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1 **Chemical degradation of thermally altered silicified organic matter during acid**  
2 **maceration: a case study from the Lower Devonian Rhynie chert**

3

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16

## 17 **ABSTRACT**

18 The effect of standard acid maceration on organic matter (OM) from ancient silicified  
19 sediments remains undocumented. Early silicification favours preservation of organic  
20 moieties against thermal alteration over time. In this study, we investigated the effects of acid  
21 maceration on the structure of OM isolated from the Lower Devonian Rhynie chert. The  
22 structure of OM was investigated by combining Rock-Eval pyrolysis and Raman  
23 spectroscopy. Besides a loss of thermolabile organic matter owing to solvent extraction,  
24 Rock-Eval pyrolysis showed that standard acid maceration also causes a loss of C-H  
25 emissions at high pyrolysis temperature ( $> 500\text{ }^{\circ}\text{C}$ ). The standard acid maceration procedure  
26 was also associated with the disappearance of the D4 and D5 Raman spectrum shoulders  
27 assigned to C–H bonds in aliphatics and bitumens, respectively, entrapped in the  
28 macromolecular network. Taken together, Rock-Eval pyrolysis and Raman spectroscopy  
29 indicate that standard acid maceration can lead to the chemical degradation of syngenetic  
30 hydro-carbonaceous moieties of OM isolated from ancient silicified and thermally altered  
31 sediments. In sediments having experienced early silicification, which hampers bitumen  
32 migration and favours pyrobitumen formation, we suggest that novel in situ molecular  
33 analytical techniques are required to provide a thorough examination of the syngenetic  
34 molecular content independent of the soluble/insoluble operational definition.

35

## 36 **1. Introduction**

37 The chemical composition of ancient organic matter (OM) attests to turning points in  
38 the evolution of life during the history of the Earth (Summons et al., 1988; Edwards et al.,  
39 1997; Love et al., 2008; Duda et al., 2016; Nguyen et al., 2019; Love et al., 2020). However,  
40 investigating the structural and chemical compositions of ancient OM is still a challenging  
41 issue as thermal alteration during geological times results in losses of pristine molecular and

42 elemental content. Since ancient sediments and metasediments are generally depleted in OM,  
43 isolation procedures have typically been employed before applying analytical techniques to  
44 investigate the chemical composition of ancient OM, notably at the molecular scale.  
45 Historically, in the field of organic geochemistry, isolation of organic-insoluble and mineral-  
46 free OM is performed through a standard acid maceration procedure involving maceration  
47 followed by successive organic solvent extractions (e.g., dichloromethane [DCM] and  
48 methanol [MeOH]) and demineralisation with acid (e.g., hydrochloric [HCl] and hydrofluoric  
49 [HF]). This physical and chemical maceration procedure (HMM) yields kerogen, which is  
50 defined as insoluble macromolecular OM (Durand, 1980; Vandenbroucke and Largeau,  
51 2007). Several investigations have suggested that acid maceration does not significantly  
52 modify the structure of kerogens from ancient rocks (Larsen et al., 1989; Vandenbroucke and  
53 Largeau, 2007; Aboulkas and El Harfi, 2009). However, there is contrasting evidence that  
54 acid maceration of coals can lead to either a rise in carbon structural order (Zhang et al., 2016)  
55 or a decrease in hydrocarbon content (Tekely et al., 1987). Moreover, Kebukawa et al. (2019)  
56 demonstrated the contrasting effects of acid maceration by comparing the chemical structure  
57 of bulk chondrites to their corresponding insoluble OM fractions. Insoluble OM from type 3  
58 chondrite was depleted in aliphatic moieties in comparison to the bulk type 3 chondrite  
59 starting material. However, the effect of acid maceration varied among the different chondrite  
60 groups studied (Kebukawa et al., 2019). Thus, according to current literature, it therefore  
61 seems that the chemical stability of thermally altered OM to standard acid maceration cannot  
62 simply be assumed.

63         Among ancient sedimentary rocks, silicified sediments are of interest as they are  
64 essential geological archives of biological evolution throughout Earth's history. However,  
65 there is often very little OM remaining in these sediments and acid maceration procedures are  
66 required to concentrate it before studying its molecular content. Occurring prior to cell lysis

67 and early degradation of organic matter, rapid silicification leads to a closed chemical system  
68 by reducing sediment porosity (Boyce et al., 2002; Ledevin et al., 2014), which favours  
69 preservation of organic remnants against thermal alteration (Alleon et al., 2016). Using  
70 geochemical micrometre-scale analysis tools, it was demonstrated that some silicified  
71 Precambrian organic-walled microfossils are composed of significant amounts of carbonyl,  
72 phenolic, carboxylic, hydroxyl, and amide functional groups, despite being subjected to peak  
73 temperatures of up to 300 °C (Alleon et al., 2016). However, standard acid maceration  
74 procedures applied to ancient silicified sediments have the potential to degrade syngenetic  
75 OM through solvent extraction and acid hydrolysis of bitumens and chemically labile organic  
76 moieties, respectively. Such degradation, if it occurs, implies that a substantial amount of the  
77 initial molecular content depicting the evolution of life, past environmental conditions, and/or  
78 thermal maturation may be lost in the process. Further investigations on the effect of standard  
79 acid maceration on the chemical structure of ancient thermally altered silicified OM are  
80 therefore required.

81         Our aim in this study was to investigate the effect of the standard acid maceration  
82 procedure on the chemical structure of ancient silicified OM. We selected the iconic Lower  
83 Devonian Rhynie chert, the earliest preserved terrestrial ecosystem owing to a rapid and  
84 complete silicification through siliceous hot-spring deposits (Trewin, 2003; Preston and  
85 Genge, 2010). Despite the exceptional preservation of fossil plants, fungi, insects, and other  
86 organisms, the overall organic carbon content of Rhynie chert is very low (Summons et al.,  
87 1996). It therefore typically requires acid maceration to access the molecular signature(s). The  
88 Rhynie chert therefore constitutes a case study to evaluate the impact of standard acid  
89 maceration on the chemical structure of ancient thermally altered silicified OM. To this end,  
90 OM was isolated according to two acid maceration procedures: (1) a high-manipulation  
91 maceration (HMM), which followed the standard treatments generally applied to geological

92 samples, and (2) a low-manipulation maceration (LMM), which minimized chemical  
93 degradation by avoiding organic solvents and HCl. Rock-Eval pyrolysis and Raman  
94 spectroscopy were used to assess the chemical structure of the OM isolated by each  
95 procedure. Comparison of the so-obtained chemical structures allowed us to estimate the  
96 minimum amount of chemical degradation imparted by HMM maceration.

97

## 98 **2. Material and methods**

### 99 *2.1. The Rhynie chert*

100 Situated in Aberdeenshire (Scotland), the Rhynie chert is a Konservat-Lagerstätte. It is  
101 hosted by the Dryden Flags Formation, which is characterised by a succession of Lower  
102 Devonian sedimentary and volcanic rocks (Rice et al., 2002). Pragian–earliest Emsian in age  
103 ( $407.1 \pm 2.2$  Ma; Mark et al., 2011), the Rhynie chert is composed primarily of  
104 microcrystalline silica and was deposited as siliceous sinter from subaerial hot springs  
105 systems (Rice et al., 1995).

106

### 107 *2.2. High- and low-manipulation maceration procedures*

108 HMM and LMM procedures were performed on similar portions of the same rock  
109 sample. HMM was performed on ~30 g of crushed rock. Solvent extraction was first  
110 performed on rock powder using a mixture of DCM and MeOH (2:1; v:v). Carbonates were  
111 then removed at room temperature using HCl (37%; reagent grade) to minimise the formation  
112 of fluorides during subsequent HF/HCl maceration. Samples were then centrifuged and  
113 washed with ultrapure water until reaching neutrality. Concentration of OM was achieved  
114 through acid maceration at room temperature in a mixture of HF (40%, reagent grade) and  
115 HCl (2:1, v/v; reagent grade). Samples were centrifuged and washed with ultrapure water to  
116 reach neutrality. Finally, HCl (37%; reagent grade) at 60 °C (24 h) was used to degrade

117 neoformed fluorides. After an additional step of solvent extraction using a mixture of DCM  
118 and MeOH (2:1, v/v), the isolated OM was again centrifuged/washed with ultrapure water  
119 until reaching neutrality. Samples were then air-dried at 60 °C after final rinsing in acetone.  
120 Hereafter, OM isolated by the HMM procedure is referred to as OHMM.

121 LMM was performed by first fragmenting ~30 g of rock samples into ~3 g rock chips.  
122 Rock chips were cleaned using ultrapure water and a mixture of DCM and MeOH (2:1, v/v)  
123 and were then placed directly in a Teflon vessel filled with HF (40%, reagent grade) at room  
124 temperature. After 48 h, successive centrifugation and rinsing steps using ultrapure water  
125 were performed until reaching neutrality. Samples were then air-dried at 60 °C. Hereafter,  
126 OM isolated by the LMM procedure is referred to as OLMM.

127

### 128 *2.3. Rock-Eval pyrolysis*

129 OHMM and OLMM were analysed using Rock-Eval 6 (Vinci Technologies) following  
130 the standard pyrolysis protocol described in Behar et al. (2001). Performed in a N<sub>2</sub>  
131 atmosphere, Rock-Eval pyrolysis comprises two steps: an isothermal phase held for 3 minutes  
132 followed by a rise in pyrolysis temperature from 300 to 650 °C at a rate of 25 °C/min. After  
133 pyrolysis, the residual material was then heated from 300 °C to 850 °C under purified air in an  
134 oxidation oven in order to calculate total organic carbon (TOC) value (see Behar et al., 2001  
135 for further details about calculation procedure). Released hydrocarbons (HC) were  
136 continuously quantified by a flame ionisation detector (S1 and S2, for the first and second  
137 pyrolysis steps, respectively, in mg HC/g of sample) while released CO and CO<sub>2</sub> were  
138 continuously and simultaneously monitored by infrared detectors during both pyrolysis  
139 (S3CO and S3CO<sub>2</sub>) and combustion (S4CO and S4CO<sub>2</sub>). Quantification of the amount of  
140 effluents led to the determination of the TOC (wt%), of the Hydrogen Index (HI, defined as  
141  $S2 \times 100 / \text{TOC}$ , in mg HC/g of TOC) and of the Oxygen Index (OI; defined as  $S3 \times 100 / \text{TOC}$ , in

142 mg CO<sub>2</sub>/g of TOC). The pyrolysis temperature associated with the maximum release of  
143 hydrocarbons, called “TpK<sub>S2</sub>”, was also determined.

144

#### 145 *2.4. Raman spectroscopy*

146 Raman spectroscopy analysis was performed using a Renishaw inVia micro-  
147 spectrometer equipped with a 532 nm argon laser. The spectrometer was first calibrated using  
148 a silicon standard before each session. For each sample analysis, the laser was focused using a  
149 Leica microscope with a ×50 objective and the spectra were recorded in the 1000–1900 cm<sup>-1</sup>  
150 first order spectral window including the defect (D) and graphite (G) peaks (Fig. 1). The laser  
151 power at the sample surface was kept below 1 mW to prevent thermal alteration of isolated  
152 OM (Everall et al., 1991). Spectra acquisition was achieved after two iterations using a time  
153 exposure of 40 s. After linear correction of the baseline between 1000 and 1900 cm<sup>-1</sup> and  
154 spectra normalization, we identified Raman peaks according to the nomenclature defined in  
155 Romero-Sarmiento et al. (2014).

156 The heights of the sub-bands D1 (~1365 cm<sup>-1</sup>), D4 (~1285 cm<sup>-1</sup>), D5 (~1445 cm<sup>-1</sup>) and  
157 G+D2 (~1600 cm<sup>-1</sup>; Fig. 1) were then determined to compute the I<sub>D1</sub>/I<sub>G+D2</sub>, I<sub>D4</sub>/I<sub>G+D2</sub> and  
158 I<sub>D5</sub>/I<sub>G+D2</sub>, ratios. Slopes α<sub>D4</sub> (determined between 1265 and 1300 cm<sup>-1</sup> Raman shift) and α<sub>D5</sub>  
159 (determined between 1415 and 1445 cm<sup>-1</sup> Raman shift) were determined to geometrically  
160 evaluate the expression of D4 and D5 shoulders on the D band.

161

### 162 **3. Results and discussion**

163 The TOC of OHMM and OLMM samples was measured to determine the efficiency  
164 of rock mineralization through HMM and LMM, respectively. The TOC in all OHMM and  
165 OLMM samples was low (25.4% and 27.5% respectively; Table 1) suggesting that the  
166 preservation and/or neoformation of substantial mineral content had occurred during both



167 procedures. The OI was approximately 15 mg and 59 mg CO<sub>2</sub>/g TOC in OHMM and OLMM,  
168 respectively (Table 1). The higher OI in OLMM is partially explained by the higher level of  
169 CO<sub>2</sub> emissions during the first isothermal step, showing that they are mostly related to  
170 desorbable OM (Fig. 2). Such a result is in line with previous investigations on coals  
171 suggesting that acid maceration can lead to a rise in oxygen content resulting possibly from  
172 the neoformation of carboxylic groups replacing carboxylate through ion exchange (Larsen et  
173 al., 1989). Higher levels of CO<sub>2</sub> emissions were also recorded for OLMM during the rise in  
174 pyrolysis temperature from 300 to 650 °C (Fig. 2). In this pyrolysis temperature range, CO<sub>2</sub>  
175 emissions appeared to be independent of hydrocarbon emissions suggesting that they do not  
176 correspond to the thermal cracking of the macromolecular network.

177 OHMM and OLMM isolated from the Rhynie chert were characterised by a TpkS2 of  
178 481 °C and 504 °C, respectively (Table 1). In every type of kerogen, these pyrolysis  
179 temperatures are commonly assigned to OM that has been subjected to high thermal  
180 alteration. Consistent with high TpkS2 values, pyrolysis of OHMM and of OLMM released  
181 low amounts of HC as evidenced by HI of 46 HC/g TOC and 68 mg HC/g TOC, respectively.  
182 Together, high TpkS2 and low HI imply that OM from the Rhynie chert falls at the transition  
183 between the oil and gas windows registered by TpkS2 ranging between 470 °C and 505 °C in  
184 analogous Type III kerogens (Espitalié et al., 1986). Hydrocarbon release during Rock-Eval  
185 pyrolysis was higher in OLMM than in OHMM (Table 1). The S1 value for OLMM was  
186 about 320% higher than for OHMM (Table 1), which suggests that thermolabile OM was  
187 more abundant in OLMM than in OHMM. This is consistent with the fact that OLMM was  
188 not subjected to organic solvent extractions, which have been shown to dramatically reduce  
189 S1 values (Delvaux et al., 1990). Between 300 and 350 °C, no release of hydrocarbons was  
190 observed suggesting a virtual absence of residual heavy oil and/or of pyrobitumen in both  
191 OHMM and OLMM (Fig. 2; Clementz, 1979; Sanei et al., 2015). The S2 curves for OHMM

192 and OLMM differ at high pyrolysis temperature ( $> 500\text{ }^{\circ}\text{C}$ ), at which point a broad shoulder  
193 is only observed in OLMM (Fig. 2). The occurrence of this thermorecalcitrant OM in OLMM  
194 mostly explains its higher HI, in the absence of solvent extractions and/or hydrochloric acid  
195 hydrolysis (Table 1). In turn, this suggests that standard acid maceration can lead to a  
196 modification of the macromolecular chemical structure in this ancient silicified OM by  
197 promoting a loss of hydrocarbonaceous moieties.

198 Determined on 15 random targets in both OHMM and OLMM, Raman spectra  
199 exhibited two broad D and G+D2 bands at around  $1365\text{ cm}^{-1}$  and  $1600\text{ cm}^{-1}$  in each  
200 preparation (Fig. 1). The D band presents a complex structure resulting from the presence of  
201 several sub-bands including here, the D4, D1, and D5 bands. In addition to the contribution of  
202 amorphous carbon, the D band is dominated by the D1 sub-band corresponding to the  
203 breathing mode of the  $\text{sp}^2$  aromatic ring within polyaromatic clusters and is attributed to  
204 defects in these aromatic structures (Ferrari and Robertson, 2000). The D2 band corresponds  
205 to defects in aromatic structure but in contrast to the D1 band, does not include amorphous  
206 carbon. A distinct D2 peak (usually centered at about  $1620\text{ cm}^{-1}$ ) is mainly observed in highly  
207 mature OM (Kouketsu et al. 2014) and is not observed in OM studied here. Therefore, the  
208 G+D2 band is mostly related to the G band corresponding to in-plane C-C bond stretching in  
209 polyaromatic layers from thermally altered materials (Marshall and Marshall, 2014). As the  
210 height of the D1 band increases with temperature, the  $I_{\text{D1}}/I_{\text{G+D2}}$  ratio is often used to probe the  
211 structural order of OM in the course of carbonization (Table 2; Wopenka and Pasteris, 1993;  
212 Quirico et al., 2005; Kouketsu et al., 2014; Delarue et al., 2016). In this study, OHMM and  
213 OLMM are characterized by  $I_{\text{D1}}/I_{\text{G+D2}}$  ratios of  $0.58 \pm 0.02$  and  $0.52 \pm 0.02$ , respectively  
214 (Table 2). This result would imply a higher carbon structural order in OHMM compared to  
215 OLMM, which is in agreement with previous findings indicating that standard acid  
216 maceration can yield a rise in carbon structural order (Zhang et al., 2016).

217           The D band also exhibits two shoulders corresponding to the D4 (1285 cm<sup>-1</sup>) and D5  
218 (1445 cm<sup>-1</sup>) bands (Fig. 1). The D4 peak is generally assigned to C–H bonds in aliphatics as  
219 C-H in aromatics does not seem to directly contribute to the D4 region (Ferralis et al., 2016).  
220 The D5 band indicates the presence of hydrocarbons trapped within the organic porosity and  
221 has been detected in a few oil and gas shale samples (Romero-Sarmiento et al., 2014;  
222 Rouzaud et al., 2015). In the current study,  $\alpha_{D4}$  and  $\alpha_{D5}$  slopes in Raman spectra were lower in  
223 OLMM than in OHMM, consistent with the more pronounced D4 and D5 shoulders in the  
224 Raman line of OLMM (Fig. 1; Table 2). However, the more pronounced D4 and D5 shoulders  
225 in OLMM were not associated with higher  $I_{D4}/I_{G+D2}$  and  $I_{D5}/I_{G+D2}$  ratios (Table 2). This could  
226 be explained by the higher carbon structural order in OHMM implying a bias in the  
227 determination of  $I_{D4}/I_{G+D2}$  and  $I_{D5}/I_{G+D2}$  ratios. The occurrence of more pronounced D4 and D5  
228 shoulders in OLMM indicate that OLMM is indeed enriched in hydrocarbonaceous moieties,  
229 due to the C-H bonds in aliphatics and other entrapped hydrocarbons, relative to OHMM. The  
230 presence of entrapped hydrocarbons in the macromolecular network of OLMM likely explains  
231 the broad shoulder cracking at high temperature (> 500 °C) as revealed by Rock-Eval  
232 pyrolysis (Fig. 2). Both Raman spectroscopy and Rock-Eval pyrolysis suggest that standard  
233 acid maceration results in a loss of hydrocarbons in OM isolated from the Rhynie chert. As  
234 LMM does not involve the organic solvent extraction used in HMM, part of this hydrocarbon  
235 loss is likely due to solvent-extractible OM, which is reflected by the respective S1 values. In  
236 contrast to OHMM, OLMM did not involve extraction with HCl, which when mixed with HF  
237 has been demonstrated to favour hydrolysis leading to a loss of aliphatic CH and/or CH<sub>2</sub>  
238 functional groups in coal (Tekely et al., 1987). Even though our experimental design did not  
239 allow us to test the singular effect of HCl hydrolysis, such an effect can be hypothesized to  
240 partly explain the loss in aliphatic moieties with HMM treatment.

241           Although the Raman spectroscopy results support the notion that hydrocarbons are lost  
242 during HMM, it does not directly allow the extent of this loss to be determined. Following the  
243 relationship between H/C and HI (Espitalié et al., 1977), we determined that H/C atomic  
244 ratios were 0.53 and 0.57 in OHMM and OLMM, respectively (Table 1). Standard acid  
245 maceration therefore led to a reduction of at least about 7–8% of the hydrocarbon content of  
246 Rhynie OM. We use “at least” because chemical degradation of hydrocarbons occurring  
247 during the isolation of OLMM cannot be straightforwardly excluded. To evaluate this point,  
248 Raman spectra were also acquired on in situ OM from thin sections of Rhynie chert  
249 (Supplementary information). These in situ Raman spectra were acquired with another Raman  
250 spectroscope and on polished thin sections, which can modify the apparent carbon structural  
251 order by yielding an increase in the D band (Ammar et al., 2011; Maslova et al., 2012). These  
252 issues therefore prevent a straightforward comparison between Raman-derived ratios from in  
253 situ OM and from OLMM/OHMM. Nonetheless, the  $\alpha_{D4}$  and  $\alpha_{D5}$  parameters are independent  
254 of the height of the D band and can be used to track a potential effect of LMM procedure. In  
255 situ OM presents a mean  $\alpha_{D4}$  and  $\alpha_{D5}$  of about  $31 \pm 4$  and  $-24 \pm 23$ , respectively ( $n = 10$ ),  
256 indicating more pronounced D4 and D5 shoulders in the in situ OM compared to OLMM  
257 (Supplementary information). This suggests that simple treatment with HF and water also led  
258 to a slight loss in aliphatic content. In addition to these hydrocarbon losses related to acid  
259 maceration procedures, the estimated 7–8% loss in hydrocarbons can also be considered as  
260 minimum estimation because of thermolabile hydrocarbons (S1 parameter), which are not  
261 taken into account in the calculation of HI and were more abundant in OLMM than in  
262 OHMM.

263           Thermolabile hydrocarbons are tightly linked to soluble OM (Delvaux et al., 1990).  
264 However, this soluble OM in ancient sediments is often ignored because its syngeneity can be  
265 difficult to prove due to the potential for post-deposit contamination (Brocks et al., 2003a,

266 2003b; Derenne et al., 2008). However, silicified sediments can be seen as unconventional  
267 dual source/reservoir rocks. Indeed, rapid silicification drastically reduces porosity leading to  
268 a closed chemical system (Boyce et al., 2002; Ledevin et al., 2014). In such source/reservoir  
269 rocks, migration of bitumens was very restricted implying that bitumens and/or pyrobitumens  
270 coexisted in close proximity with residual kerogens (Vandenbroucke and Largeau, 2007).  
271 Hence, in ancient silicified sediments, bitumens and pyrobitumens can be considered  
272 syngenetic.

273 In contrast to soluble OM, insoluble OM is often considered less prone to late  
274 contamination (Derenne et al., 2008). Involving no chemical treatment or low-manipulation  
275 procedures, numerous studies have investigated the molecular structure of OM – at the scale  
276 of plant fossils – to assess the effects of fossilization or to depict plant evolution and affinities  
277 (Ewbank et al., 1996; Edwards et al., 1997; Abbott et al., 1998; Czaja et al., 2009; Quijada et  
278 al., 2016). Because a large proportion of the hydrocarbon content of these samples is  
279 preserved, these investigations minimized secondary hydrothermal and endolithic  
280 contaminations – which can also yield insoluble OM – by studying insoluble OM directly  
281 from plant fossils. To avoid contamination and to optimize the study of syngenetic  
282 hydrocarbons that can be degraded in the course of acid maceration, the development of in  
283 situ molecular micrometre-scale analyses techniques (e.g., time-of-flight secondary ion mass  
284 spectrometry and laser micropyrolysis) are of interest. Such techniques have the potential to  
285 directly probe the molecular structure of organic fossils independent of the soluble/insoluble  
286 operational definition. In addition to insightfully assessing chemical heterogeneity within  
287 macrofossils (Boyce et al., 2002; Abbott et al., 2018), in situ molecular micrometre-scale  
288 analyses also offers the possibility to study the molecular composition of micrometric to sub-  
289 micrometric diffuse particulate OM (Stout, 1993; Greenwood et al., 2001; Yoshioka and

290 Takeda, 2004; Silva et al., 2016). This includes remnants of microorganisms, such as organic-  
291 walled microfossils, which are essential organic components of the early geological record.  
292 In the specific context of silicified rocks – where silicification prevented migration of  
293 bitumens and favoured formation of pyrobitumen in the close vicinity of the residual kerogen  
294 – the use of in situ molecular micrometre-scale analyses offers promise in the investigation of  
295 the molecular composition of early life found within Archean silicified sediments. Indeed, the  
296 emergence of micrometre-scale *in situ* analyses have provided compelling evidence  
297 supporting an unexpected and wide chemical heterogeneity among Archean putative organic-  
298 walled microfossils (Delarue et al., 2017, 2020; Hickman-Lewis et al., 2020). Overlooked by  
299 bulk extraction approaches, this chemical heterogeneity defines a chemically-well preserved  
300 end product among the earliest traces of life. Therefore, the precise study of such geological  
301 archives requires further development of in situ molecular micrometre-scale analyses Such  
302 techniques may pave the way to document biological and abiotic processes and affinities of  
303 the earliest putative remnants of life by: (i) focussing on chemically well preserved  
304 specimens, (ii) without the use of chemical extraction and maceration techniques, which can  
305 degrade aliphatic moieties preserved in sediments subjected to early and prompt silicification.

306

#### 307 **4. Conclusions**

308 In this study, we investigated the impact of standard acid maceration on the chemical  
309 structure of silicified OM from the Lower Devonian Rhynie chert. Using Rock-Eval pyrolysis  
310 and Raman spectroscopy, we assessed the chemical structure of OM isolated by two different  
311 physical and chemical manipulation procedures. Results from Rock-Eval pyrolysis and  
312 Raman spectroscopy converged to demonstrate that the standard procedure of OM isolation  
313 led to a significant and substantial degradation of hydrocarbons. These chemical alterations  
314 indicate that a significant amount of molecular content is lost as a result of standard acid

315 maceration procedures, thus preventing a thorough examination of the molecular content of  
316 organic remnants in thermally altered silicified sediments.

317

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325

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496

#### 497 **Figure captions**

498

499 **Fig. 1.** (a) Typical Raman spectra measured on OHMM (black line) and OLMM (grey line).

500

501 **Fig. 2.** Emissions of (a) hydrocarbons and (b) CO<sub>2</sub> during Rock-Eval pyrolysis of OHMM  
502 (black line) and OLMM (grey line). The red curve indicates the pyrolysis temperature  
503 program. The green area in (b) indicates the temperature interval, for which CO<sub>2</sub> emissions  
504 are included in the calculation of OI.

505

Table 1: Rock-Eval parameters determined in OHMM and OLMM. \*H/C atomic ratio was estimated using the relationship between H/C atomic ratio and HI ( $H/C = 0.0017 \times HI + 0.453$ ) published by Espitalié et al. (1977).

	<b>S1</b> (mg HC/g)	<b>S2</b> (mg HC/g)	<b>TpkS2</b> (°C)	<b>TOC</b> (%)	<b>HI</b> (mg HC/g TOC)	<b>OI</b> (mg CO <sub>2</sub> /g TOC)	<b>H/C</b> <b>atomic ratio*</b>
OHMM	0.59	11.76	481	25.4	46	59	0.53
OLMM	2.48	18.83	504	27.5	68	15	0.57

**Table 2:** Raman-derived parameters (mean value  $\pm$  S.D.) determined in OHMM and OLMM (n= 15; Wilcoxon rank test).

	$I_{D1}/I_{G+D2}$	$I_{D4}/I_{G+D2}$	$I_{D5}/I_{G+D2}$	$\alpha_{D4}$	$\alpha_{D5}$
OHMM	$0.58 \pm 0.02$	$0.38 \pm 0.02$	$0.43 \pm 0.04$	$359 \pm 35$	$-288 \pm 39$
OLMM	$0.52 \pm 0.02$	$0.36 \pm 0.02$	$0.31 \pm 0.01$	$202 \pm 37$	$-240 \pm 28$
<i>p</i> -value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001





