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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 CO₂-rich atmosphere during the Hadean and Archean to the
45 O₂-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. CO₂ versus N₂/O₂). 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 CO₂ vs dominated by heterocycles under
55 N₂/O₂). 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 O₂ and was rather dominated by N₂ and CO₂ (Marty *et al.*,
127 2013; Kasting, 2014), as suggested by studies of Hadean magmas (Trail *et al.*, 2011). The
128 concentration of CO₂ 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 CO₂ (simulating a primitive atmosphere) or with a gas phase composed of N₂ and O₂
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_{0.4}(Mg₃)(Al_{0.4},Si_{3.60})O₁₀(F_{0.05},OH_{0.95})₂. 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⁻¹ of aqueous CaCl₂, 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 %_{wt} and 0.0 %_{wt},
154 respectively.

155

156 2.2. Fossilization experiments

157

158 The fossilization experiments were conducted in closed system in titanium Parr[®] reactor of 100 mL
159 at 200°C for 7 days with 1 bar (at 25°C) of a mixture of N₂ and O₂ or 1 bar of pure CO₂. 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 CO₂ (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° , 1° , 0.04° 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 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 O₂ to remove carbon contamination. Energy calibration was done using the
203 well-resolved 3p Rydberg peak of gaseous CO₂ 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 ≤ 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 μ 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 N₂/O₂ (0.13) were higher than for those of experiments conducted under CO₂ (0.11) (Table 1).
239 This was similar for residues of experiments conducted in the presence of Mg-smectites (0.18 under
240 N₂/O₂ vs 0.14 under CO₂ – 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 CO₂, 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 N₂/O₂ (Table 1). In contrast, in the presence of Mg-smectites, more C and N remained in the
244 residues obtained under CO₂ (i.e. 36.5 wt.% of the initial C and 10.8 wt.% of the initial N under CO₂
245 versus 20.4 wt.% of the initial C and 7.6 wt.% of the initial N under N₂/O₂) (Table 1).

246 The XRD patterns of the residues reveal that NH₄⁺-rich phosphates formed during the
247 experiments conducted in the absence of Mg-smectites under N₂/O₂ (Fig. 1). In addition, low
248 amounts of Ca-rich phosphates formed under N₂/O₂, while low amounts of Mg hydrated carbonates
249 formed under CO₂. 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 cm^{-1}), to CH in alkanes (bands at 1457 and 1374 cm^{-1}), C=O
259 and N-H in amides (bands at 1585, 1680 and 3200 cm^{-1}), to CH_2 and CH_3 moieties (bands at 2924,
260 2860, 2871 and 2960 cm^{-1} , plus the band at 1275 cm^{-1} for the residues of experiments conducted
261 under N_2/O_2). Of note, residues of experiments conducted under CO_2 exhibited well defined bands
262 corresponding to C-O in alkoxy at 1050, 1100 and 1150 cm^{-1} , while only a large feature centered at
263 1200 cm^{-1} attributed to C-O in acyl or phenol or alkoxy could be observed for residues of experiments
264 conducted under N_2/O_2 . Bands at 740 and 1440 cm^{-1} could be attributed to P-C in aromatic
265 compounds (NIST Office of Data, 2019) while the one at 1014 cm^{-1} for residues of experiments
266 conducted under N_2/O_2 could be attributed to 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 cm^{-1}). The formation of phosphates, evidenced by XRD, was confirmed by
270 FTIR (cf. bands at 560 and 600 cm^{-1} attributed to PO_4^{3-} in phosphates). The adsorption band at 1381
271 cm^{-1} in spectra of residues of experiments conducted under CO_2 were attributed to CO_3^{2-} in
272 carbonates, while the adsorption bands at 1440 and 1560 cm^{-1} corresponded to R-NH_3^+ or 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 N_2/O_2), attributed to
275 carboxylic C=O, and bands at 2852, 2928, 2870 and 2960 cm^{-1} (more intense for experiments
276 conducted under 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_x 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 CO₂.

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 CO₂, 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 N₂/O₂
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 N₂/O₂
301 and CO₂. Under N₂/O₂ 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 CO₂ 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 CO₂, even though the N/C values were slightly
336 higher under N₂/O₂. Of note, an higher amount of residue is obtained under CO₂, as a result of the
337 lower dissolution rate of smectite under CO₂ (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 CO₂ while they were O-rich
343 within residues of experiments conducted under N₂/O₂, 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 (3×10^{-4} atmosphere), the 001 reflections of the
352 experimental residues and of reference Mg-smectites saturated with NH₄⁺ 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 H₂O molecules.

357 XANES and FTIR data revealed that the organic compounds trapped within the Mg-smectites
358 of the residues of experiments conducted under CO₂ were mainly composed of aliphatic carbons and
359 amide groups, suggesting that cationic exchange occurred during the experiments, Ca²⁺ 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 N₂/O₂ 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 Ca²⁺
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 O₂-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 CO₂-rich atmosphere during the Hadean and Archean to the O₂-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 (CO₂ or N₂/O₂) 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 N₂/O₂ than under CO₂. 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 CO₂ gas, while they are dominated by heterocycles such as piperidine
418 or pyrrolidine un residues of experiments conducted under N₂/O₂. 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 CO₂). 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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448

449 **7. References**

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- 771

772 **Table caption**

773

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

Experiments	RNA	RNA- CO₂	RNA- N₂/O₂	Smectite-RNA- CO₂	Smectite-RNA- N₂/O₂
Initial mass of Mg-smectites (mg)	-	-	-	300 (± 0.1)	300 (± 0.1)
Initial mass of RNA (mg)	-	150	150	150	150
Final mass of residue (mg)	-	7.0	26.6	270	193.2
%wtC (± 0.07)	31.6	52.9	63.3	6.4	5.0
Initial mass of C (mg)		47.4	47.4	47.4	47.4
Final mass of C (mg)	-	3.7	16.8	17.3	9.7
% of C preserved		7.8	35.4	36.5	20.4
%wtN (± 0.02)	14.9	5.7	8.1	0.9	0.9
Initial mass of N (mg)		22.3	22.3	22.3	22.3
Final mass of N (mg)	-	0.4	2.1	2.4	1.7
% of N preserved		1.7	9.4	10.8	7.6
N/C (± 0.02)	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 N₂/O₂ and under CO₂.

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 N₂/O₂ and under CO₂.

784

785 **Figure 3:** Spatially resolved characterization of the residues of experiments conducted in the
786 presence of Mg-smectites under N₂/O₂ and under CO₂. a, STEM image of a residue of experiments
787 conducted under CO₂ 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 N₂/O₂ 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 NH₄⁺ saturated Mg-smectites and of the
796 residues of experiments conducted in the presence of Mg-smectites under N₂/O₂ and under CO₂ at
797 atmospheric pressure and under vacuum.









