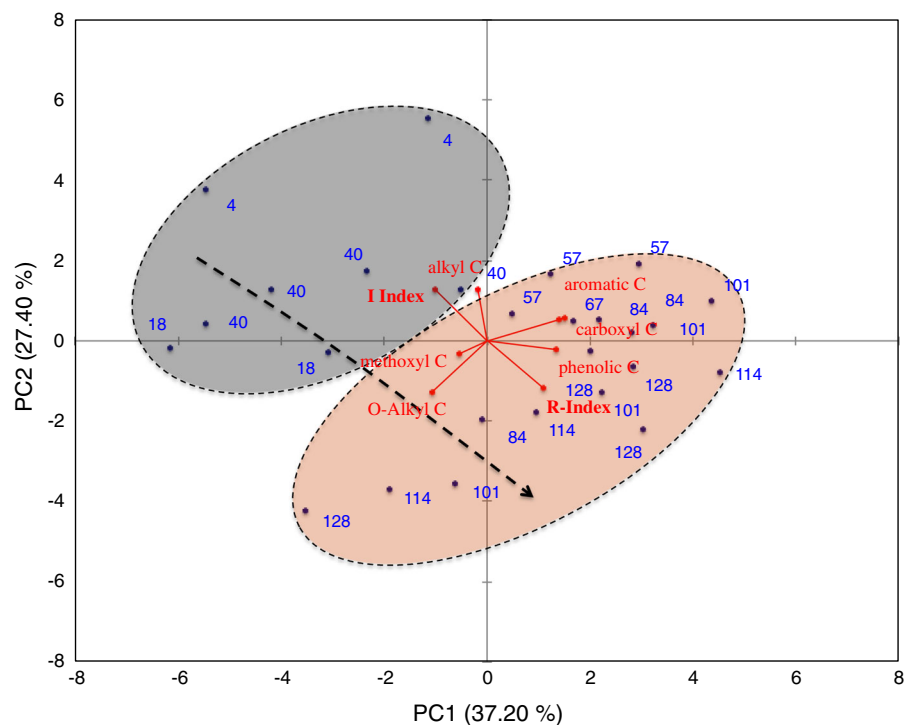
a second cluster (middle/right hand side of the diagram). The third cluster is composed by oldest composts of 114 and 128 days at the right hand side of the plot. From this specific ordination of samples, a chronological distribution of composts on PC1 is clearly emphasized. On PC1, A1 and A2 factors have an opposite trend compared to A3 and A4: A1 and A2 point to youngest composts, whereas A3 and A4 point to the oldest ones.

## Discussion

### Composting processes

Numerous chemical and biological parameters (moisture, pH,  $\text{C}_{\text{org}}$ ,  $\text{N}_{\text{org}}$ , C/N, OM, HA and FA, respiration, cellulase, protease, and phenoloxidase activities) were

**Fig. 2** Principal component analysis (PCA) using Rock–Eval pyrolysis indices (I and R) and  $^{13}\text{C}$  NMR data (functional chemical groups). The percentage for each axis of the explained variance is given in axis labels. The black dotted arrow expresses the time variable (number of days of composting) on the PCA plane and numbers refer to these numbers of days



monitored on samples during a complete composting process of lasting six months (Albrecht et al. 2009). Results revealed the existence of two phases within the composting processes. The initial phase (4 to 50–60 days) has been characterized by an intensive degradation and a rapid increase in temperature. Both C/N ratio and OM content sharply decreased. The second phase (up to 146 days) was characterized by the stabilization of C/N ratio and OM content, a decrease in all biological activities (respiration, cellulase, phenoloxidase, and protease activities), and an increase in the humification process occurring within the OM with notably a high increase of HA/FA ratio, which tripled from 0.54 to 1.61 between 4 and 146 days. This decline in biological activity was explained by a quantitative and qualitative reduction of nutrient sources, which became a limiting factor. Indeed, as shown by  $^{13}\text{C}$  CPMAS NMR on the same samples (Albrecht et al. 2008b), peaks at 40 and 35 ppm, assigned to the  $\text{CH}_2$  groups of proteins and lipids, which are easily degradable compounds, decrease rapidly during composting. Similarly, an increase in aromaticity (aromatic C + phenolic C by  $^{13}\text{C}$  NMR) emphasizes a clear preference of easily biodegradable C compounds for microorganisms.

Lignin transformation and its structural changes, observed through the S/G (syringyl to guaiacyl) ratio by  $^{13}\text{C}$  NMR, revealed the same inclination (Albrecht et al. 2008b).

Statistical analyses of  $^{13}\text{C}$  NMR and NIRS data using principal components analysis (PCA) show, in both cases, a chronological distribution of composts according to the degree of compost maturation and clearly indicate two distinct phases. To summarize, previous works on the same samples revealed a clear link between the degree of transformation of OM during composting processes and a succession of specific bacterial communities within two phases (Albrecht et al. 2010). The first phase is characterized by a fast decomposition of non-humic biodegradable OM and the second phase by the modification of recalcitrant biopolymers (cellulose, lignin and hemicelluloses), accompanied by the formation of humic-like substances (Albrecht et al. 2008b; 2009). Consequently, compost represents a perfect system to study OM evolution (mineralization and humification processes) and is considered in this study as a model with clear and well identified transformations of OM without the complex “interference” due to pedogenetic processes occurring in soils.

## Contribution of RE pyrolysis

### *Behaviour of elementary components*

RE pyrolysis analysis of compost samples showed two opposite trends for the four main elementary components (A1–A4) generated by deconvolution of the S2 curves (Table 1). While A1 and A2 decreased during composting, A3 and A4 increased. Sebag et al. (2006) proposed that these four components are related to major classes of organic constituents differing in origin and their resistance to pyrolysis: labile biological constituents (A1), resistant biological constituents (A2), immature non-biotic constituents (A3), and a mature refractory fraction (A4).

Regarding RE pyrolysis parameters, it appears that labile and resistant biological constituents (A1 and A2) decrease as observed by Albrecht et al. (2008b, 2009). Indeed, these authors found a decrease in the C/N ratio linked to the decrease of biopolymers during the composting process. Similarly, they showed an intensive biological activity (respiration, cellulase, phenoloxidase, and protease activities) during the first phase of composting. This was explained by the abundance of labile OM and plant biopolymers, such as cellulose and hemicelluloses in the sewage sludge and green wastes used. This labile OM component has also been identified and characterized by  $^{13}\text{C}$  NMR as alkyl-C, which are commonly assigned to amino acids, lipids, and waxes (Inbar et al. 1991; Venceslas-Akpa & Loquet, 1997). Moreover, specific peaks at 40 and 35 ppm were assigned to the  $\text{CH}_2$  groups of proteins and lipids. Alkyl C, known to be easily degradable compounds (Albrecht et al. 2008b), may be linked to the first component identified by RE pyrolysis: labile biological constituents (A1). The Pearson correlation coefficient corroborates this hypothesis with a significant and positive correlation between A1 and alkyl C fraction with  $r = 0.478$  (Table 3).

According to Disnar et al. (2003) and Sebag et al. (2006), thermoresistant biopolymers during RE pyrolysis may be found in the A2 component. To verify this hypothesis, correlation between O-alkyl C and A2 fractions was tested according to the postulate that the O-alkyl C fraction with a  $^{13}\text{C}$  NMR chemical shift ranging between 45 and 110 ppm is assigned to cellulose and hemicelluloses (Inbar et al. 1991; Venceslas-Akpa & Loquet, 1997). Although O-alkyl C—A2 correlation is positive, it is not significant

(Table 3). It is possible that the above-noted decreasing trend for both A1 and A2 throughout composting (Table 1) could be at the source of this lack of significant correlation. The principle of RE pyrolysis is based OM segregation depending on its thermal resistance and not directly on its biomolecular composition. Thus, plant biopolymers (cellulose, hemicelluloses, etc.) may occur in both A1 and A2 components at the same time, depending of their thermal resistance. Based on this hypothesis, alkyl C and O-alkyl C fractions can be combined to build a new “labile OM and plant biopolymers” component. This component is significantly, highly and positively correlated with A1 and A2 components ( $r^2$  of 0.621 and 0.709), respectively. Consequently, these results confirmed the hypothesis about the nature of A1 and A2 components being easily degradable fractions of OM.

Moreover, both correlation coefficients between “labile OM and plant biopolymers” and A3 and A4 components have negative signs ( $r = -0.431$  and  $-0.756$ , respectively), compared to the positive correlation coefficients with A1 and A2. As shown by results in Table 1, these opposite trends in correlation coefficients confirm the hypothesis by Albrecht et al. (2010) on the adaptation of metabolic capacities by compost microbial communities between the two well-known phases of composting process. These authors showed that composting process operated into two phases with the same samples. The first phase was characterized by a fast decomposition of non-humic biodegradable OM (<67 days), as it is observed with decreasing A1 and A2 and emphasized by their relationships with alkyl and O-alkyl C, which are easily degradable compounds. The second phase corresponds to the formation of humic-like substances by the adaptation of microbial communities. More precisely, the first phase is focused on easily degradable substrate utilization, whereas the maturation phase consists of multiple metabolisms, which induce the release of metabolites and humification processes (Albrecht et al. 2008b).

These humification processes have been identified through the behavior of the more thermal resistant biomolecules comprised in both A3 and A4 components. According to Disnar et al. (2003) and Sebag et al. (2006), A3 and A4 are designed for immature non-biotic constituents (A3) and mature refractory fractions (A4). A3 and A4 are both positively correlated with aromatic



and phenolic C (Table 3) during the time of composting. Moreover, significant correlation coefficients only existed for A4 ( $r = 0.623$  and  $0.422$ , respectively). Similarly to A1–A2 and aromatic–phenolic C, A3–A4 were negatively correlated with alkyl–O–alkyl C (Table 3). Consequently, it is assumed that aromatic–phenolic C compounds increase during the composting process. In addition, increasing A3 and A4 during composting (Table 1) can be related to a trend showing the increase of several indices noted in previous studies on the same samples and used to assess the humification processes, *i.e.* increase of humic substances or degradation of plant biopolymers (Albrecht et al. 2008b). The first index is the monitoring of HA, and more precisely, the monitoring of the HA/FA ratio during composting. HA/FA is a tool usually used to assess humification processes. Indeed, increase of HA and decrease of FA raise the HA/FA ratio from values of 0.5 after 4 days to 1.1 after 128 days (Albrecht et al. 2008b). This change can be attributed to the selective preservation of aromatic compounds and high molecular size compounds and/or to the formation of humic-like substances with molecular structures and properties similar to that of HA. Thus, in this case, both increasing A3 and A4 may be related to humification processes during composting. Similarly, the aromaticity index provides an overall view of the evolution of aromatic compounds and enables humification in compost to be characterized with respect to the accumulation of aromatic compounds (Vincelas-Akpa and Loquet, 1997). This index increased (Albrecht et al. 2008b) in direct proportion to A3 and A4 components.

One point to clarify is, even if the prevalence of humic substances as polymers in soils has been assumed for decades, a large body of evidence shows instead an alternative understanding of their conformational nature. Humic substances should be regarded as supramolecular associations of self-assembling heterogeneous and relatively small molecules deriving from the degradation and decomposition of dead biological material (Piccolo 2001). Recent studies underlined the importance of ecosystem properties in SOM stabilization processes, such as physical disconnection between SOM and microbes or organo-mineral associations (Schmidt et al. 2011). This is indeed supported by progress in analytical and visualization techniques. Methods used to classify soil OM into active, slow, and passive pools are no longer chemically, but rather physically performed, based on

the various degrees of physical protection of SOM by either aggregates, association force between SOM and minerals, or different particle sizes (von Lützow et al. 2007). However, in compost science, the ecosystem properties of OM remain totally different and determining humic-like substance contents is a frequently used tool to assess the degree of maturity during the composting process (Sanchez-Monedero et al. 1999; Tomati et al. 2000). This is why significant and positive correlations between HA/FA ratio and A3 and A4 give additional evidence that RE pyrolysis is capable of providing information about changes of OM during the composting process.

Even if it is often difficult to assess changes in content of resistant biopolymers, several indices reveal their transformations. Increase of cellulose crystallinity expressed by the ordered cellulose (89 ppm)/disordered cellulose (84 ppm) ratio and the S/G (syringyl to guaiacyl) ratio revealed transformations of cellulose and lignin, respectively (Albrecht et al. 2008b). Thus, increasing A3 and A4, related to immature non-biotic constituents (A3) and mature refractory fraction (A4) by Disnar et al. (2003) and Sebag et al. (2006), during the composting process, can be related to these transformations. Therefore, these results confirm the nature of A3 and A4 components as being complex OM, still under humification or having started to undergo humification processes.

#### *Rock–Eval pyrolysis indices*

TpS2 is a well-known maturity indicator for OM (Espitalie et al. 1985a, b; Disnar 1994; Carrie et al. 2012) and its increase ( $312.5$ – $327.3$  °C between 4 and 128 days—Table 1) is a good parameter of OM maturity produced by aforementioned humification processes. According to Saenger et al. (2013), TpS2 can be regarded as a proxy of the thermal energy required by microorganisms to decompose OM. Increase of TpS2 during the composting process confirms the presence of easily degradable components and/or plant biopolymers in both A1 and A2 components and the presence of more complex and/or humified and/or recalcitrant compounds in A3 and A4.

To have a more explicit view of these results, a PCA on RE pyrolysis (I and R- indices) and  $^{13}\text{C}$  NMR data was performed (Fig. 2). Time of composting (*i.e.* humification of OM) is shown by a black dashed arrow

in Fig. 2. This chronological distribution of composts is negatively linked with the I-index (left hand side of the diagram) but positively linked with R-index related to the oldest compost (right hand side of the diagram). Among all PCA parameters, I- and R-indices accounted for 10.6 and 12.4 %, respectively, of the variance explained on PC1 and 23.4 and 19.9 % on PC2. These large percentages of variance explained on both axes reinforce the above-mentioned trend. This representation of data highlights links between the I-index and easily degradable fractions of OM, which both decrease during composting (negatively correlated), and R-index and complex OM, which both increase (positively correlated).

In order to follow OM evolution using RE pyrolysis and during the composting process, study results have been projected on a I versus R plot built using a soil database (in grey, Fig. 1). R and I have two opposite behaviors along the timeline of composting. The R-index increases from a value of 0.66 after 4 days to 0.75 after 128 days of composting. In contrast, the I-index decreases during the same time span from 0.27 to 0.24 (Table 1). By using both indices, a comparison of SOM evolution between different horizons in a given profile, as well as between profiles from different contexts, can be conducted (Copard et al. 2006; Sebag et al. 2006; Marchand et al. 2008; Poot et al. 2009; Tambach et al. 2009; Graz et al. 2011; Saenger et al. 2013). A similar comparison can be drawn with compost samples: they are distributed along the curve referring to organic soils and horizons (in grey), and all compost samples are ranked according to their degree of maturity. Young composts (4–67 days) are plotted on the left hand side of the diagram, near organic tissues and forest litter samples. They are characterized by fresh and/or slightly evolved OM. More mature composts (67–128 days) are plotted on the right hand side of the diagram, among organic horizons characterized by more complex and/or evolved OM. This chronological distribution of compost samples along the curve (in grey) characterizing the degree of transformation of OM (from organic tissues/forest litter samples to organic horizons on the right hand side of the diagram) confirms the pertinence of RE pyrolysis to assess OM transformation, i.e. humification and mineralization processes. Indeed, an IR plot may be a valuable tool for monitoring bio-wastes composting maturation. In addition, box plots (Fig. 1) display a gap, delimited by

a threshold after 57/67 days, in the I-index as well as in the R-index. This threshold is related to the increase of maturity by changes in the compost chemical composition: a decrease of aliphatic compounds and a concomitant increase of humic substances and aromaticity (Albrecht et al. 2008b), as well as a change in the microbial community structures and functional diversity (Albrecht et al. 2010). To conclude, these gaps, observed as well by NIRS (Albrecht et al. 2008a) and  $^{13}\text{C}$  NMR (Albrecht et al. 2009) confirm that RE pyrolysis and the associated I and R-indices constitute a valuable approach to an accurate and rapid estimation of the degree of OM transformation.

## Conclusions

Rock–Eval pyrolysis and the use of I (for immature OM) and R (for recalcitrant OM) indices constitute a valuable approach to a fast and accurate assessment of transformation degrees of OM. This has been demonstrated by using a model of composting as it is a revealing model to study OM evolution (mineralization and humification processes) and its by-products are well documented. In addition, in a first step to explore the pertinence of RE pyrolysis for SOM, compost excludes the complex “interferences” due to pedogenetic processes occurring in soils. The precision of RE pyrolysis is good enough to identify thresholds (57/67 days). These thresholds have been demonstrated to be related to an increase in maturity due to changes in chemical composition, as well as in microbial community structures and functional diversity changes. The presented results show that relationships between indices from RE pyrolysis and the main classes of OM characterized by  $^{13}\text{C}$  NMR can be significantly correlated. The R index (contribution of bio-macromolecules) is mainly correlated with phenolic and aromatic C, whereas the I index refers to labile—easily degradable components (alkyl C et O-alkyl C). In other words, these results confirm the pertinence of RE pyrolysis to monitor OM dynamics.

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