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1 **The amending potential of vermicompost, compost and digestate from urban biowaste:**  
2 **evaluation using biochemical, Rock-Eval® thermal analyses and transmission electronic**  
3 **microscopy**

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18 **Abstract:** Organic fraction of municipal solid waste can be recovered using a variety of  
19 processes including composting, anaerobic digestion and vermicomposting. The impact of  
20 these products was evaluated using an innovative approach combining quantitative and  
21 qualitative analyses. Chemical analysis showed that the organic matter content of dry compost,  
22 digestate and vermicompost was 76%, 67.2% and 41.7%, respectively. Index of Recalcitrant  
23 Organic Carbon (I<sub>ROC</sub>) was higher for vermicompost (87.2%) than for compost (77.9%) or  
24 digestate (66.9%). The Rock-Eval® approach was correlated to the I<sub>ROC</sub> with its R-index  
25 (R<sup>2</sup>=0.97). Transmission electron microscopy was used to describe the microbial activity, the  
26 decomposition state and we observed more advanced maturity from vermicompost, compost to  
27 digestate. Finally, the digestate is predicted to have a fertilizing effect whereas the compost  
28 should have more an amending effect in the medium/long term. Due to a higher degree of  
29 stability, the vermicompost could have both a fertilizing and amending effect.

30  
31 *Keywords: organic fraction of municipal solid waste, organic matter quality, carbon dynamics,*  
32 *biological potential, methodological approach.*

33 **Abbreviations:**

34 AD: Anaerobic Digestion

35 Cmin: Carbon mineralization

36 EOM: Exogenous Organic Matter

37 HC: Hydrocarbon Compounds

38 MinC: Mineral Carbon

39 OFMSW: Organic Fraction of Municipal Solid Waste

40 OM: Organic Matter

41 I<sub>ROC</sub>: Index of Recalcitrant Organic Carbon

42 PCA: Principal Component Analysis

43 RE: Rock-Eval®

44 SOM: Soil Organic Matter

45 TEM: Transmission Electron Microscopy

46 TOC: Total Organic Carbon

## 47 **1. Introduction**

48 Population growth and urbanization (55% will live in urban areas in 2050) (European Union et  
49 al., 2021) as well as changing consumption patterns have led to an explosion in the quantity of  
50 waste, which currently represents a real social, economic and environmental challenge (Sharma  
51 and Jain, 2020). Among this urban waste, 20-30% and 50-80% is organic waste in high-income  
52 and middle- to low-income countries, respectively (Lohri et al., 2017). The organic fraction of  
53 municipal solid waste (OFMSW) is a potential source of organic matter (OM) for the  
54 development of agriculture (Sharma et al., 2019) and many institutions such as the European  
55 Union agree that it should be separated from other wastes for recycling and soil amendment  
56 (European Union, 2018). OM losses from agricultural soils are estimated at 12Gt of carbon  
57 from 1850 to 2000, thus many researchers are looking for techniques to restore this OM (Lal,  
58 2009; Sparling et al., 2006). Exogenous organic matter (EOM) is one of the solutions for  
59 restoring and/or preserving the level of soil OM (SOM) (Barral et al., 2009; Moinard et al.,  
60 2021) and contributing to soil carbon storage (Peltre et al., 2017). However, the quality of these  
61 inputs varies greatly depending on the recycling technique used (Komakech et al., 2015) and  
62 waste typologies (Fisgativa et al., 2016) despite generally positive effects on soils and crops  
63 (Obriot et al., 2016). To evaluate the contribution of EOM on agricultural soils, it is necessary  
64 to precisely characterize these products as they are very variable (Stewart-Wade, 2020),  
65 especially for OFMSW (Hanc et al., 2011; Mendes et al., 2013).

66 There are many recycling processes that can stabilize OFMSW and transform it into energy,  
67 fertilizer or amendment (Ahmed et al., 2019; Lv et al., 2018). Among these techniques,  
68 composting, vermicomposting, and anaerobic digestion (AD), are the most commonly used  
69 processes to transform OFMSW into an agriculturally usable product (Lohri et al., 2017). In  
70 composting, the organic constituents of plant and animal origin are degraded in a controlled  
71 process involving a succession of microbial communities under aerobic conditions, which leads  
72 to an increase in temperature and the development of a humified and stabilized OM: the  
73 compost (Pergola et al., 2018; Sharma et al., 1997). Vermicomposting, like composting is also  
74 a controlled OM decomposition process (Alshehrei and Ameen, 2021). However, earthworms  
75 upstream of the decomposition process assist and accelerate the waste stabilization process  
76 (Lim et al., 2016). The earthworms used are usually epigeous (naturally living in the litter)  
77 because they reproduce quickly and consume a large amount of OM (Blouin et al., 2013;  
78 Bouché, 1977). AD is a biochemical process in which a series of microbial reactions convert  
79 solid OM into biogas ( $\text{CH}_4$  and  $\text{CO}_2$  along with a small fraction of other gases such as  $\text{H}_2$ ,  $\text{NH}_3$ ,  
80  $\text{H}_2\text{S}$ ,  $\text{N}_2$ , etc.) and a highly concentrated slurry, the digestate (Chatterjee and Mazumder, 2016).

81 This digestate generally has a solid content of less than 6% and the solid and liquid phases both  
82 have applications once separated with a decanter or separator (Bauer et al., 2009).

83 In order to predict the impact of organic products on agricultural soils, the characterization of  
84 products is a crucial issue for both researchers and farmers. Actually there are many different  
85 techniques to evaluate the maturity of organic products such as scanning electron microscopy  
86 (SEM), fourier transform infrared (FT-IR) spectroscopy, thermogravimetry, ultraviolet-visible  
87 (UV-vis) spectroscopy, evaluation of physico-chemical parameters and biological indicators  
88 (germination index) (Bhat et al., 2017). Each analysis method has advantages and disadvantages  
89 and the objective of this study was to characterize different products by comparing two classical  
90 and two innovative methods to highlight their complementarity and/or redundancy for assessing  
91 the potential effects of the products on soil.

92 The first classical method used was chemical elementary analyses, which estimates the  
93 agronomic parameters of organic amendments (organic carbon content, C:N ratio, nitrogen,  
94 phosphorus, potassium). However, the C:N ratio which is a good indicator of nutrient supply  
95 for crops (Tejada et al., 2009), does not appear to explain all the differences in N mineralization,  
96 since OM with similar C:N ratios can mineralize different amounts of N (Diacono and  
97 Montemurro, 2010). To specify the mineralization potential of the product, the Index of  
98 Recalcitrant Organic Carbon ( $I_{ROC}$ ), based on kinetics of C mineralization (three days) and Van  
99 Soest fractionation under controlled conditions, was also determined (Lashermes et al., 2009).  
100 However, this index does not consider the quality and the complexity features of each organic  
101 fraction and other complementary protocols were thus investigated such as Rock-Eval®  
102 analysis (RE) (Albrecht et al., 2015; Barré et al., 2016; Jimenez et al., 2015; David Sebag et al.,  
103 2006 and 2022). Rock-Eval® thermal analysis was originally designed for petroleum evaluation  
104 (Espitalie et al., 1986) and is a tool that can also be used for soil issues (Gregorich et al., 2015;  
105 Saenger et al., 2015; Sebag et al., 2022 and 2016; Soucémarianadin et al., 2018). With this  
106 method, the quantity of organic and inorganic carbon in a product is estimated, but its quality  
107 in terms of labile and resistant or refractory organic carbon is also defined and its degree of  
108 thermal stability is evaluated. Another complementary approach is the evaluation of the  
109 amending and microbial potential of the product through morphological characterization of  
110 microstructures using transmission electron microscopy (TEM) analysis (Watteau and  
111 Villemin, 2018). This second innovative method is a non-quantitative technique allowing us to  
112 predict the mineralization potential of OM in the soil specifically by describing the microbial  
113 activity (active bacteria and fungi, or in form of a necromass). TEM characterization of OM  
114 microstructure provides information regarding the heterogeneity in size and composition of

115 organic constituents, features that are taken into account when attempting to specify compost  
116 quality parameters such as maturity and stability (Watteau and Villemin, 2011).

117 RE analysis was never previously applied on organic material derived from OFMSW and we  
118 chose to compare it to classical analysis models (chemical and I<sub>ROC</sub>) to determine whether it  
119 could provide additional information on the carbon degradation dynamics on a long term basis,  
120 while reducing analysis time, costs and sample multiplication. To confirm the RE findings, we  
121 chose to deepen the analysis with the TEM approach. To apply this characterization strategy,  
122 we selected different products already used in the Lyon region which all contain at least 50%  
123 of urban OFMSW but different bulking agents (shredded wood, cardboard or manure). The  
124 characterization focused on optimized products rather than comparing them since the waste  
125 inputs are well known to be extremely variable for OFMSW (Fisgativa et al., 2016; Ho and  
126 Chu, 2019). The objective of this study was therefore to (i) test RE analysis for the first time on  
127 OM from urban waste, (ii) compare the analysis methods from the European standards  
128 (chemical and I<sub>ROC</sub>) which are the references for farmers, to the RE and TEM results, to specify  
129 the degree of maturity of the products and (iii) define the types of uses for composts, digestates  
130 and vermicomposts resulting from the OFMSW.

131

## 132 **2. Materials and methods**

### 133 **2.1. Origin of different products**

134 Three products generated from OFMSW were studied : (i) food waste compost produced from  
135 a selective collection of waste from professionals, (ii) digestate from an agricultural AD made  
136 with a mix of OFMSW and (iii) vermicompost from a mixture of four different collective  
137 vermicomposters.

138 The compost was made using an electromechanical composter (TIDY PLANET - A900) mixed  
139 with food waste and shredded wood as the bulking agent (volume 50/50). The waste remained  
140 in the composter for an average of 12-14 days at 50-70°C before the compost matured in bins  
141 for three months and was sifted at 20 mm and then stored in a big bag.

142 The digestate was produced by AD without phase separation in the form of sludge. At the time  
143 of digestate collection, the daily mixture consisted of 23 tons of slurry, five tons of cattle  
144 manure, five tons of paper sludge, one ton of caramel process residues, one ton of cereal, six  
145 tons of biowaste (in the form of soup) and seven tons of slaughterhouse grease. In total there  
146 was a mixture of 29 tons of agricultural residues (60%) and 19 tons of waste from the food and  
147 paper industry (40%).

148 The mixture of vermicompost was from collective vermicomposters in the same district of the  
149 city of Lyon and collected at maturity (stage ready for standardization and sale by producers).  
150 The vermicompost is made by citizens from biowaste and cardboard as the bulking agent  
151 (volume 60/40) and managed by an association and site referents. The earthworm litter (*Eisenia*  
152 *fetida* in this case) is deposited and the first bin filled to 80% with organic waste (maximum 1.5  
153 m high). Then, the second bin (glued to the first one with space for earthworm circulation) is  
154 opened and once it is filled to 80%, the mature vermicompost is collected, from the first bin,  
155 without worms, and then waste is once again added to the first bin (principle of migration to  
156 separate vermicompost and earthworms). Once the first bin is refilled, it is then possible to  
157 harvest the vermicompost from the second bin and so on. The process of transformation in a  
158 vermicomposter takes four to five months for degradation and one month for maturation  
159 (migration to the other bin) on average. After harvesting, the vermicompost is homogenized by  
160 sifting it with a 50 mm screen.

161 For each product, we took three samples of approximately 20 to 40 liters at random before  
162 taking about 20 subsamples at different depths of the heap for compost and vermicompost and  
163 liquid samples at different levels of the tank for digestate). We then performed all the analyses  
164 on each of these replicates.

165

166 Table 1 summarizes the purpose of each analysis performed in this study as well as the expected  
167 data obtained.

168  
169

Table 1: Analytical comparison of the different methods applied in the study for the characterization of organic material from OFMSW.

	Index of Recalcitrant Organic Carbon (I <sub>ROC</sub> )	Rock-Eval® (RE)	Transmission Electron Microscopy (TEM)
Analysis type	Chemical fractionation	Thermal analysis	Morphological analysis
Measurement	Indicator derived from the fractionation of organic matter and the mineralization of carbon after 3 days to evaluate its potential storage in the soil at a long term (indicator of the amending potential).	Quantify the organic and inorganic carbon during the thermal cracking of molecules over time in order to evaluate the dynamics of mineralization and carbon stabilization.	Qualitatively characterize the organic microstructures and the microbiological potential (presence, nature and activity of microorganisms) of the products.
Available (or labile) OM (weight %)	Amount of easily degradable organic matter (not be stored in the soil as humic material).	Amount of thermally labile OM released at relatively low temperature (< 400°C) during pyrolysis.	Observed available organic matter, more or less degraded by microbial activity.
Stable OM (weight %)	Amount of organic matter likely to remain in the soil as humic matter over the long term.	Amount of thermally refractory OM released at relatively high temperature (> 400°C) during pyrolysis.	Observed stable organic matter, either due to its recalcitrant nature (i.e. polyphenolic compounds) or its evolution stage (humified microstructures).

170



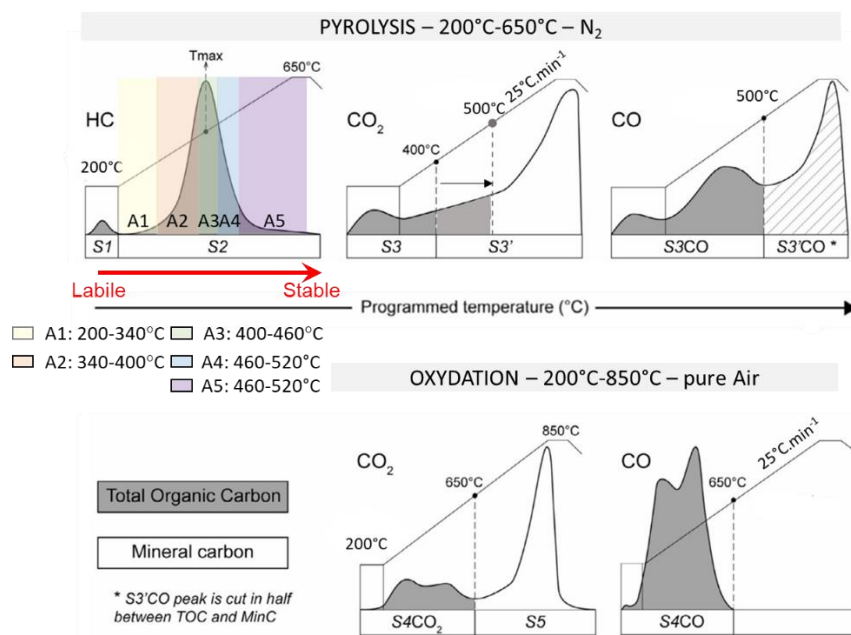
171 **2.2. Physico chemical characterization, Index of Recalcitrant Organic Carbon (I<sub>ROC</sub>) and**  
172 **sample preparation**

173 For each of the samples we performed agronomic quality analysis according to the European  
174 standards of organic amendments NFU44-051 (compost and vermicompost) and organic  
175 fertilizers NFU42-001 (digestate). We also calculated the I<sub>ROC</sub> based on the biochemical  
176 fractionation of organic matter according to Van Soest, (i.e. into the soluble fraction (SOL),  
177 cellulose (CEL), hemicellulose and lignin and cutin (LIC)) and the carbon mineralization  
178 (C<sub>min</sub>) after three (3) incubation days (C<sub>3j</sub>) for each product according to French standards FD  
179 U44-162 (AFNOR, 2016) and FD U44-163 (AFNOR, 2018), respectively. The following  
180 formula allowed us to calculate the I<sub>ROC</sub>:  $44.5 + 0.5 \text{ SOL} - 0.2 \text{ CEL} + 0.7 \text{ LIC} - 2.3 \text{ C}_{3j}$ . For  
181 the two innovative methods (Rock-Eval® and transmission electron microscope), the digestate  
182 was sieved (28.4% solids (still wet) and 71.6% liquid) in order to isolate the solids needed for  
183 the analyses.

184  
185 **2.3. Thermal analysis: Rock-Eval® (RE)**

186 RE thermal analyses were performed with a Rock-Eval 6 device manufactured by Vinci  
187 Technologies (Behar et al., 2001; Disnar et al., 2003; Espitalie et al., 1986, 1985a, 1985b, 1977;  
188 Lafargue et al., 1998; Sebag et al., 2006). For each replicate (three composts, three digestates  
189 and three vermicomposts), the RE analysis was replicated three times on 50 mg of dried and  
190 shredded sample (a similar amount of sample was precisely weighed for each analysis). The  
191 RE analysis is based on the continuous measurement of carbonaceous compounds during a  
192 progressive thermal degradation with an increasing temperature programmed at 25°C/min. The  
193 thermal degradation is performed in two successive steps: a pyrolysis under inert N<sub>2</sub> atmosphere  
194 (without O<sub>2</sub>) at a temperature of 200°C for 3 min then from 200°C to 650°C at 25°C/min  
195 followed by a pyrolysis of the pyrolyzed residue in an oxidized atmosphere (oxidation with air)  
196 at a temperature at 200°C for 1 min then from 200 to 850 °C at 25°C/min. During pyrolysis the  
197 labile and pyrolysable organic compounds released from the samples are analyzed by a flame  
198 ionization detector (FID) while the CO<sub>2</sub> and CO gases released are analyzed by an infrared  
199 detector (IR). Then during the oxidation phase, the infrared detector analyses CO<sub>2</sub> and CO  
200 produced by the combustion of pyrolysed residue (refractory organic carbon for T < 650°C and  
201 inorganic carbon for T > 650°C). The conventional RE parameters : Total organic carbon  
202 (TOC), mineral carbon (MinC), hydrogen index (HI), and oxygen index (OI) were calculated  
203 by integrating the different signal areas as described in Lafargue et al. (1998) and Behar et al.  
204 (2001). However as far as SOM and even more fresh OM are concerned, corrections have to be  
205 applied for a better quantification of TOC and MinC (see Disnar et al., 2003; Sebag et al., 2022).

206 The RE method analyzes temporal follow-up of OM cracking with deconvolution in five main  
 207 peaks: S1 when hydrocarbons are released between 0 and 200°C); S2 showing hydrocarbons  
 208 produced between 200 and 650°C: S3 obtained from pyrolysis of OM and composed of S3CO<sub>2</sub>  
 209 (CO<sub>2</sub> from 200 to 500°C) and S3CO (CO from 200 to 500°C); S4 released from residual OM  
 210 during the oxidation step of S4CO<sub>2</sub> (CO<sub>2</sub> from 200 to 650°C) and S4CO (CO from 200 to  
 211 650°C). MinC decomposition is defined during the pyrolysis by the S3'CO<sub>2</sub> peak (> 500 °C),  
 212 the S3'CO peak (> 500 °C), and the S5 peak and during the oxidation by the S5 peak (CO<sub>2</sub> >  
 213 650 °C) (Albrecht et al., 2015). To characterize OM quality defined by the thermal stability and  
 214 described by the thermolabile and thermostable carbon pools of our samples, we focused on the  
 215 S2 peak. This S2 peak was divided into five thermal ranges (A1: 200-340 °C, A2: 340-400 °C,  
 216 A3: 400-460 °C, A4: 460-520 °C and A5: 520-650 °C) according to Sebag et al. (2016),  
 217 characterizing three thermal SOC pools: labile (A1 and A2) and stable (resistant (A3) and  
 218 refractory (A4 and A5). Based on the integration of these five S2 sub-signals, Sebag et al. (2016)  
 219 proposed two new indices: the I-index reflecting the amplitude of mineralization (I index = log  
 220 (A1+A2/A3) and the R-index reflecting the carbon stability (R index = (A3+A4+A5)/100).  
 221 Figure 1 summarizes the RE principle. All RE data (parameters table and thermal profiles) are  
 222 presented in Annex 1.



223  
 224 Figure 1: Schematic diagram of Rock-Eval principle modified from Behar et al. (2001).  
 225

## 226 2.4. Transmission Electron Microscopy

227 To morphologically characterize the products, we first defined the granulometric fraction  
 228 (fraction < 2 mm and > 2mm) for compost and vermicompost on wet and dry matter (three

229 replicates each). This fractionation could not be carried out on the digestate because it was  
230 drained before handling and could not be further suitably dried and sieved. The samples were  
231 then observed with a stereomicroscope, to take into account heterogeneities and select as many  
232 subsamples as there were recognized phases (particles, fine phase, wood pieces and all other  
233 particular components) within each product. Five replicates for each subsample were then  
234 processed for morphological characterization in TEM. All subsamples were fixed in 2%  
235 osmium tetroxide ( $\text{OsO}_4$ ) in cacodylate buffer (pH 7) for 1 hour. They were then rinsed in  
236 cacodylate buffer and dehydrated with acetone in increasing concentration (10%, 20%, 40%,  
237 60%, 80%, 95% and 100%). The acetone was then progressively replaced with epoxy resin.  
238 The subsamples in the resin were then placed in molds and once the resin was polymerized  
239 ( $60^\circ\text{C}$  for 16 hours), solidified blocks were cut into ultra-thin sections (80nm thick) with a  
240 Reichert Ultracut S ultra-microtome. The 80 nm sections were collected on 200 mesh copper  
241 grids of 5 mm diameter, then stained with uranium acetate (Valentine, 1961) and lead citrate  
242 (Reynolds, 1963), in order to increase the contrast of the organic material when viewed under  
243 the electron beam. The sections were observed using a JEOL EXII transmission electron  
244 microscope (80 kV) equipped with an Erlangshen camera. A minimum of 20 observation fields  
245 at a magnification of 4,000 was observed before other magnifications were used to fully specify  
246 the origin (plant or microbial) as well as the constitution and biodegradation state of organic  
247 matter.

248

## 249 **2.5. Statistical analysis**

250 We did not carry out statistical tests on chemical results because the primary objective was to  
251 verify if the products were within the standards applied to commercial soil amendments. For  
252 the  $I_{\text{ROC}}$  values, a one-way ANOVA was used to compare the different products after verifying  
253 the requirements of normality and independence with a Shapiro and Bartlett test. For the RE  
254 analysis, we also chose to compare the products graphically to each other. Indeed, the statistical  
255 comparisons of the  $I_{\text{ROC}}$  were sufficient to express the differences between the products (even  
256 if they are different from each other). All computations were carried out in RStudio software  
257 (version 1.4.17.17) (RStudio Team, 2020).

258

## 259 **3. Results**

### 260 **3.1. Results of classical methods**

#### 261 *3.1.1. Chemical elementary analysis*

262 The results of the chemical analysis (Table 2) validated the hypothesis that the products from  
263 OFMSW are usable in agriculture because they comply with European standards. Indeed, none  
264 of the products were found to contain pathogens, pollutants or heavy metals exceeding the limits  
265 of the standards. They also did not contain either inerts or plastics above 2%. Only the  
266 vermicompost had an OM content below (15.6%) the standard limit (20% OM of raw product).  
267 The sieved digestate used for RE and TEM analysis was also analyzed and the results are  
268 presented in table 3 (28.4% of solid product).

269 Table 2: Physico-chemical analyses of dry products; values are the average of the three analyses per product with their standard deviation; nd: not  
 270 determined; abs: absence.

Samples (n=3)	Compacted density	dry weight %	pH	Ntot	N-NH4	N-NO3	Norg	OM	TOC
	kg.l <sup>-1</sup>								
Compost	<b>0.37</b> ± 0.03	<b>51.93</b> ± 7.14	<b>9.40</b> ± 0.1	<b>39.80</b> ± 1.42	<b>0.18</b> ± 0.03	<b>0.47</b> ± 0.40	<b>39.20</b> ± 1.45	<b>760.33</b> ± 18.58	<b>424.33</b> ± 8.39
Digestate	nd	<b>7.31</b> ± 0.33	<b>7.97</b> ± 0.25	<b>65</b> ± 3.4	<b>23.73</b> ± 0.42	<b>0.05</b> ± 0.01	<b>41.21</b> ± 2.99	<b>672</b> ± 2	<b>378.33</b> ± 1.53
Vermicompost	<b>0.59</b> ± 0.01	<b>37.40</b> ± 2.2	<b>8.67</b> ± 0.75	<b>22.13</b> ± 1.34	<b>&lt;0.05</b> ± 0	<b>1.09</b> ± 0.94	<b>21</b> ± 2.1	<b>417.33</b> ± 44.52	<b>219</b> ± 25.36
Samples (n=3)	C/N	P	K	Ca	Mg	Helminth and Salmonella	Fluo- ranthene	Benzo(b) fluoranthene	Benzo(a) pyrene
	g kg <sup>-1</sup>						mg kg <sup>-1</sup>		
Compost	<b>10</b> ± 0	<b>17.07</b> ± 1.40	<b>30.57</b> ± 3.62	<b>75.53</b> ± 2.15	<b>5.25</b> ± 0.19	abs	<b>&lt;0.05</b> ± 0	<b>&lt;0.05</b> ± 0	<b>&lt;0.05</b> ± 0
Digestate	<b>5.83</b> ± 0.30	<b>26.57</b> ± 1.52	<b>50.73</b> ± 0.60	<b>50.87</b> ± 0.60	<b>10.11</b> ± 0.69	abs	<b>&lt;0.05</b> ± 0	<b>&lt;0.05</b> ± 0	<b>&lt;0.05</b> ± 0
Vermicompost	<b>9.67</b> ± 0.42	<b>13.37</b> ± 0.35	<b>23.37</b> ± 6.12	<b>165.67</b> ± 22.03	<b>7.93</b> ± 0.39	abs	<b>0.08</b> ± 0,05	<b>&lt;0.05</b> ± 0	<b>&lt;0.05</b> ± 0
Samples (n=3)	As	Cd	Cr	Cu	Hg	Ni	Pb	Se	Zn
	mg.kg <sup>-1</sup>								
Compost	<b>0,85</b> ± 0,12	<b>0.27</b> ± 0.02	<b>6.17</b> ± 0.56	<b>25.57</b> ± 0.68	<b>&lt;0.02</b> ± 0	<b>4.58</b> ± 0.32	<b>9.34</b> ± 2.30	<b>&lt;1.5</b> ± 0	<b>113.33</b> ± 7.02
Digestate	nd	<b>0.25</b> ± 0	<b>18.77</b> ± 1.81	<b>47.07</b> ± 1.18	<b>&lt;0.02</b> ± 0	<b>7.56</b> ± 0.50	<b>2.62</b> ± 0.21	<b>&lt;1.5</b> ± 0	<b>240.33</b> ± 5.51
Vermicompost	<b>3.62</b> ± 1.40	<b>0.32</b> ± 0.03	<b>20.63</b> ± 6.85	<b>45.70</b> ± 1.22	<b>&lt;0.02</b> ± 0	<b>12.84</b> ± 3.90	<b>50.6</b> ± 37.42	nd	<b>203.67</b> ± 61.26

271

272

273

274 Table 3: Physico-chemical analyses of liquid of sieved digestate.

dry weight	pH	Ntot	N- NH4	N- NO3	Norg	OM	TOC	C/N	P	K	Ca	Mg	As	Cd	Cr	Cu	Hg	Ni	Pb	Se	Zn
%		g.kg <sup>-1</sup>						mg.kg <sup>-1</sup>													
7.67	7.70	66	18.90	0.10	47	645	374	5.7	22.1	71.6	41.4	7.52	1.48	0.28	14.7	79.1	<0.2	23.3	2.62	<1.5	252

275

276 3.1.2. *I<sub>ROC</sub>*

277 The results for OM fractionation (soluble fraction, cellulose, hemicellulose and lignin and  
 278 cutin), *I<sub>ROC</sub>* and *C<sub>min</sub>* are shown in Table 4. A one-way ANOVA showed that the proportions  
 279 of soluble fraction (p value = 2.474e-05 and F value = 99.959), lignin and cutin (p value =  
 280 1.479e-07 and F value = 564.23) are significantly different in the three products. Indeed the  
 281 digestate has a high soluble fraction (48.5% of OM) and a low lignin and cutin content (22.6%  
 282 of OM) while the vermicompost has a high lignin and cutin content (53.4% of OM) and a low  
 283 soluble fraction content (24.8% of OM). Intermediate values were obtained for the compost,  
 284 between those of the digestate and the vermicompost, although it is closer to the vermicompost  
 285 with 30.1% soluble fraction and 40.8% lignin and cutin. Cellulose and hemicellulose, which  
 286 are the most degradable fractions, were similar in each product with relatively low quantities  
 287 compared to the other two fractions ( between approximately 16 and 21% for cellulose and  
 288 between 5 and 11% for hemicellulose). The *I<sub>ROC</sub>* (p value = 2.3e-05 and F value = 102.49)  
 289 shows that vermicompost is the most stable product (87.2% of OM) and therefore has a lower  
 290 *C<sub>min</sub>* potential (p value = 1.364e-05 and F value = 122.56) than the other products (1.7% of  
 291 OM after three days of incubation). The digestate is the least stable (*I<sub>ROC</sub>* = 66.9% of OM and  
 292 *C<sub>min</sub>* 6.2%) while the compost showed intermediary stability (*I<sub>ROC</sub>* = 77.9% of OM and *C<sub>min</sub>*  
 293 2.7%).

294 Table 4: Biochemical fractionation and biological stability (% of OM) of the three products.  
 295 Values are the average of the three analyses per product with their standard deviation and  
 296 different letters are at the 5% level (one-way anova).

Samples (n=3)	Soluble fraction	Cellulose	Hemicellulose	Lignin and Cutin	<i>I<sub>ROC</sub></i>	<i>C<sub>min</sub></i> (3 days incubation)
Compost	<b>30.06</b> ± 2.56 <b>b</b>	<b>21.90</b> ± 2.23	<b>6.77</b> ± 1.21	<b>40.80</b> ± 0.23 <b>b</b>	<b>77.90</b> ± 1.57 <b>b</b>	<b>2.67</b> ± 0.45 <b>b</b>
Digestate	<b>48.50</b> ± 0.78 <b>a</b>	<b>17.60</b> ± 1.76	<b>11.20</b> ± 2.20	<b>22.60</b> ± 1.64 <b>c</b>	<b>66.90</b> ± 2.22 <b>c</b>	<b>6.17</b> ± 0.38 <b>a</b>
Vermicompost	<b>24.80</b> ± 2.57 <b>c</b>	<b>16.30</b> ± 3.70	<b>5.50</b> ± 5.07	<b>53.40</b> ± 1.04 <b>a</b>	<b>87.20</b> ± 1.30 <b>a</b>	<b>1.67</b> ± 0.25 <b>c</b>

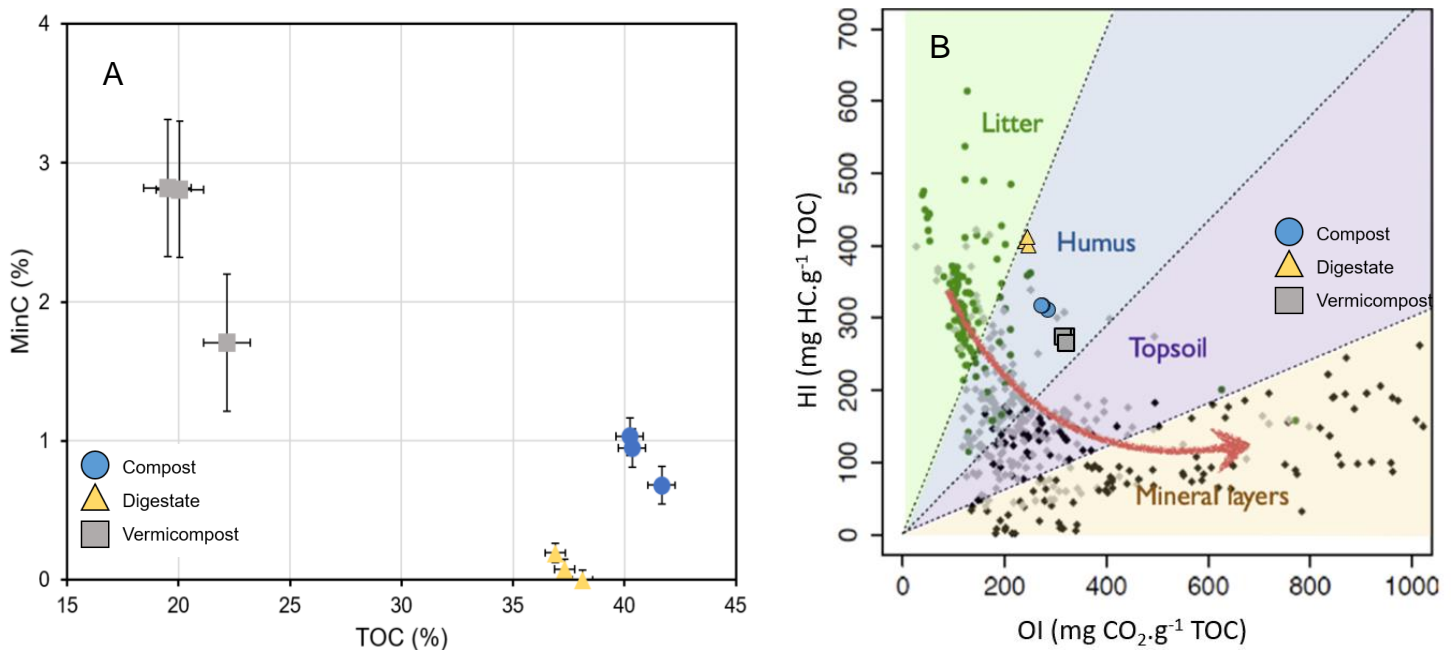
297

298

299 **3.2. Rock-Eval® thermal analysis**

300 The RE analysis revealed that the TOC content (expressed as a percentage of weight) is similar  
 301 for the digestate (37.5 ± 0.6 %) and compost (40.8 ± 0.8 %) but much lower for the  
 302 vermicompost (20.6 ± 1.4 %) which also had the most heterogeneous OM (highest TOC  
 303 variability) (see Annex 1). Moreover, by normalizing the percentage of carbon in the sieved  
 304 digestate with the RE analysis, total carbon amount reached 36.6 ± 0.5%. Highly labile organic  
 305 compounds (peak S1 - see Annex 1) are present in greater quantities in the digestate (0.5  
 306 mgHC.g<sup>-1</sup> sample) than in the compost (0.1 mgHC.g<sup>-1</sup> sample) and vermicompost (<0.1  
 307 mgHC.g<sup>-1</sup> sample), even though they remain relatively small in all samples. The MinC content

308 (expressing the amount of carbon bonded to carbonate species and calculated from the S5 and  
 309 part of the S3 peaks) is nearly absent in the digestate and accounts for  $0.9 \pm 0.2\%$  in compost.  
 310 However the highest inorganic carbon content is observed in the vermicompost ( $2.4 \pm 0.6\%$ ).  
 311 If we assume that all the inorganic carbon in the S5 peak is associated with  $\text{CaCO}_3$  species, this  
 312 represents roughly 0.3% Ca in the digestate, 3% in compost and 8.1% in vermicompost. These  
 313 differences in calcium content are similar to those observed with chemical analysis (Table 2)  
 314 and represents 5.8, 39 and 49% of the total calcium, respectively. Furthermore, only the S3CO<sub>2</sub>  
 315 and S3CO profiles of the compost show a peak at 520°C which could be attributed to the  
 316 presence of specific mineral forms of calcium such as calcium oxalate (CaOx). Figure 2A shows  
 317 a higher TOC and MinC variability for vermicompost compared to the other two products. The  
 318 HI vs. OI plot (Figure 2B) shows that HI ( $\text{S}_2 \cdot 100 \cdot \text{g}^{-1} \text{ TOC}$  expressed in  $\text{mg HC} \cdot \text{g}^{-1} \text{ TOC}$ ),  
 319 reflecting the hydrogen richness of the organic compounds of the samples, is higher for  
 320 digestate (mean value = 403  $\text{mg HC} \cdot \text{g}^{-1} \text{ TOC}$ ) followed by compost (mean value = 312  $\text{mg}$   
 321  $\text{HC} \cdot \text{g}^{-1} \text{ TOC}$ ) and vermicompost (mean value = 269  $\text{mg HC} \cdot \text{g}^{-1} \text{ TOC}$ ). The oxygen index OI  
 322 ( $\text{S}_3 \cdot 100 \cdot \text{g}^{-1} \text{ TOC}$  expressed in  $\text{mg CO}_2 \cdot \text{g}^{-1} \text{ TOC}$ ) is fairly consistent across the products with  
 323 a slight increase in the average value from digestate, to compost to vermicompost (245.2  $\text{mg}$   
 324  $\text{CO}_2 \cdot \text{g}^{-1} \text{ TOC}$ , 280.1  $\text{mg CO}_2 \cdot \text{g}^{-1} \text{ TOC}$  and 321  $\text{mg CO}_2 \cdot \text{g}^{-1} \text{ TOC}$  respectively).

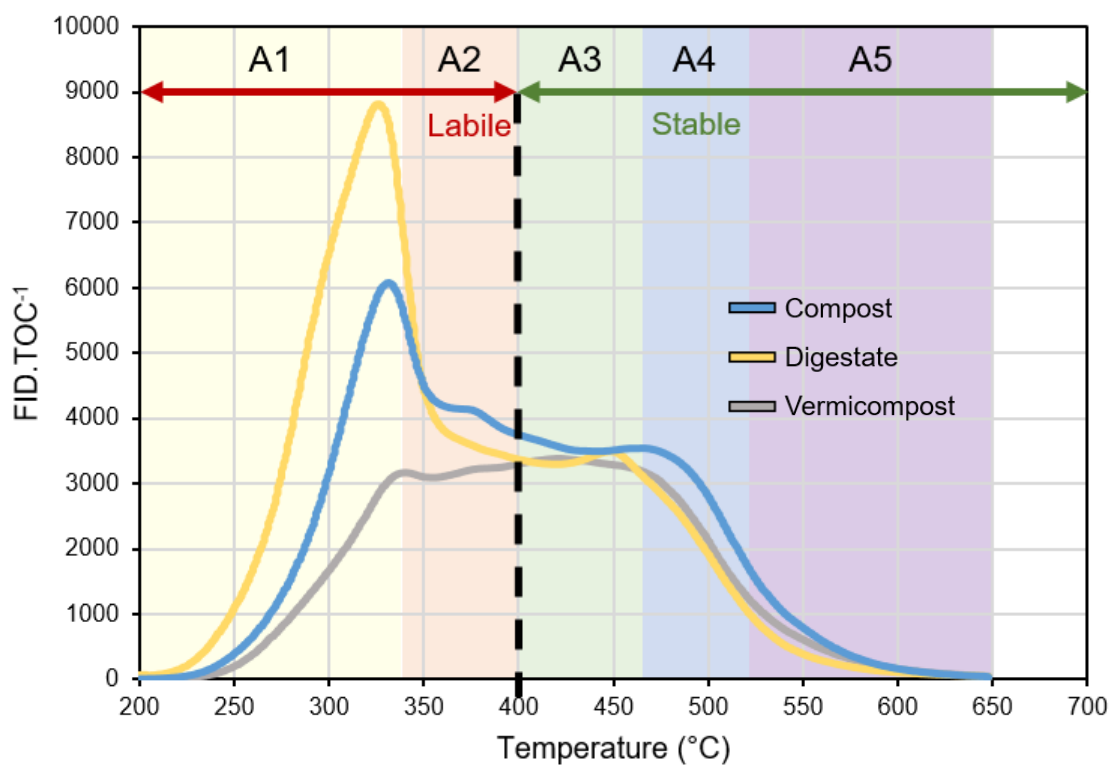


325 Figure 2: Overview of classic Rock-Eval® parameters with A. MinC vs TOC diagram. B. HI  
 326 vs OI diagram (“pseudo Van Krevelen diagram”).

327

328 The continuous analysis of thermal degradation of OM was used to characterize more precisely  
 329 the quantity and quality of the different organic products from the OFMSW ( $\text{mgHC}$  and  $\text{CO}_2 \cdot \text{g}^{-1}$

330 <sup>1</sup> of the organic products decomposed during S1 to S5 peaks). The pyrolysis profile, which  
 331 represents the amount of HC containing compounds released by thermal cracking (pyrolysable  
 332 OM), is shown in Figure 3. This pyrolysis S2 plot shows the ratio of the FID signal (pico amp)  
 333 to the TOC (%) in relation to the temperature (pyrolysis phase from 200 to 650°C) and  
 334 illustrates the resistance of carbon compounds related to the energy required for its cracking  
 335 release (Figure 3); the higher the temperature, the higher thermal stability of the carbon. To  
 336 evaluate the thermal stability using RE, the I and R indices were calculated based on the  
 337 integration of the S2 peak into five range temperature areas (A1 to A5) (Figures 1 and 3). The  
 338 S2 A1-A2 profiles were very different between the organic products but the S2 A3 to A5  
 339 profiles were relatively similar (Figure 3). These S2 profiles indicate that digestate contains  
 340 organocompounds with a significantly higher carbon mineralization potential (labile carbon  
 341 pool content) than compost and vermicompost; the contribution of areas A1-A2 into the S2  
 342 peak was much higher in the digestate (Figure 3). This carbon pool is even more important if  
 343 we consider that the TOC is also higher in the digestate.



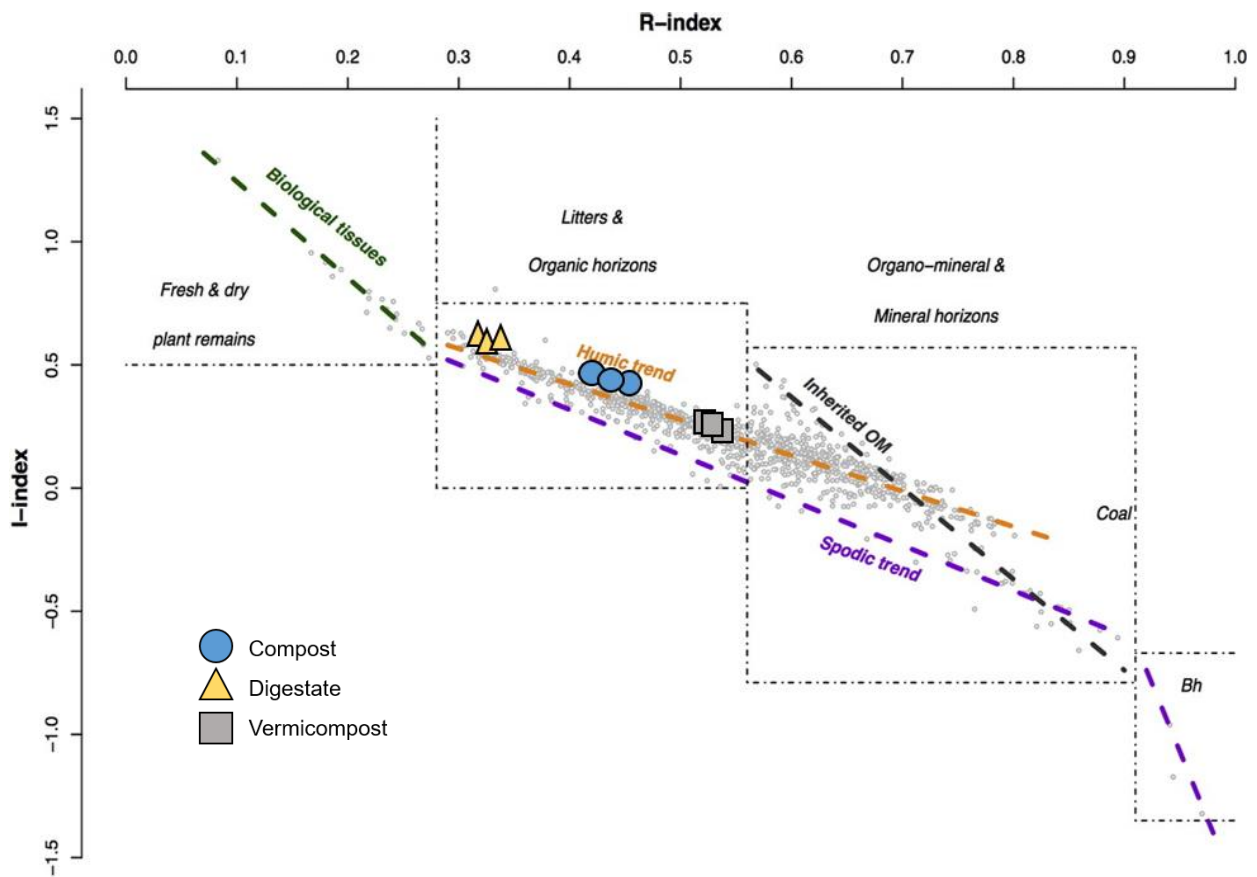
344  
 345 Figure 3: Pyrolysis S2 signal ( $\text{FID.TOC}^{-1} = \text{pA.TOC}^{-1}$ ) vs temperature for the three products  
 346 with different thermal limits (A1 to A5) according to Sebag et al. (2016).  
 347

348 This carbon stability is expressed by the I/R diagram shown in Figure 4. The I-index represents  
 349 the amplitude of SOM mineralization and the R-index the SOM thermal stability. The samples  
 350 are distributed along a regression line (“humic trend”) that reflects a progressive microbial  
 351 decomposition model for SOM. At the stage of the degradation process reached by each



352 OFMSW, the digestate has the highest remaining mineralization potential (0.58) followed by  
353 the compost (0.42) and finally the vermicompost (0.24) (Figure 4). Conversely, the R-index  
354 shows that vermicompost is composed of the most stable products (0.54) ahead of compost  
355 (0.44) and digestate (0.34). When the I/R diagram is superimposed on the Sebag et al. (2016)  
356 model (Figure 4), all the products fall within the OM category for soil litter and the organic  
357 horizon . Digestate is the most easily mineralizable product, closer to the undegraded organic  
358 tissue category. Vermicompost falls close to the junction of the litter and organo- layer  
359 categories while compost is perfectly centered within it.

360  
361 With regards to oxygen content, the pyrolysis S3 peaks showed that the compost and digestate  
362 release more CO<sub>2</sub> (S3) and CO (S3CO) (mean value = 125.7 and 109.5 mg CO<sub>2</sub> + CO.g<sup>-1</sup> organic  
363 product respectively) than the vermicompost (mean value = 71.2 mg CO<sub>2</sub> + CO.g<sup>-1</sup> organic  
364 product) (see Annex 1 for the value and Annex 2 for the profiles). There are more oxygen-  
365 containing compounds in the compost than in the digestate and vermicompost (S3 peaks were  
366 not normalized to the TOC). Finally, the combustion peaks (S4 and S5 peaks), obtained during  
367 the oxidation step under air of the remaining pyrolysed OM, revealed carbon species mainly  
368 refractory to thermal cracking and suggests that compost and digestate contain more residual  
369 organic carbon (S4 peaks mean value = 774.2 and 689.9 mgCO<sub>2</sub>+CO.g<sup>-1</sup> organic product  
370 respectively) than vermicompost (S4 peaks mean value = 437.7 mgCO<sub>2</sub>+CO.g<sup>-1</sup> organic  
371 product). The same trend was observed after normalization to TOC, which is calculated via RC  
372 (in %) parameters (see annex 1): 57.3% for compost, 59.2% for digestate against 44% for  
373 vermicompost. However, the S4 profiles were very different and clearly indicate different  
374 chemical families and/or organo-mineral interactions impacting the combustion temperature  
375 range (see annex 1). The residual carbon pool is less thermally stable for the digestate compared  
376 to the compost and vermicompost, as the organic carbon was oxidized at a lower temperature  
377 (see Annex 1 and 3). The compost appears to contain the most thermally stable organo-  
378 compounds that may be affected by the presence of calcium oxalate crystals.



379

380 Figure 4: **The** I-index vs R-index for the three products modified from Sebag et al. (2016).

381

### 382 3.3. Transmission electron microscopy analysis

383

384 The fractionation of wet matter showed that the compost and vermicompost contain  
 385 approximately the same percentage of fine particles (<2mm) of 29.7% and 28.8%, respectively.  
 386 However, after drying (water content of 60.62% for vermicompost versus 47.9% for compost),  
 387 the compost had a larger fine particle than vermicompost (46.4% versus 38.1%). The  
 388 observation of each product under the stereomicroscope defined five types of subsamples for  
 389 the compost (fine particle, aggregates, white wood, wood associated with white rot fungi, small  
 390 branches), three types of subsamples for the digestate (woody fragments associated with the  
 391 fine particle, unidentified black beads and unidentified black residues) and six types of  
 392 subsamples for the vermicompost (fine particle, eggshells, aggregates, wood fragments, seeds,  
 393 avocado skin - presumed origin). Each of these subsamples was analyzed by TEM (see Annex  
 394 1) and only examples of the fine particle results are shown in Figure 5.

395

#### 396 3.3.1 TEM characterization of the compost fine particles

397 The fine particle represented more than half the weight of the dry product since particle < 2 mm  
 398 was evaluated at 46% of the dry mass. The majority of the aggregates, accounting for an

399 important proportion of the > 2 mm particle also consisted of fine particles associated with  
400 woody residues. The fine particle mainly consisted of woody residues (tissular or cellular) from  
401 the biodegradation of plant tissues and intracellular coatings of condensed substances of  
402 polyphenolic origin were frequently observed (Figure 5 - A). Parietal fragments could be  
403 reduced to the middle lamella and some woody cushions were persistent. We also found many  
404 active bacteria, including actinomycetes, as well as some fungi which were active or in the form  
405 of cellular residues. Some micro quartz and minerals were also observed. The aggregates were  
406 also composed of parietal remains, residues of polyphenolic nature, some strongly degraded  
407 woody residues, associated with abundant microflora, mainly bacteria still active in the walls  
408 remaining to be degraded and also active fungi (Figure 5 - B). Within the fine particle and the  
409 aggregates, numerous bacterial spores attest to the rise in temperature during the composting  
410 process. The other particle of the product (white wood + wood associated with white rot fungi  
411 + small branches) was composed of more or less degraded lignin elements (Annex 4 - A). These  
412 particles are recalcitrant to degradation because of their biochemical nature (lignin and  
413 polyphenolic substances) compared to the particles of the fine fraction (pecto-cellulosic walls).  
414 The biodegradation of the woody parts is mainly due to fungi (some still active) still more or  
415 less visible (Annex 4 – B). The wood fragments are coated with the previously described fine  
416 particle, with bacteria and actinomycetes present in the residues of the woody cells.

417

### 418 3.3.2 TEM characterization of the digestate fine particle

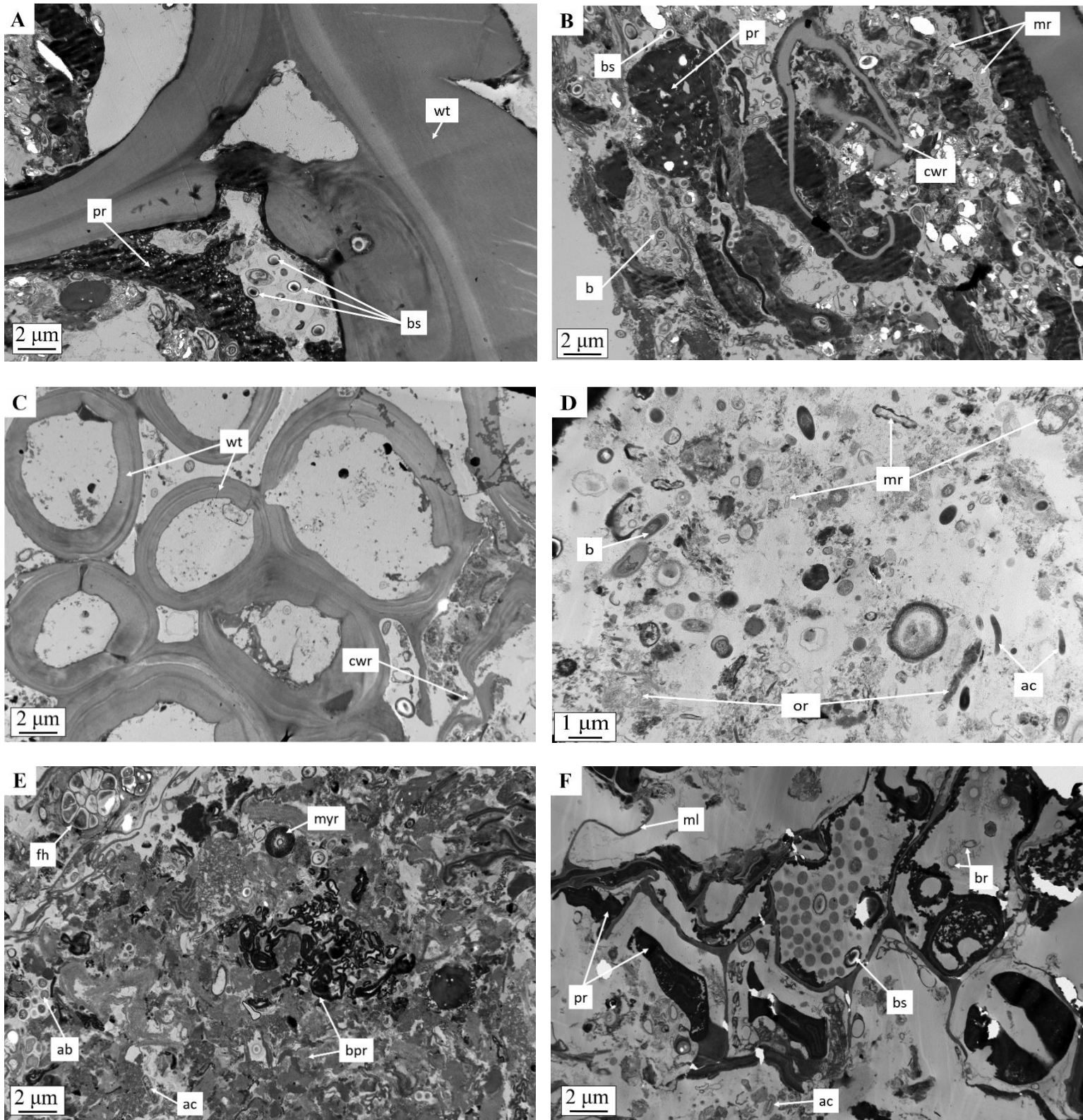
419 The sieved digestate (28.41% of the original product) was mainly composed of wood fragments  
420 and a fine particle. The wood fragments presented different stages of degradation. Some were  
421 relatively intact (Figure 5 - C), others appeared to be colonized with active fungi and a  
422 significant proportion were highly degraded (i.e. without woody thickening and cells reduced  
423 to the middle lamella). The fine particle was mainly composed of cellular residues of plant and  
424 microbial origin (Figure 5 - D). Some bacteria and rare but still active fungi were observed in  
425 wood tissues (Annex 4 – C). We also noted the presence of some minerals. A small part of the  
426 product was composed of black beads and fragments, both of which appeared to be of plant  
427 origin. The black beads and residues were composed of large cells with an intracellular content  
428 of polyphenolic origin (Annex 4 – D). Black residues also contained chloroplasts, identified by  
429 the presence of thylakoids, in some of the cells. The shape of these chloroplasts suggests the  
430 presence of algae in small quantities in the product. This part of the product was coated with  
431 the fine particle, i.e. colloidal OMs and microbial residues. The high electron-contrast of the  
432 black beads and residues suggests that these structures are recalcitrant to degradation. In

433 addition, we observed numerous holes in the resin in the chloroplast areas which may be due to  
434 the presence of starch grains, the hardness of which leads to tearing during ultra-thin  
435 sectioning.

436

### 437 3.3.3 TEM characterization of the vermicompost fine particle

438 The vermicompost was essentially composed of a fine fraction (38% of dry product) and  
439 aggregates (62% dry product) which were themselves mainly constituted of this same fine  
440 particle. The fine particle was composed of highly evolved organic residues in the form of an  
441 organic matrix envelopped some parietal or polyphenolic residues (Figure 5 - E). This fraction  
442 is very rich in microorganisms with the presence of fungi and many isolated bacteria or colonies  
443 which degraded the plant remains. Microbial residues were also present, attesting to the past  
444 activity of these microorganisms. We noted the presence of some chloroplasts and just a few  
445 unidentified plant structures, as well as some minerals. The aggregates, which mainly resulted  
446 from the aggregation of the fine particle, had a higher proportion of polyphenolic and tissue  
447 residues (probably due to the protective effect of the aggregation) (Figure 5 - F). There appeared  
448 to be less microbial activity than in the fine particle and the only living fungi were within the  
449 still biodegradable rare woody residues. The other parts of the vermicompost were essentially  
450 eggshells, seeds and wood fragments. The woody structure of the seeds was more or less  
451 degraded by fungi and actinomycetes which were still largely present (Annex 4 - E). These  
452 tissues were covered by the fine particle. The wood fragments were highly degraded and the  
453 majority of the woody thickenings had disappeared. Only the middle lamella remained and were  
454 packed on each other and cellular contents of a polyphenolic nature (Annex 4 - F). Fungi and  
455 bacteria remained very present and active within the still biodegradable plant structures and  
456 bacterial spores were also observed.



457

458 Figure 5: Transmission electron microscope observation of (A) and (B) the compost fine particle, (C)  
 459 the digestate fine particle composed of relatively intact plant residues, (D) the digestate fine particle  
 460 composed of microbial residues, (E) the vermicompost fine particle composed of plant residues with  
 461 high biological activity and (F) the vermicompost fine particle within aggregates composed of  
 462 contrasted cellular residues. ac, actinomycete; ab, active bacteria; b, bacteria; bpr, biodegraded plant  
 463 residues (the whole matrix of (E) is mainly composed of these residues); bs, bacterial spores; cwr,  
 464 cell wall remain; fh, fungal hyphae; ml, middle lamella; mr, microbial residues; myr, mycelial residue; or,  
 465 organic residues; pr, polyphenolic residues; wt, woody thickening.

## 466 **4. Discussion**

### 467 **4.1. Complementarity of the analysis methods**

#### 468 *4.1.1. Chemical elementary analysis, I<sub>ROC</sub> and interest to farmers*

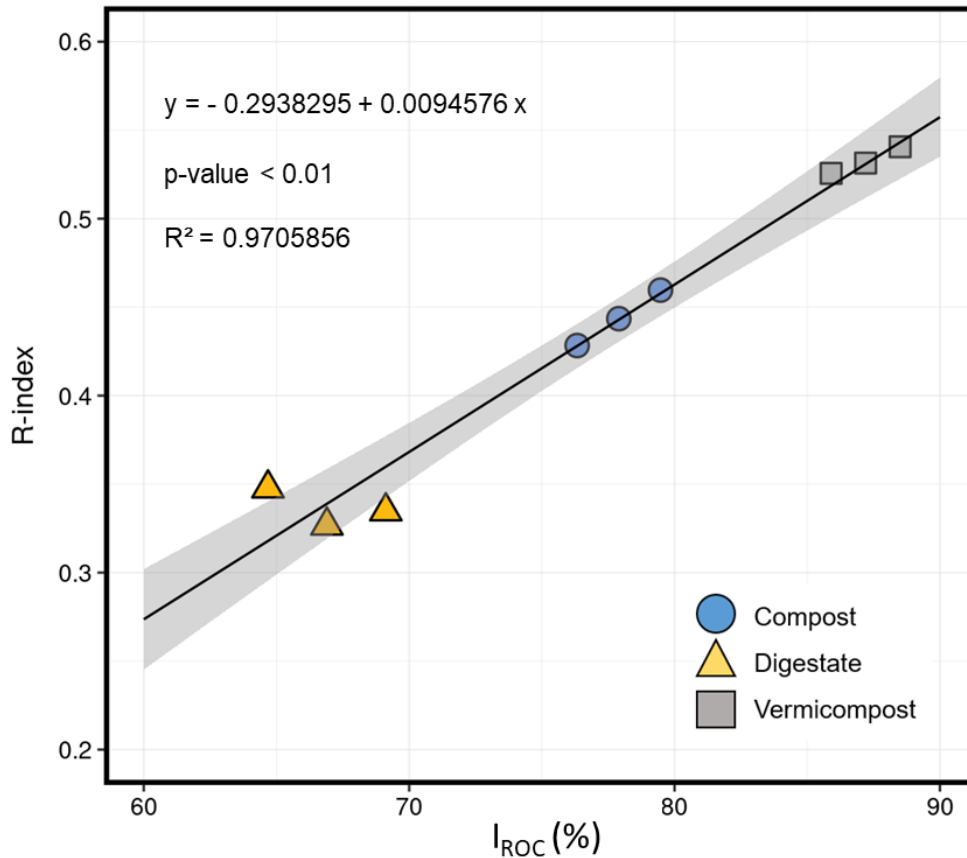
469 All three products contained different bulking agents and were generated from different  
470 processes leading to products with different overall characteristics. For example, for the same  
471 C or N input, farmers will need to apply much higher quantities of digestate than compost or  
472 vermicompost considering the C contents, but less if we consider the N content in the dry  
473 products. It is worth noting that for research, products are compared on a dry basis whereas in  
474 practice, farmers generally compute application doses on raw products without taking into  
475 account their water content. If we consider analyses of raw products, the digestate has the lowest  
476 contribution in C or N equivalent (requiring the supply of a larger quantity of raw material by  
477 weight) followed by the vermicompost and the compost (requiring the smallest amount of raw  
478 material by weight in C or N equivalent). For example, larger inputs of vermicompost than  
479 compost will lead to higher recalcitrant carbon inputs on the soil. Chemical analysis alone is  
480 not sufficient to predict the effects of a product when applied to the soil. However, these  
481 chemical analyses can be used to define the maturity of a product and help professionals to stop  
482 the recovery process (Silva et al., 2022). To define the amending potential of OM in the soil,  
483 the I<sub>ROC</sub> was developed to estimate the proportion of stable carbon potentially accumulating in  
484 the soil. However, this method is simply complementary to the European standard and is  
485 therefore not mandatory for the sale of a product. Although this technique is widely recognized  
486 and reliable, it is long to set up, expensive and generates polluting chemical waste, thus it would  
487 be desirable to develop faster and more sustainable methods. The reliability of the I<sub>ROC</sub> (created  
488 from a database of 400 Van Soest biochemical fractionation analyzes of different organic  
489 products) is however very useful for developing models on the fate of carbon in soils (Peltre et  
490 al., 2009).

491

#### 492 *4.1.2. Redundancy and complementarity between classical and Rock-Eval® analyses*

493 In this study, the RE analyses were performed on products from recycling of OFMSW which  
494 are enriched in fresh OM. This method applied to this type of heteroelement-enriched OM (in  
495 O and N in particular) gives new perspectives on the characterization of this category of  
496 products (organic amendment) compared to classical methods. Indeed, the results of RE ramped  
497 thermal analysis provide insights which go beyond trends observed for soil samples, such as,  
498 the S2 profile addressing the thermally range repartition of soil C pools. This method is

499 particularly encouraging because all the results in our study were consistent with the results of  
500 the classical method. For example, the TOC found in RE analysis in all products were in good  
501 agreement with the TOC values from chemical analysis (Tables 2). Since a higher R-index is  
502 related to preferential decomposition of more labile organic compounds (Sebag et al. 2016,  
503 2022), it can be thus correlated to the degree of OM mineralization, as demonstrated by a  
504 comparison of incubation experiments and RE analysis (Domeignoz-Horta et al., 2021).  
505 Furthermore, a link was established for the first time between I<sub>ROC</sub> measuring mineralization  
506 potential of the product and the RE R-index measuring the preferential decomposition of more  
507 labile organic compounds, as shown in figure 6. Although this correlation needs to be further  
508 investigated and validated on more products, our results are promising as they are based on  
509 three very different types of OM. The data are well correlated ( $R^2=0.97$ ) with a higher  
510 dispersion of the data for the digestate. Furthermore, the carbon thermal resistance used in the  
511 RE method is consistent with the resistance of carbon to mineralization as demonstrated by,  
512 among others, Domeignoz-Horta et al. (2021) showed direct evidence of a relationship between  
513 thermal stability and soil microorganism decomposition potential. Based on these preliminary  
514 results, the R index calculated from RE ramped thermal analysis could be used as a proxy to  
515 predict the amending capacity of an organic product, equivalent to the I<sub>ROC</sub>, without requiring  
516 complex chemical analysis or sample manipulation. One limitation compared to the elementary  
517 chemical analysis method is that the RE relies only on the HC component and not on the  
518 elementary composition of the products, which can be essential for farmers (N, P and K for  
519 crop growth).



520

521 Figure 6: Correlation of the I<sub>ROC</sub> and the R-index (Rock-Eval®) for the three products.

522

523 *4.1.3. Additional insight provided by the transmission electronic microscopy (TEM)*

524 The TEM method is a very different approach for characterizing the nature of OM and its  
 525 decomposition state at the micrometer scale and provides useful information on the status of  
 526 microorganisms. With TEM, the heterogeneity of OM can be specified and the status (living or  
 527 not) of bacteria and fungi (e.g. intact state of living organisms, spores, residues) at the time of  
 528 sampling can also be determined. For example, in our case, although the RE data obtained for  
 529 the digestate suggested the presence of microbial derived compounds, the TEM showed that it  
 530 was a necromass and thus we can expect limited further transformation of the product. In  
 531 addition, micrometric characterization combined with other methodologies such as soil and OM  
 532 fractionation (such as Van Soest for the OM), can be used to study both the incorporation of  
 533 biodegraded litter into soil aggregates and the impact of microbial dynamics on soil  
 534 aggregation, notably through the production of extracellular polymeric substances (Watteau and  
 535 Villemin, 2018). Our ability to predict the effect that these organic products will have when  
 536 applied to the soil was improved by combining the findings from the different analyses .

537



## 538 **4.2. Characterization and comparison of the different organic products from the OFMSW**

### 539 *4.2.1. Characterization of compost*

540 RE analysis revealed the possible presence of calcium oxalate (CaOx) in the compost, a  
541 common bio-mineral present in plant tissue (Costa et al., 2009; Franceschi and Nakata, 2005;  
542 Xi-Min et al., 2019), which probably came from the shredded wood chips used as an bulking  
543 agent. Indeed, such addition to OFMSW allows a better control of the process but impacts the  
544 quality of the final product (Maxianova et al., 2019). TEM highlighted that the microflora of  
545 the compost is still very active with the presence of fungi degrading lignin and also bacteria  
546 present in all OM constituents. The presence of these microorganisms shows that the product  
547 is still decomposing and that some of the recalcitrant materials are made accessible by microbial  
548 activity. It also shows that the product does not yet have an optimum biochemical stability in  
549 spite of the presence of microbial spores which attests to the past biological activity during the  
550 composting process. The compost, as revealed by the RE and TEM analyses, is a partially  
551 degraded product which has an amending capacity (undegraded recalcitrant matter) with a  
552 portion directly accessible to plants thanks to the active microflora. Indeed, the compost may  
553 not have reached maturity and may have been sold too soon. This problem is really common  
554 in food waste composting which is difficult to monitor (Cerda et al., 2018). At the same time,  
555 with the increase in decentralized management of urban waste (collective composting) (Pai et  
556 al., 2019), compost quality control will become more complex (multiplication of analysis costs).  
557 In our case, this compost from an urban electromechanical composter would be suitable for  
558 carbon storage or in soil bioremediation.

559

560

### 561 *4.2.2. Characterization of digestate*

562 The digestate is rich in N and poor in C, as generally described in the literature (particularly in  
563 liquid phase) (Guilayn et al., 2019), because anaerobic digestion aims to extract carbon (CH<sub>4</sub>)  
564 for producing energy (Bastabak and Kocar, 2020; Leung and Wang, 2016). The RE analysis  
565 confirmed that it contains a significant portion of labile organic C, suggesting that the product  
566 is at a limited stage of decomposition. Indeed, most of digestate is currently treated with phase  
567 separation to recompose the solid particle (majority of carbon) and use the liquid as fertilizer  
568 (Angouria-Tsorochidou et al., 2022). Since the product had not undergone this separation, it  
569 was not very mineralized and can be categorized as a fertilizer (Nkoa, 2013). Moreover,  
570 although the RE analysis detected the presence of microorganisms (through higher HI index),  
571 the TEM showed that they were not active. The RE measurements therefore took into account

572 a necromass probably due to previous activity of microorganisms with few traces of organisms  
573 still living. However, all the OMs (decomposed woody elements and residues in the liquid  
574 fraction) seem to be available for plants. In our case, the AD was carried out after the waste had  
575 been sterilized at 70 degrees for three days which can explain the absence of organisms. The  
576 AD process is still developing (O'Connor et al., 2022) and it would be interesting to use these  
577 analyses to investigate the differences between solid digestate (rich in carbon) and liquid  
578 digestate (fertilizer) which can both have different applications (Ronga et al., 2019).

579

#### 580 *4.2.3. Characterization of vermicompost*

581

582 The RE applied to vermicompost revealed a higher TOC and MinC variability for  
583 vermicompost compared to the other two products. This can be explained by the higher sample  
584 heterogeneity due to the mixture of four different vermicomposters. The presence of lignin and  
585 eggshell derivatives (Annex 3) was detected with RE oxidation thermograms (S4 and S5 peaks).  
586 The residual organic C content was lowest for vermicompost (RC% = 44%) and its  
587 carbonaceous macromolecules belong to apparently much more homogeneous chemical  
588 families compared to those present in compost or digestate. Thus this product appears to have  
589 undergone the most advanced mineralization (lowest I and highest R index). The TEM  
590 confirmed that vermicompost is a highly evolved product where the lignolytic activity of the  
591 microorganisms has degraded the majority of the woody fragments. The plant residues were  
592 either resistant OM (middle lamina and polyphenolic residues) or accessible almost colloidal  
593 OM. The microbial potential was mainly present in the form of residues but there was still some  
594 activity mainly associated with remaining biodegradable residues. To evaluate the potential of  
595 vermicompost it may be interesting to carry out quantitative microbiological analyses such as  
596 enzyme assays (Li et al., 2020) or microbiome analysis with 16S ribosomal DNA (Hashimoto  
597 et al., 2021). Our approach confirms that the more interesting potential of vermicompost is its  
598 biological potential rather than its chemical quality. Indeed, the combination of vermicompost  
599 with plant growth promoting rhizobacteria had promising results on yields due to its strong  
600 biological potential (Karagoz et al., 2019; Moridi et al., 2019; Zahra et al., 2019). The  
601 vermicompost in our study was produced by citizens in collective systems and we can assume  
602 that with a better control of the system (less variability) it would be possible to increase the  
603 quality of the product.

604

#### 605 *4.2.4. Comparison of different products*

606 First of all, based on the C/N of the products, the digestate is the most mature product , with a  
607 C/N of 5.83 compared to close to 10 for the compost and vermicompost; a lower C/N indicates  
608 more N and, in theory, a more mature product. Indeed, compared to the compost and  
609 vermicompost, the digestate is richer in N and poorer in C. The biochemical fractioning analysis  
610 found that vermicompost contains more lignin and cutin and has a higher  $I_{ROC}$ , while digestate  
611 has a higher soluble fraction associated with the lowest  $I_{ROC}$  of the products (Table 4). The  
612 compost and the vermicompost are more similar in terms of C/N but the complementary  
613 analyses highlighted their differences.  $I_{ROC}$  showed less lignin and cutin in the compost than in  
614 the vermicompost. This is linked to the fact that the first was made from wood chips while the  
615 latter was made from cardboard (and thus cellulose). Thus other compounds such as condensed  
616 polyphenols can be found in this "non-extractable" fraction (typically the condensed  
617 polyphenols of the vermicompost revealed by TEM). According to these results, the digestate  
618 appears to be more of a fertilizer (low  $I_{ROC}$  and a low OM rate: 67.8% of OM in 7.31% of the  
619 dry product; Table 2) whereas the compost and the vermicompost are amendments. Our  
620 findings also suggest that compared to the compost, the vermicompost has a higher fertilization  
621 potential. Further information on the quality of organic content was provided by RE in terms of  
622 hydrogen (S2, HI) and oxygen (S3 and S4 peaks, OI) as well as of mineral carbon (S5 peak)  
623 contents. The decrease in HI between the three products highlights either a variable initial  
624 biomass and/or microbial input or a different degradation state. However, the most probable  
625 hypothesis to explain a higher hydrogen content in the digestate is the presence of a higher  
626 amount of microbial necromass (which was confirmed with TEM analysis) related to the  
627 anaerobic digestion process. The TEM confirmed that vermicompost is more advanced than  
628 compost because half of the OM is very stable and the other half is mineralizable. If we compare  
629 it to the other products, vermicompost has both the boost effect of digestate (fertilizer) and an  
630 amendment effect like compost (carbon storage in the longer term). Finally, TEM also  
631 characterized the nature of the recalcitrant woody fraction which was more polyphenolic in the  
632 vermicompost than in the compost, the latter implying more biological activity able to degrade  
633 the lignin of the compost (already degraded in the vermicompost). The vermicompost has  
634 therefore a higher proportion of very available carbon to plants in its fine colloidal fraction  
635 (boost effect) and the higher reserves in more humified OM would be amending in the long  
636 term. Note that in our case it is still difficult to compare products since the input materials are  
637 very different and were not controlled.

638

### 639 **4.3. Practical implications of this study**

640

641 The quality of products from the OFMSW varies greatly depending on the recycling technique  
642 used (Komakech et al., 2015) and waste typologies (Fisgativa et al., 2016) despite generally  
643 positive effects on soils and crops (Obriot et al., 2016). Indeed, OFMSW recycling can lead to  
644 the production of fertilizers in liquid digestates (Tampio et al., 2016) or amendments for  
645 composts (Diacono and Montemurro, 2010). It is therefore necessary to precisely characterize  
646 exogenous OM to evaluate their potential contribution on agricultural soils (Stewart-Wade,  
647 2020), especially for OFMSW (Hanc et al., 2011; Mendes et al., 2013). Our study found that  
648 the three studied products would have different properties for soils with a fertilizing role for the  
649 digestate, an amending role for the compost and a fertilizing and amending role for the  
650 vermicompost. Our work emphasises that the classical chemical analyses imposed in the  
651 standards (NFU44-051 for organic amendments and NFU42-001 for organic fertilizers in  
652 Europe) are not sufficient to predict the behavior of a product. It is at least necessary to perform  
653  $I_{ROC}$  analyses which is not mandatory in NFU44-051. Our study highlights the interest of RE  
654 analysis to specify the fate of carbon in parallel with the  $I_{ROC}$ . However, only TEM will provide  
655 accurate information on the state of decomposition, even though it is qualitative. Given the  
656 diversity of products, it may be attractive to create standards for each process with specific  
657 analyses for each one. For example, vermicompost has a high biological activity. It may  
658 therefore be interesting to promote biological analyses for this type of product and not to focus  
659 on the analysis of its chemical components and its higher moisture content than compost. For  
660 compost, carbon analysis on a long term basis (RE) may be of interest for following carbon  
661 dynamics and predicting its storage capacity. For the digestate (liquid), chemical analyses seem  
662 quite appropriate because it is essentially a fertilizer.

663

664

### 665 **5. Conclusion**

666 Our study demonstrates a complementarity between the methods of quantitative ( $I_{ROC}$  and RE)  
667 and qualitative (TEM) evaluations. In addition, we showed for the first time that it is potentially  
668 possible to determine the  $I_{ROC}$  from R index obtained by Rock-Eval® analysis to predict the  
669 biochemical composition of the products ( $R^2=0.97$ ). This complementarity of analyses can be  
670 used to predict more precisely the fate of organic matter in the field. In our case, the (liquid)  
671 digestate would be preferentially applied to soils already rich in organic matter, to quickly

672 increase crop yields. If this product is to be applied in a recurrent way, it would be necessary to  
673 compensate with carbonaceous organic matter to avoid the depletion of soil C. Compost could  
674 perfectly fulfill this function because, as in our study, it has a good potential for carbon storage  
675 in the soil with an incomplete mineralization (medium term amending potential compared to  
676 vermicompost). On the other hand, compost would have no effect on yields in the short term  
677 (first year). Finally, vermicompost, which is very degradable, could have a double effect: an  
678 increase in yields and a carbon storage over a very long term. It can therefore be used in any  
679 situation since the product seems to be well balanced. For future research it would be interesting  
680 to compare Rock-Eval® with other techniques such as infrared, which are generally also  
681 inexpensive. TEM analysis is a time-consuming and expensive technique but remains very  
682 accurate and should be applied to more products from OFMSW in the future to better  
683 understand the functioning of OM in the products and in the soil. Indeed, TEM analysis  
684 characterizes the microbiological status of the products which is often neglected in the chemical  
685 analysis of OM. Finally, it would be very constructive to confirm the hypotheses described in  
686 these analyses by field and/or laboratory tests and to apply this approach to other products with  
687 a view to developing relevant indexes correlating characterization and use of these organic  
688 products.

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695

696 **Conflicts of interest/Competing interests**

697 The authors have no conflict of interests or competing interests to declare.

698

699 **Ethics approval**

700 Not applicable.

701

702 **Consent to participate**

703 Not applicable.

704

705 **Availability of data and material**

706 The datasets generated and analyzed during the current study are available on demand.

707

708 **Code availability**

709 The code used in this article is available from the corresponding author upon reasonable  
710 request.

711 **Authors' contributions**

712 Conceptualization, VD, FW, IK, YC, JP ; Methodology, VD, FW, IK, HR, YC, JP ;  
713 Investigation, VD, FW, IK, YC, JP ; Formal analysis, VD, FW, IK ; supervision, project  
714 administration, YC, JP ; writing and editing VD, FW, IK, YC, JP; Funding acquisition, VD,  
715 FW , IK, YC, JP. All authors read and approved the final manuscript.

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