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1 **Influence of the nature of the gas phase on the degradation of RNA**  
2 **during fossilization processes**

3  
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13 **Keywords:** Biosignatures, Fossilization, RNA world, Clay Minerals, Gas Phase

14  
15 **Highlights:**

- 16  
17 • The composition of the gas phase impacts fossilization processes.  
18 • Organic compounds are mainly fixed within the interlayer space of the Mg-smectites  
19 • Heterogeneous organo-mineral assemblage could potentially be a biosignature  
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38 **Abstract**

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40 The search for the most ancient traces of life on Earth has always been fraught with controversies  
41 because of the inevitable degradation undergone by fossilized biomolecules. Laboratory experiments  
42 may provide unique clues to achieve a better mechanistic understanding of the key processes  
43 involving (biogenic or abiotic) organic carbon during a geological history. The Earth atmosphere has  
44 changed over geological times, from a CO<sub>2</sub>-rich atmosphere during the Hadean and Archean to the  
45 O<sub>2</sub>-rich atmosphere of the present day, with a direct impact on the nature of the gas phase trapped  
46 within the sediment porosity. Yet, the influence of the nature of this gas phase on fossilization  
47 processes has almost never been investigated. Here, we conducted a series of fossilization  
48 experiments using an emblematic biomolecule (e.g. RNA) and clay minerals at 200°C for 7 days in  
49 closed systems in equilibrium with two different gas phases (e.g. CO<sub>2</sub> versus N<sub>2</sub>/O<sub>2</sub>). The multiscale  
50 characterization of experimental residues using a suite of advanced microscopy and spectroscopy  
51 techniques showed that the final organo-mineral assemblages strongly depend on the nature of the  
52 gas phase. In addition to the nature of the mineral phases, results showed that the nature of the gas  
53 phase impacts the chemistry of the residual N-rich organic compounds trapped within the interlayer  
54 spaces of Mg-smectites (e.g. mainly aliphatic-rich under CO<sub>2</sub> vs dominated by heterocycles under  
55 N<sub>2</sub>/O<sub>2</sub>). Altogether, the present study demonstrates the necessity to take into account the nature of  
56 the gas phase composition when experimentally simulating fossilization processes aiming at better  
57 constraining which biosignatures may be preserved in ancient rocks. Finally, the experimental results  
58 reported here may serve to identify the potential biosignatures that should be searched for on other  
59 planetary bodies.

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## 65 1. Introduction

66

67         There is still no consensus on how life originated on Earth nor on what the primordial living  
68 cells were (Mann, 2012; Szostak, 2017). Only the fossil record may offer the necessary clues to form  
69 a proper appreciation of both the diversity and disparity of ancient life, as well as key information on  
70 its origins (Summons & Hallmann, 2014; Knoll *et al.*, 2016). For instance, inspection of the modern  
71 biosphere conspicuously fails to anticipate the existence of morphologically curious fossil groups  
72 such as Ediacarian biota, trilobites or sauropods (Serenio, 1999; Hughes, 2007; Erwin *et al.*, 2011).  
73 The study of the ancient fossil record may thus help better constrain the emergence of primordial life  
74 in terms of patterns, rates and processes, as well as the evolution of early terrestrial  
75 paleoenvironments.

76         But this is not that facile. Although the ancient fossil record may contain fundamentally  
77 important biogeochemical signals, its poor quality makes it not that easy to decode (McMahon *et al.*,  
78 2018). The search for the earliest traces of life has to take place in the most ancient, and thus disturbed,  
79 geological record. Difficulties do not only pertain to the inevitable degradation of fossilized  
80 biomolecules occurring during the geological history of their host rocks (Bernard & Papineau, 2014;  
81 Briggs & Summons, 2014), but also to the possibility that abiotic chemical pathways yield organic  
82 microstructures exhibiting morphological and geochemical signatures very similar to fossilized  
83 biogenic ones (Grotzinger & Rothman, 1996; Garcia-Ruiz *et al.*, 2003; Horita, 2005; McCollom &  
84 Seewald, 2006; Cosmidis & Templeton, 2016; Rouillard *et al.*, 2018).

85         Although extrapolating laboratory results to natural settings remains difficult, notably  
86 because geological timescales cannot be replicated in the laboratory, fossilization experiments  
87 conducted under carefully controlled and reproducible conditions can provide unique clues on the  
88 degradation/generation processes of (biogenic or abiotic) organic carbon (Bernard & Papineau, 2014;  
89 Javaux, 2019). Of note, such laboratory experiments do not aim at reproducing the full complexity of  
90 natural settings (this will never be achieved), but rather aim at creating simple systems to evidence  
91 anticipated processes, verify/test thermodynamics predictions or qualitatively and/or quantitatively



92 investigate the influence of specific parameters.

93           Following early studies (e.g. Oehler & Schopf, 1971; Oehler, 1976; Briggs & Kear, 1993;  
94 Briggs, 1995), many experimental studies have recently been conducted to document the chemical  
95 changes of (biogenic or abiotic) organic compounds during a geological history (Ruhl *et al.*, 2011;  
96 Schiffbauer *et al.*, 2012; Watson *et al.*, 2012; Li *et al.*, 2013, 2014; McNamara *et al.*, 2013a, 2013b;  
97 Fraser *et al.*, 2014; Bernard *et al.*, 2015; Colleary *et al.*, 2015; Picard *et al.*, 2015, 2016; Alleon *et al.*,  
98 2016a, 2017; Miot *et al.*, 2017; Igisu *et al.*, 2018; Saitta *et al.*, 2018; Vinogradoff *et al.*, 2018, 2020).  
99 Altogether, these studies highlighted that the degradation of organic molecules can be more abstruse  
100 than is generally believed. Besides temperature conditions, these studies documented the influence  
101 of the nature of the organic compound, the nature of the mineral matrix and the nature of the fluid on  
102 the organo-mineral evolution.

103           Yet, most of the experiments reported in the literature were conducted using either modern  
104 microorganisms or modern biomolecules resistant to fossilization, even though primordial living cells  
105 were not as evolved as modern ones and likely did not produce complex resistant macromolecules.  
106 In fact, according to the RNA World concept, the primary ‘living’ substance on the early Earth was  
107 RNA (ribonucleic acid) or any compound chemically similar (Hud *et al.*, 2013; Higgs & Lehmann,  
108 2015). Yet, it has long been known that nucleic acids cannot withstand diagenetic conditions (Lindahl,  
109 1993; Levy & Miller, 1998; Mathay *et al.*, 2012), hence drastically limiting the chances of finding  
110 preserved fragments within ancient rocks. Conducting fossilization experiments using RNA would  
111 offer unique clues on the traces potentially left by the RNA world in the fossil record.

112           Plus, despite their high potential for biopreservation (e.g. Ehlmann *et al.*, 2008; Summons *et*  
113 *al.*, 2011; Cai *et al.*, 2012; Kennedy *et al.*, 2014; Wacey *et al.*, 2014; McMahon *et al.*, 2018) and  
114 despite their continuous formation at the surface of the Earth since the Hadean (Hazen *et al.*, 2008,  
115 2013), smectitic clay minerals have rarely been used in fossilization experiments (Wilson &  
116 Butterfield, 2014; McMahon *et al.*, 2016; Naimark *et al.*, 2016; Newman *et al.*, 2019; Viennet *et al.*,  
117 2019a). Because of their ability to adsorb, protect, concentrate, and transform biomolecules, clay  
118 minerals such as smectites are believed to have played a major role in the origin of life (Bernal, 1951;

119 Cairns-Smith, 1966; Hazen *et al.*, 2008, 2013; Meunier *et al.*, 2010; Brack, 2013). Yet the exact  
120 impact of clay minerals on biomolecules during fossilization processes remains to be carefully  
121 investigated experimentally.

122         Last, with the exception of experiments conducted by Schiffbauer *et al.* (2012) which aimed  
123 at investigating the effect of redox conditions, the influence of nature of the gas phase has never been  
124 investigated. While the exact composition and density of the atmosphere of the early Earth have long  
125 been debated (Walker, 1985; Sleep *et al.*, 2001; R. Lowe & Tice, 2004; Tian *et al.*, 2005), it is now  
126 widely accepted that it contained no free O<sub>2</sub> and was rather dominated by N<sub>2</sub> and CO<sub>2</sub> (Marty *et al.*,  
127 2013; Kasting, 2014), as suggested by studies of Hadean magmas (Trail *et al.*, 2011). The  
128 concentration of CO<sub>2</sub> likely declined from multibar levels during the early Hadean to a few tenths of  
129 a bar by the mid- to late Archean (Marty *et al.*, 2013; Kasting, 2014). Such evolution obviously  
130 impacted the nature of the gas phase trapped within the sediment porosity. Investigating the influence  
131 of the gas phase on the preservation/degradation of molecular biosignatures during advanced  
132 fossilization processes thus appears critically needed to properly decode the fossil record.

133         Here, we experimentally submitted RNA in the presence of Mg-smectites to hydrothermal  
134 conditions in pure bi-distilled water at 200°C for 7 days in equilibrium with a gas phase only  
135 composed of CO<sub>2</sub> (simulating a primitive atmosphere) or with a gas phase composed of N<sub>2</sub> and O<sub>2</sub>  
136 (simulating a modern atmosphere). We characterized the water-insoluble solid residues of  
137 experiments at a multiple length scale using X-ray diffraction and advanced spectromicroscopy tools.

138

## 139 **2. Materials and methods**

140

### 141 *2.1. Starting materials*

142

143 Powder of pure RNA from torula yeast (Sigma-Aldrich) was used for the present experiments. Mg-  
144 smectites were synthesized in the lab from a hydrogel of theoretical half-unit-cell formula of saponite:  
145 Na<sub>0.4</sub>(Mg<sub>3</sub>)(Al<sub>0.4</sub>,Si<sub>3.60</sub>)O<sub>10</sub>(F<sub>0.05</sub>,OH<sub>0.95</sub>)<sub>2</sub>. The hydrogel was obtained by mixing reagents under air

146 in the following order: pure water, hydrofluoric acid, sodium acetate, magnesium acetate tetrahydrate,  
147 aluminium acetate basic and aerosol. The hydrogel was mixed at room temperature for 3 hours and  
148 then introduced in a PTFE-lined stainless steel autoclave under air. The autoclave was heated at 220°C  
149 for 3 days. After cooling down at room temperature, the Mg-smectites were Ca-saturated by putting  
150 in contact 1 mol.L<sup>-1</sup> of aqueous CaCl<sub>2</sub>, renewed 5 times, for a total duration of ~60 H. Then, the Ca-  
151 saturated Mg-smectites were dried at 60°C after centrifugation within pure water to remove salts.  
152 Note that, the initial content of carbon and nitrogen (measured by CHNS elemental analysis see  
153 section 2.4.1 below) within the Ca-saturated Mg-smectites are below 0.4 %<sub>wt</sub> and 0.0 %<sub>wt</sub>,  
154 respectively.

155

## 156 2.2. Fossilization experiments

157

158 The fossilization experiments were conducted in closed system in titanium Parr<sup>®</sup> reactor of 100 mL  
159 at 200°C for 7 days with 1 bar (at 25°C) of a mixture of N<sub>2</sub> and O<sub>2</sub> or 1 bar of pure CO<sub>2</sub>. Fifteen mL  
160 of pure water were mixed either with 300 mg ( $\pm$  0.1%) of Mg-smectites and 150 mg of RNA ( $\pm$  <  
161 0.5%). Pure CO<sub>2</sub> (purity > 99.7%) was flushed into the reactors for 10 min at 20°C. Then the reactor  
162 was sealed and heated to 200°C. After 7 days, the sealed reactors were cooled down at ambient  
163 temperature. The liquid and solid phases of the residues were separated by centrifugation within pure  
164 water before drying at 50°C for ~12 hours. All experiments were triplicated to ensure reproducibility.

165

## 166 2.3. Ultracryomicrotomy

167

168 Cryo-ultramicrotome sections (70 nm thick) were prepared for X-ray absorption near edge structure-  
169 scanning transmission X-ray microscopy (XANES-STXM) and transmission electron microscopy  
170 (TEM) characterization using the Leica ultramicrotome available at UMET (Lille, France).  
171 Experimental residues were mixed with 0.1 mL of water-ethanol (50/50 % Vol) before being frozen  
172 in liquid nitrogen at -160°C. The sections of frozen residues were then deposited on holey carbon

173 film TEM grids.

174

#### 175 2.4. CHNS estimations

176

177 The total carbon and nitrogen contents of the solid fractions of the residues were determined using a  
178 Flash 2000 Thermo CHNSO elemental analyser operating at IStEP (France). About 2 to 3 mg of each  
179 residue were combusted under oxygen/helium flux at 960°C. Uncertainties are about 0.02 wt% for N  
180 and 0.07 wt% for C.

181

#### 182 2.5. X-ray diffraction (XRD)

183

184 Powder XRD patterns were obtained on a Panalytical X'pert Pro MPD 2 circles operating at IMPMC  
185 (Paris, France), with a step size of  $0.033^\circ 2\theta$  and a counting time per step of 250s over the  $3\text{--}65^\circ 2\theta$   
186  $\text{CuK}\alpha_{1,2}$  angular range. Additional measurements were done using oriented preparations at both  
187 atmospheric pressure or under vacuum ( $3.10^{-4}$  atmosphere) using an Anton Parr HTK 1200 oven and  
188 a temperature monitor TCU1000N coupled to a EDWARDS RV3 pump. The divergence slit, the anti-  
189 scatter slit and the two Soller slits were  $0.5^\circ$ ,  $1^\circ$ ,  $0.04^\circ$  and 0.04 radiant, respectively.

190

#### 191 2.6. Fourier-transform infrared (FTIR) spectroscopy

192

193 FTIR spectra were recorded over the  $400\text{--}4000\text{ cm}^{-1}$  range with a  $4\text{ cm}^{-1}$  resolution using a Nicolet  
194 6700 FTIR spectrometer fitted with a KBr beamsplitter and a DTGS-KBr detector operating at  
195 IMPMC (Paris, France). Spectra were obtained using an attenuated total reflectance (ATR) geometry  
196 using a Specac Quest ATR device fitted with a diamond internal reflection element.

197

#### 198 2.7. X-ray absorption near edge structure (XANES) spectroscopy

199

200 XANES data were collected using the scanning transmission X-ray microscope (STXM) of the  
201 HERMES beamline at SOLEIL (Belkhou et al., 2015). Beamline optical elements were exposed to a  
202 continuous flow of pure O<sub>2</sub> to remove carbon contamination. Energy calibration was done using the  
203 well-resolved 3p Rydberg peak of gaseous CO<sub>2</sub> at 294.96 eV. XANES data were extracted from image  
204 stacks collected at energy increments of 0.1 eV over the carbon (270–350 eV) absorption range with  
205 a dwell time of  $\leq 1$  ms per pixel to prevent irradiation damage (Wang et al., 2009). Alignment of  
206 images of stacks and extraction of XANES spectra were done using the latest version aXis2000  
207 software (Hitchcock, 2018). The C-XANES spectra shown in the present contribution correspond to  
208 homogeneous carbon-rich areas of several hundreds of square nanometers and were normalized to  
209 the carbon quantity by integrating the spectra (after subtraction of a power law background) from the  
210 pre-edge region up to the mean ionization energy (e.g. 282–291.5 eV at the C K edge) using the  
211 QUANTORXS freeware available online (Le Guillou *et al.*, 2018). This freeware also allowed  
212 estimating the N/C values of the organic compounds found within the residues, with a precision of  $\pm$   
213 0.02 (Alleon *et al.*, 2015).

214

## 215 2.8. Scanning transmission electron microscopy (STEM)

216

217 STEM investigations were performed using a Thermofisher Titan Themis 300 microscope operated  
218 at 300 keV, located at the “centre commun de microscopie – CCM” at the university of Lille.  
219 Hyperspectral EDS data were obtained using the super-X detector system equipped with four  
220 windowless silicon drift detectors. These detectors have a high sensitivity for light elements and allow  
221 a high counting rate of the carbon, nitrogen and oxygen X-rays. The probe current was set at 600 pA  
222 with a dwell time at 10  $\mu$ s per pixel. The post-processing of the hyperspectral data was performed  
223 using Hyperspy (De la Pena *et al.*, 2017). The signal was first denoised using PCA, then fitted by a  
224 series of Gaussian functions and a physical model for background/bremsstrahlung. The integrated  
225 intensities of the Gaussian functions were used to quantify the compositions thanks to the Cliff-  
226 Lorimer method, using experimentally determined k-factors. Absorption correction was taken into

227 account, which is mandatory to correct for the re-absorption within the sample of the carbon, nitrogen  
228 and oxygen X-rays. Finally, end-member phases (smectites, phosphates, amorphous silicon oxides,  
229 organic compounds) were identified and their spectra used as inputs for linear combination fitting  
230 (multiple linear least square fits). Pixels of similar composition were given the same colors scaled as  
231 a function of the proportion of each phase.

232

### 233 **3. Results**

234

#### 235 3.1. Bulk investigations

236

237 In the absence of Mg-smectites, the final N/C values of the residues of experiments conducted  
238 under N<sub>2</sub>/O<sub>2</sub> (0.13) were higher than for those of experiments conducted under CO<sub>2</sub> (0.11) (Table 1).  
239 This was similar for residues of experiments conducted in the presence of Mg-smectites (0.18 under  
240 N<sub>2</sub>/O<sub>2</sub> vs 0.14 under CO<sub>2</sub> – Table 1). In the absence of Mg-smectites, 7.8 wt.% of the initial C and  
241 1.7 wt.% of the initial N were preserved in residues of experiments conducted under CO<sub>2</sub>, while 35.4  
242 wt.% of the initial C and 9.4 wt.% of the initial N were preserved in residues of experiments conducted  
243 under N<sub>2</sub>/O<sub>2</sub> (Table 1). In contrast, in the presence of Mg-smectites, more C and N remained in the  
244 residues obtained under CO<sub>2</sub> (i.e. 36.5 wt.% of the initial C and 10.8 wt.% of the initial N under CO<sub>2</sub>  
245 versus 20.4 wt.% of the initial C and 7.6 wt.% of the initial N under N<sub>2</sub>/O<sub>2</sub>) (Table 1).

246 The XRD patterns of the residues reveal that NH<sub>4</sub><sup>+</sup>-rich phosphates formed during the  
247 experiments conducted in the absence of Mg-smectites under N<sub>2</sub>/O<sub>2</sub> (Fig. 1). In addition, low  
248 amounts of Ca-rich phosphates formed under N<sub>2</sub>/O<sub>2</sub>, while low amounts of Mg hydrated carbonates  
249 formed under CO<sub>2</sub>. Of note, the Mg and Ca cations came from the pristine RNA salts to compensate  
250 the negative charge from the phosphorus backbone. Minor amount of Anatase came from the reactor  
251 itself that was released during the residue sampling. During the experiments involving Mg-smectites  
252 and RNA, the structure of the Mg-smectites did not evolve that much as indicated by the XRD patterns  
253 of the residues (Fig. 1), even though the slight variations of the position and width of the 001 reflection

254 indicate some disturbance along the c-axis. These residues also contained Al- and Mg-phosphates,  
255 whatever the nature of the gas phase (Fig. 1).

256 None of the residues displayed IR absorption features of RNA (Fig. 2). All the residues of  
257 experiments conducted in the absence of Mg-smectites exhibited adsorption bands attributed to CH  
258 in alkenes (bands at 983, 930 and 908  $\text{cm}^{-1}$ ), to CH in alkanes (bands at 1457 and 1374  $\text{cm}^{-1}$ ), C=O  
259 and N-H in amides (bands at 1585, 1680 and 3200  $\text{cm}^{-1}$ ), to  $\text{CH}_2$  and  $\text{CH}_3$  moieties (bands at 2924,  
260 2860, 2871 and 2960  $\text{cm}^{-1}$ , plus the band at 1275  $\text{cm}^{-1}$  for the residues of experiments conducted  
261 under  $\text{N}_2/\text{O}_2$ ). Of note, residues of experiments conducted under  $\text{CO}_2$  exhibited well defined bands  
262 corresponding to C-O in alkoxy at 1050, 1100 and 1150  $\text{cm}^{-1}$ , while only a large feature centered at  
263 1200  $\text{cm}^{-1}$  attributed to C-O in acyl or phenol or alkoxy could be observed for residues of experiments  
264 conducted under  $\text{N}_2/\text{O}_2$ . Bands at 740 and 1440  $\text{cm}^{-1}$  could be attributed to P-C in aromatic  
265 compounds (NIST Office of Data, 2019) while the one at 1014  $\text{cm}^{-1}$  for residues of experiments  
266 conducted under  $\text{N}_2/\text{O}_2$  could be attributed to  $\text{PO}_4^{3-}$  in phosphates.

267 The Mg-smectites structures of the tetrahedral and octahedral layers did not evolve during the  
268 experiments as attested by XRD and FTIR (cf. the Si-O; -OH and  $\text{Mg}_3\text{-OH}$  absorption features at  
269 981, 790; 3675 and 668  $\text{cm}^{-1}$ ). The formation of phosphates, evidenced by XRD, was confirmed by  
270 FTIR (cf. bands at 560 and 600  $\text{cm}^{-1}$  attributed to  $\text{PO}_4^{3-}$  in phosphates). The adsorption band at 1381  
271  $\text{cm}^{-1}$  in spectra of residues of experiments conducted under  $\text{CO}_2$  were attributed to  $\text{CO}_3^{2-}$  in  
272 carbonates, while the adsorption bands at 1440 and 1560  $\text{cm}^{-1}$  corresponded to  $\text{R-NH}_3^+$  or  $\text{NH}_4^+$  and  
273 NH, respectively. In addition, the residues of experiments conducted in the presence of Mg-smectites  
274 displayed a band at  $\sim 1700 \text{ cm}^{-1}$  (more intense for experiments conducted under  $\text{N}_2/\text{O}_2$ ), attributed to  
275 carboxylic C=O, and bands at 2852, 2928, 2870 and 2960  $\text{cm}^{-1}$  (more intense for experiments  
276 conducted under  $\text{CO}_2$ ), corresponding to C-H bonds.

277

### 278 3.2. Spatially-resolved investigations

279

280 STEM investigations allowed mapping mineralogical heterogeneities within the residues of

281 experiments conducted in the presence of Mg-smectites (Fig. 3). Although Mg-smectites remained  
282 the main component of the final residues, Al- and Mg- phosphates were observed, confirming XRD  
283 results. In addition, although not detectable using XRD, C-free amorphous Mg,Ca phosphates, C-free  
284 amorphous SiO<sub>x</sub> particles as well as N-poor organic particles were observed within the residues. The  
285 Mg-smectites appeared closely associated with N-rich organic compounds whatever the atmosphere  
286 (Fig. 3.B., C. E. and F.), while Ca-carbonates were only observed within residues of experiment  
287 conducted under CO<sub>2</sub>.

288 STXM-based XANES investigations revealed that RNA was no more present within the  
289 residues (Fig. 4.). The XANES spectrum of RNA presents a series of peaks attributed to nucleobases  
290 (aromatic and olefinic carbons (285 eV), heterocycles (285.9 eV), ketone and phenol groups (286.7 -  
291 287.4 eV)) and ribose (saturated carbons (288 eV) and hydroxyl groups (289.3 eV)) (Le Guillou et  
292 al. (2018)) (Fig. 4). The organic residues of experiments conducted in the absence of Mg-smectites  
293 appeared very homogeneous and significantly more aromatic than RNA. While under CO<sub>2</sub>, RNA  
294 evolved into a polyaromatic residue (peak at 285.4 eV attributed to conjugated aromatic cycles –  
295 Bernard & Horsfield (2014)), the organic residues of the experiments conducted under N<sub>2</sub>/O<sub>2</sub>  
296 displayed olefinic or aromatic carbons (peak at 285 eV), C=O or C=N or C≡N moieties (peak at 286.7  
297 eV) as well as carboxylic groups (peak at 288.4 eV) (Alleon *et al.*, 2017; Vinogradoff *et al.*, 2018).

298 In contrast, the organic fraction of the residues of experiments conducted in the presence of  
299 Mg-smectites was heterogeneous (Fig. 4). Despite similar N/C (~ 0.1), the organic compounds  
300 associated to the Mg-smectites were not identical in residues of experiments conducted under N<sub>2</sub>/O<sub>2</sub>  
301 and CO<sub>2</sub>. Under N<sub>2</sub>/O<sub>2</sub> these compounds mainly contained aromatic or olefinic carbons (peak at 285  
302 eV), conjugated aromatic cycles (peak at 285.4), heterocycles at 285.8 eV, the compounds that formed  
303 under CO<sub>2</sub> mainly exhibited aliphatic carbons (peak at 288 eV) and amide groups (peak at 288.2 eV)  
304 with a relatively low amount of aromatic or olefinic carbons (peak at 285 eV). Nanoscale aromatic  
305 particles (N/C < 0.05) mainly composed of aromatic and olefinic carbons (peak at 285 eV),  
306 conjugated aromatic cycles (285.4 eV) and C=O and C=N groups were observed in the residues  
307 whatever the nature of the gas phase.



309 **4. Discussion**

310

## 311 4.1. Impact of the nature of the gas phase

312

313 The nature of the gas phase ( $N_2/O_2$  vs  $CO_2$ ) impacted the chemistry of the residues in the  
314 absence of Mg-smectites. Although, the N/C values of the solid residues were relatively similar  
315 whatever the nature of the gas phase, it was not the case of the final amounts of C and N (Table 1).  
316 In fact, the residues of experiments conducted under  $N_2/O_2$  were roughly 5 times richer in C and N  
317 than the residues of experiments conducted under  $CO_2$ . This was possibly related to the precipitation  
318 of  $NH_4^+$ -phosphates (that only occurred during the experiments conducted under  $N_2/O_2$ ) and their P-  
319 C bonds preserving the N and C content. The precipitation of such phosphates pinpointed alkaline  
320 conditions (Babić-Ivančić *et al.*, 2006; Birnhack *et al.*, 2015) having likely impacted the chemical  
321 structure of RNA (cf XANES and FTIR data – Figs. 2 and 4).

322 Although the aromaticity of the organic fraction increased during all experiments, as expected  
323 (Schiffbauer *et al.*, 2012; Li *et al.*, 2014; Bernard *et al.*, 2015; Alleon *et al.*, 2016a, 2017; Vinogradoff  
324 *et al.*, 2018; Viennet *et al.*, 2019a), its oxygen content varied with the nature of the gas phase. In fact,  
325 the organic fraction of the residues of experiments conducted under  $N_2/O_2$  displayed a higher  
326 concentration of oxygen-bearing functional groups, including phenol, alkoxy and amide groups (cf.  
327 XANES and FTIR data – Figs. 2 and 4), in good agreement with the experimental study of Schiffbauer  
328 *et al.* (2012).

329 The nature of the gas phase also impacted the nature of the final residues of the experiments  
330 conducted in the presence of Mg-smectites. Besides the precipitation of Ca-carbonates under  $CO_2$   
331 and of Ca-phosphates under  $N_2/O_2$ , similar mineral phases formed whatever the nature of the gas  
332 phase as a result of the dissolution of Mg-smectites and the degradation of RNA: amorphous silica  
333 particles and Al-, Mg- and Mg-Ca-phosphates. Yet, the organic fractions of the residues are different.  
334 In contrast to results obtained in the absence of Mg-smectites, the final concentrations of C and N

335 trapped within the solid residues were higher under CO<sub>2</sub>, even though the N/C values were slightly  
336 higher under N<sub>2</sub>/O<sub>2</sub>. Of note, an higher amount of residue is obtained under CO<sub>2</sub>, as a result of the  
337 lower dissolution rate of smectite under CO<sub>2</sub> (Viennet *et al.*, 2017, 2019b).

338         Although the free aromatic-rich particles produced during the experiments were similar  
339 whatever the nature of the gas phase, the organic compounds associated to the Mg-smectites were  
340 not. This is a quite important point given that these compounds constituted the main organic reservoir  
341 of the residues, as confirmed by their N/C values similar to those of the bulk residues. These organic  
342 compounds were N-rich within residues of experiments conducted under CO<sub>2</sub> while they were O-rich  
343 within residues of experiments conducted under N<sub>2</sub>/O<sub>2</sub>, in good agreement with the experimental  
344 results of Schiffbauer *et al.* (2012). In fact, other things being equal, Schiffbauer *et al.* (2012) showed  
345 that the presence of oxygen results in a much more aggressive and accelerated degradation of organic  
346 compounds. Here, the main driver of the final N/C values was likely the pH. Acidic conditions are  
347 known to enhance the thermal degradation of RNA (Matoba *et al.*, 1988), thereby impacting organo-  
348 mineral interactions.

349         The organic compounds associated with Mg-smectites might be either adsorbed onto or  
350 bonded to surfaces (lateral or basal) or trapped within their interlayer space (Lagaly *et al.* (2013), and  
351 references therein). Exposed to high vacuum ( $3 \times 10^{-4}$  atmosphere), the 001 reflections of the  
352 experimental residues and of reference Mg-smectites saturated with NH<sub>4</sub><sup>+</sup> exhibited a markedly  
353 different behaviour (Fig. 5), attesting that the interlayer space of the Mg-smectites of the residues  
354 were full of organic compounds (Ferrage *et al.*, 2005; Gautier *et al.*, 2017; Viennet *et al.*, 2019a). In  
355 fact, if no organic compounds were present, the layer to layer distance would have decreased under  
356 vacuum as a result of the release of H<sub>2</sub>O molecules.

357         XANES and FTIR data revealed that the organic compounds trapped within the Mg-smectites  
358 of the residues of experiments conducted under CO<sub>2</sub> were mainly composed of aliphatic carbons and  
359 amide groups, suggesting that cationic exchange occurred during the experiments, Ca<sup>2+</sup> having been  
360 replaced by the positively charged organic compounds that efficiently compensated the permanent  
361 charge of smectites. In contrast, the organic compounds trapped within the Mg-smectites of the

362 residues of experiments conducted under N<sub>2</sub>/O<sub>2</sub> did not display any amide groups, but rather  
363 heterocycles (conjugated or not), carboxylic groups and C=N or N-H bonds. Because they can be  
364 positively charged as well, heterocycles such as piperidine or pyrrolidine may have replaced Ca<sup>2+</sup>  
365 within the interlayer space via cationic exchange (Byrne, 1953; Oades & Townsend, 1963; Brindley  
366 & Thompson, 1966; Gournis *et al.*, 2004, 2006; Park *et al.*, 2008; Viennet *et al.*, 2019a).

367

#### 368 4.2 Implications for decoding the ancient fossil record.

369

370         The experimental results reported here have strong implications for our comprehension of  
371 the ancient fossil record. Because Archean paleontology relies on imperfect, degraded signals,  
372 interpretations have always been and are still, by essence, subject to controversy (Schopf, 1975;  
373 Alleon & Summons, 2019). Over the years, many authors have highlighted the equivocal nature of  
374 the main criteria used to discuss the biogenicity of putative remnants of life in rocks (Craig, 1954;  
375 Buick, 1990; Altermann & Kazmierczak, 2003; Pasteris & Wopenka, 2003; Tice & Lowe, 2004;  
376 Awramik & Grey, 2005; Sugitani *et al.*, 2007; Brasier *et al.*, 2015; Wacey *et al.*, 2016, 2018; Rouillard  
377 *et al.*, 2018; Alleon & Summons, 2019). With the exception of the recently described objects that  
378 have yet to be critically evaluated (Delarue *et al.*, 2017, 2020; Dodd *et al.*, 2017; Hassenkam *et al.*,  
379 2017; Tashiro *et al.*, 2017; Alleon *et al.*, 2018), none of the plethora of ‘microfossils’ reported from  
380 Archean rocks have been fully accepted as authentic biological remains (Schopf, 1975; Alleon &  
381 Summons, 2019).

382         The advanced chemical characterization of ancient potential microfossils using spatially  
383 resolved spectroscopy techniques can provide some clues regarding their molecular structure (Javaux  
384 & Marshal, 2006; Bernard *et al.*, 2007, 2010; Schiffbauer *et al.*, 2007; Igisu *et al.*, 2009; Steemans *et*  
385 *al.*, 2010; Galvez *et al.*, 2012; Dutta *et al.*, 2013; Alleon *et al.*, 2016b, 2018; Bobroff *et al.*, 2016;  
386 Delarue *et al.*, 2017, 2020; Loron *et al.*, 2019; Javaux, 2019), but this is not sufficient to conclude on  
387 their biogenicity. In fact, what remains critically missing is information on the original chemical  
388 nature of these organic constituents, i.e. information on what these compounds were at the time of

389 their generation, before the geological history they underwent. Obtaining such information requires  
390 the nature and the extent of the transformations that biogenic compounds have undergone since their  
391 fossilization to be quantified, i.e. it requires laboratory investigations (Bernard & Papineau, 2014;  
392 Briggs & McMahon, 2016; McMahon *et al.*, 2018; Alleon & Summons, 2019; Javaux, 2019).

393 Many experimental studies investigated the degradation of organic molecules during  
394 fossilization processes (cf above), highlighting that it can be quite abstruse, depending on the nature  
395 of the organic compound, the nature of the mineral matrix and the nature of the fluid. But, as  
396 demonstrated here, special care is needed when conducting laboratory experiments. In fact, most  
397 experiments reported in the literature have been conducted either under argon or nitrogen or under  
398 the oxygen-rich present day atmosphere. Yet, Schiffbauer *et al.* (2012) demonstrated that an O<sub>2</sub>-rich  
399 atmosphere leads to a more aggressive degradation than an inert atmosphere. Worse, the experimental  
400 results reported here evidence that the nature of the gas phase impacts the nature of the organic  
401 compounds produced during fossilization processes. Given that the Earth atmosphere has changed  
402 over geological times, from a CO<sub>2</sub>-rich atmosphere during the Hadean and Archean to the O<sub>2</sub>-rich  
403 atmosphere of the present day (Marty *et al.*, 2013; Kasting, 2014), it definitely appears fundamental  
404 to take into account the nature of the gas phase trapped within the sediment porosity (which depends  
405 on the composition of the atmosphere to some extent) when experimentally simulating fossilization  
406 processes aimed at better constraining which biosignatures may potentially be preserved in ancient  
407 rocks.

408

## 409 **5. Concluding remarks**

410 The present study highlights the effect of the nature of the gas phase (CO<sub>2</sub> or N<sub>2</sub>/O<sub>2</sub>) on the  
411 thermal degradation (at 200°C and 15.6 bars) of RNA in the presence or absence of Mg-smectites.  
412 The organic fractions of the residues of experiments conducted in the absence of Mg-smectites consist  
413 in aromatic organic compounds, richer in oxygen and nitrogen under N<sub>2</sub>/O<sub>2</sub> than under CO<sub>2</sub>. The  
414 nature of the gas phase does not impact significantly the nature of the mineral assemblage of residues  
415 of experiments conducted in the presence of Mg-smectites. Yet, the N-rich organic compounds fixed

416 within the interlayer space of the Mg-smectites are dominated by amide groups in residues of  
417 experiments conducted under CO<sub>2</sub> gas, while they are dominated by heterocycles such as piperidine  
418 or pyrrolidine un residues of experiments conducted under N<sub>2</sub>/O<sub>2</sub>. Of note, whatever the nature of  
419 the gas phase, the organic compounds of the experimental residues did not carry anymore information  
420 on the original chemical structure of RNA. Yet, as shown here, the hydrothermal degradation of such  
421 a (N,P)-rich organic molecule in the presence of Mg-smectites led to the precipitation of a quite  
422 uncommon mineral assemblage comprising submicrometric (Al,Mg,Ca)-phosphates, amorphous  
423 silica particles and organic-rich clay minerals (plus Mg-carbonates for experiments conducted under  
424 CO<sub>2</sub>). Such submicrometric, heterogeneous organo-mineral assemblage could be tentatively seen as  
425 a signature of the so-called “RNA world”. Although additional experiments are required to further  
426 constrain the impact of fossilization processes, the present experimental results open new avenues for  
427 the search for potential biosignatures on other planetary bodies, including rocky and/or icy ones (such  
428 as Mars, Ceres, Enceladus or Europa) on which organic compounds, hydrothermal systems and/or N-  
429 rich clay minerals have recently been detected (Carter *et al.*, 2013; Ammannito *et al.*, 2016; Carter,  
430 2017; Choblet *et al.*, 2017; Carozzo *et al.*, 2018; Eigenbrode *et al.*, 2018; Nordheim *et al.*, 2018;  
431 Marchi *et al.*, 2019). In other words, there is no doubt that many additional laboratory experiments  
432 should be conducted to support astrobiological exploration in eventually providing evidence of the  
433 existence of extraterrestrial life.

434

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- 771

772 **Table caption**

773

774 **Table 1:** Concentrations of carbon and nitrogen within the residues.

<b>Experiments</b>	<b>RNA</b>	<b>RNA- CO<sub>2</sub></b>	<b>RNA- N<sub>2</sub>/O<sub>2</sub></b>	<b>Smectite-RNA- CO<sub>2</sub></b>	<b>Smectite-RNA- N<sub>2</sub>/O<sub>2</sub></b>
<b>Initial mass of Mg-smectites (mg)</b>	-	-	-	300 (±0.1)	300 (±0.1)
<b>Initial mass of RNA (mg)</b>	-	150	150	150	150
<b>Final mass of residue (mg)</b>	-	7.0	26.6	270	193.2
<b>%wtC (±0.07)</b>	31.6	52.9	63.3	6.4	5.0
<b>Initial mass of C (mg)</b>		47.4	47.4	47.4	47.4
<b>Final mass of C (mg)</b>	-	3.7	16.8	17.3	9.7
<b>% of C preserved</b>		7.8	35.4	36.5	20.4
<b>%wtN (±0.02)</b>	14.9	5.7	8.1	0.9	0.9
<b>Initial mass of N (mg)</b>		22.3	22.3	22.3	22.3
<b>Final mass of N (mg)</b>	-	0.4	2.1	2.4	1.7
<b>% of N preserved</b>		1.7	9.4	10.8	7.6
<b>N/C (±0.02)</b>	0.47	0.11	0.13	0.14	0.18

775

776

777 **Figure captions**

778

779 **Figure 1:** Powder XRD patterns of the residues of experiments conducted in the presence or  
780 absence of Mg-smectites under N<sub>2</sub>/O<sub>2</sub> and under CO<sub>2</sub>.

781

782 **Figure 2:** Infrared spectra in the middle range of the residues of experiments conducted in the  
783 presence or absence of Mg-smectites under N<sub>2</sub>/O<sub>2</sub> and under CO<sub>2</sub>.

784

785 **Figure 3:** Spatially resolved characterization of the residues of experiments conducted in the  
786 presence of Mg-smectites under N<sub>2</sub>/O<sub>2</sub> and under CO<sub>2</sub>. a, STEM image of a residue of experiments  
787 conducted under CO<sub>2</sub> at 200°C in the presence of Mg-smectites. b-c, Maps of minerals and organic  
788 compounds (same area as a.). d, STEM image of a residue of experiments conducted under N<sub>2</sub>/O<sub>2</sub> at  
789 200°C in the presence of Mg-smectites. e-f, Maps of minerals and organic compounds (same area as  
790 D.).

791

792 **Figure 4:** XANES spectra of organic compounds encountered in the residues. The spectrum  
793 of RNA is shown for comparison.

794

795 **Figure 5:** XRD patterns on oriented preparation of NH<sub>4</sub><sup>+</sup> saturated Mg-smectites and of the  
796 residues of experiments conducted in the presence of Mg-smectites under N<sub>2</sub>/O<sub>2</sub> and under CO<sub>2</sub> at  
797 atmospheric pressure and under vacuum.











