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The Organic Signature of an Experimental Meat-cooking Fireplace: the Identification of Nitrogen Compounds and their Archaeological Potential

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Abstract: A better understanding of the operation and use of prehistoric fires is fundamental to interpreting the organization of living spaces. Following a previous study that focused on the organic signatures of fireplaces fueled with wood and/or bones, this study targeted the completion of an experimental reference database through the addition of a wood-fueled fireplace dedicated to the cooking of meat. Different sedimentary features of this experimental fireplace were visually identified (e.g. darkening, reddening), sampled, and subjected to geochemical analysis. Corg and N contents were quantified, samples were extracted with organic solvents and analyzed through GC-MS and bulk organic matter was characterized through py(TMAH)-GC-MS. Five different samples were studied and compared with a control sample, representative of the local background. A significant increase of Corg and N contents was measured for the three samples presenting darkened or charred characteristics. The meat-cooking fireplace seems to be characterized by the strong contribution of nitrogen, which was visible in elementary analyses as well as in the molecular composition of solvent extracts, and bulk organic matter. More specifically, compounds containing nitrile functions, amides, N-heterocyclic and N-aromatic compounds could be detected in solvent extracts. Amines, amides, N-heterocyclic and N-aromatic compounds could be identified in py(TMAH)-GC-MS. Some of these compounds present a relative stability in soils and could therefore aid in our comprehension and functional interpretations of archaeological fireplaces, and may, more particularly, make it possible to highlight the use of fireplaces for the cooking of meat.

Research Data Related to this Submission

There are no linked research data sets for this submission. The following reason is given:

Data we used are already published or provided as supplementary materials

Highlights

- %Corg and %N reflect significant inputs of OM in sediments during fireplace operation
- Lipids and bulk OM mark the use of wood as fuel and the involvement of animal OM
- Distinction between meat-cooking and bone-fueled fireplaces is possible
- N-compounds are characteristic of culinary use

1 **The Organic Signature of an Experimental Meat-**
2 **cooking Fireplace: the Identification of Nitrogen**
3 **Compounds and their Archaeological Potential**

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21 with wood and/or bones, this study targeted the completion of an
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23 fireplace dedicated to the cooking of meat. Different sedimentary features of
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25 reddening), sampled, and subjected to geochemical analysis. Corg and N
26 contents were quantified, samples were extracted with organic solvents and
27 analyzed through GC-MS and bulk organic matter was characterized
28 through py(TMAH)-GC-MS. Five different samples were studied and
29 compared with a control sample, representative of the local background. A
30 significant increase of Corg and N contents was measured for the three
31 samples presenting darkened or charred characteristics. The meat-cooking
32 fireplace seems to be characterized by the strong contribution of nitrogen,
33 which was visible in elementary analyses as well as in the molecular
34 composition of solvent extracts, and bulk organic matter. More specifically,
35 compounds containing nitrile functions, amides, N-heterocyclic and N-
36 aromatic compounds could be detected in solvent extracts. Amines, amides,
37 N-heterocyclic and N-aromatic compounds could be identified in py(TMAH)-
38 GC-MS. Some of these compounds present a relative stability in soils and
39 could therefore aid in our comprehension and functional interpretations of

40 archaeological fireplaces, and may, more particularly, make it possible to
41 highlight the use of fireplaces for the cooking of meat.

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49 during fireplace operation

50 - Lipids and bulk OM mark the use of wood as fuel and the
51 involvement of animal OM

52 - Distinction between meat-cooking and bone-fueled fireplaces is
53 possible

54 - N-compounds are characteristic of culinary use

55 1.Introduction

56 Many branches of archaeological investigation borrow frequently from the
57 natural sciences, and the study of fireplaces is a prime example as these
58 structures involve numerous and diversified types of material evidence.
59 Consequently, research on this matter implies the use of a wide range of
60 methods and multiple scales of analysis (*e.g.* Mentzer, 2014 and 2017). From
61 an archaeological perspective, a better understanding of fire management
62 and use during prehistoric times is, in and of itself, a fundamental research
63 question. Moreover, these structures plays a key role in our interpretation of
64 the organization of living spaces (Olive and Taborin, 1989; Sandgathe and
65 Berna, 2017).

66 However, the complexity of the archaeological evidence frequently inhibits
67 an investigation of the specific purpose(s) of prehistoric fireplaces (Coudret
68 et al., 1989; Goldberg et al., 2017). Numerous studies have investigated the
69 type of fuel exploited by prehistoric peoples using anthracology (*e.g.* Théry-
70 Parisot, 2002) or archaeozoology (*e.g.* Pérez et al., 2017), the temperatures
71 reached through an analysis of the alteration of mineral constituents
72 (sediments or stones; Canti and Linford, 2000; Dumarçay et al., 2008), or
73 the activities occurring around the fireplace via techno-economic studies of
74 the surrounding archaeological materials (*e.g.* Leesch et al., 2010). Despite
75 these efforts, the proposed functions of archaeological fireplaces are rarely
76 more specific than the logical, yet admittedly over simplified, combination of
77 light, warmth, and protection. To overcome this hurdle, numerous
78 experimental and archeological studies now involve in-depth analyses of

79 multiple fireplace components and focus on interpreting them from a
80 holistic perspective (*e.g.* Bodu et al., 2006; Mallol et al., 2013; Mentzer,
81 2017; Sandgathe and Berna, 2017).

82 One of the many explored research avenues has been the investigation of
83 anthropogenic organic matter (OM). Since the foundational work of
84 Rottländer (1983 and 1989) the use of chromatography coupled with
85 spectrometry (*e.g.* March et al., 1989; March, 1995) in order to characterize
86 the organic content of sediment or stones associated with fireplaces has
87 become relatively frequent (*e.g.* Buonasera, 2005; Lucquin, 2007; March et
88 al., 2008 and 2017; Kedrowski et al., 2009; Sistiaga Gutiérrez et al., 2010;
89 Azemard et al., 2013; Hérison et al., 2013; March, 2013; Buonasera et al.,
90 2015; Choy et al., 2016). These different analyses highlighted the
91 preservation of organic material over long periods of time. Analyses at the
92 molecular scale can be informative about the type of fuel used (Lejay et al.,
93 2016), as well as about the possible function of the fireplace, through, for
94 example, an identification of specific markers of food products (Evershed et
95 al., 1999). Experimental research is pivotal in this context, as it provides
96 comparative data material that is essential for the interpretation of
97 archaeological evidence (March et al., 2014, Buonasera et al., 2019 and
98 references therein).

99 In the same vein as the abovementioned trailblazing studies, we have
100 presented in a previous publication (Lejay et al., 2016) the results obtained
101 from the study of an experimental dataset that was oriented towards the
102 differentiation of organic signatures resulting from the use of wood and/or

103 bone as fuel during fireplace operation. The present paper expands and
104 diversifies this reference dataset with results obtained from an additional
105 experimental wood-fueled fire that was repeatedly used to cook meat.
106 According to interpretative archaeological models and ethnographic data on
107 hunter-gatherer fire use in domestic contexts, this type of use, oriented
108 towards either direct consumption or meat preservation, is indeed one of the
109 most commonly expected uses for prehistoric fires (*e.g.* Binford, 1978, 1983;
110 Audouze, 1987; Julien et al., 1987; Olive, 1997; Beyries and Vaté, 2007;
111 Mallol and Henry, 2017). This specific function is, however, still difficult to
112 ascertain from the prehistoric record, as analyses tend to be solely focused
113 on indirect archaeological evidence (*i.e.* artefacts). As an example, the
114 archaeological site upon which this entire experimental project leans on
115 (Régismont-le-Haut, France; ca. 38-32 ky cal BP) is characterized by a wide
116 range of activities and by technological and spatial organizations that
117 indicate a relatively long-term campsite involving the use of multiple fire-
118 related structures. Consequently, repeated cooking is therefore quite
119 logically suspected to have occurred in at least some of the structures, yet
120 previous studies have failed to directly prove this hypothesis (Anderson et
121 al., 2018; Lejay, 2018; Bon et al., in press).

122 The objectives of the present study were thus to establish the organic
123 signature left in the soil by a experimental meat-cooking fireplace and to
124 compare said signature with the previously documented organic signatures
125 of experimental fireplaces that used various types of fuel. These signatures
126 were determined through the measurement of the organic carbon (C_{org}) and

127 nitrogen (N) contents, the molecular characterization of the solvent extract
128 via Gas Chromatography coupled with Mass Spectrometry (GC-MS)
129 analysis, as well as through thermochemolysis coupled with GC-MS
130 (py[TMAH]-GC-MS) for treating bulk OM. This combination of analyses was
131 designed to cover both extractible OM (*e.g.* lipids) and non-extractible OM
132 (*e.g.* black carbon/combustion products).

133 2. Material and Methods

134 2.1. Context and Sampling

135 The experiments were conducted in September 2014 near the excavation of
136 the Aurignacian prehistoric site of Régismont-le-Haut (Southern France;
137 Anderson et al. 2018). This location was chosen in order to emulate the
138 sedimentary context of the archaeological layer, a colluvic and calcaric
139 Regosol (IUSS Working Group WRB, 2015). The local sedimentary
140 background was documented in a previously reported *Control* sample (see
141 Lejay et al., 2016) and corresponds to a yellow sandy loam (Table 1). This
142 control sample was collected in the same area before any experimentation
143 had occurred.

144 The specific experiment that constitutes the subject of this article was
145 designed to mimic a wood-fueled fire used for the repeated cooking of meat.
146 The fireplace was used 7 times over a couple of weeks, consuming 5 kg of
147 dry pine wood (*Pinus pinea*) and 0.7 kg of lean beef (*Bos taurus*) during each
148 use. The typical operation happened as follows:

- 149 - Lighting and operation with wood for ~50min in order to form a bed
150 of embers;
- 151 - Cooking of meat; in order to increase the organic signature of this
152 type of use meat was placed directly on the bed of embers and was
153 left to burn (Fig. 1a and b);
- 154 - No subsequent intervention, except for flipping the meat over;
- 155 - Mean total operation time: 125 min.

156 After 4 uses, the formation of an important deposit of combustion residues
157 (ash, charcoal, carbonized meat) hindered the proper operation of the
158 fireplace. These superficial residues were thus raked out of the structure
159 before the 3 subsequent uses. After 7 uses, the resulting fireplace was
160 excavated and the sediments were sampled following the visual distinction
161 of stratigraphic layers and planimetric areas (Fig. 1c to e; Table 1). A first
162 sample, *YELLOW*, was collected 5 cm under the fireplace, in seemingly
163 unaltered sediments (Fig. 1e). Three other samples were collected in
164 different locations according to visible modifications of the sediment due to
165 the operation of the fireplace, namely reddening or darkening. The *RED*
166 sample corresponds to reddened sediments sampled at a depth of 0.5-1.5 cm
167 as observed in section (Fig. 1e). The *DARK_A* and *DARK_B* samples were
168 collected on the surface of the fireplace (0-0.5 cm depth; Fig. 1c and d) and
169 correspond to darkened sediments. Above the location of the *DARK_A*
170 sample, a superficial dark and porous residue, presumed to be a fat-derived
171 char, was also collected (*CHAR*: fig. 1d; see Goldberg et al., 2009; Miller et

172 al., 2010; Braadbaart et al., 2012; Mentzer, 2014; Mallol et al., 2013 for
173 definitions and discussions about this class of combustion residues).

174 Table 1: Samples list and description.

Sample	Description
Control (same as in Lejay et al., 2016)	Yellow sandy loam with frequent calcitic features (<i>e.g.</i> hypocoatings, nodules)
YELLOW	Yellow sandy loam with frequent calcitic features (<i>e.g.</i> hypocoatings, nodules)
RED	Reddened sandy loam with frequent calcitic features
DARK_A	Darkened sandy loam; Sampled under a fat-derived char (<i>CHAR</i> sample; See Fig. 1c and d) in the periphery of the fireplace.
DARK_B	Darkened sandy loam grains; Sampled in the center of the fireplace
CHAR	Fat-derived char and adhering darkened sandy loam (See Fig. 1c and d); Sampled in the periphery of the fireplace (above DARK_A)

175

176 The samples were then prepared and analyzed following the same protocol
177 as in Lejay et al. (2016). The samples were air-dried over two days at 35 °C
178 (Froilabo-AC120 oven). The most fragile coarse elements (calcitic nodules
179 and macroscopic organic remains such as roots and charcoals) were removed
180 manually before the samples were sieved and the >2 mm fraction was
181 discarded. The samples were then ground with a mortar and pestle and
182 subsequently further ground using a Retsch-PM200 planetary ball mill (6
183 min, 450 rpm) in order to pass a 250 µm sieve.

184 2.2.Corg and N Contents

185 Due to the naturally high carbonate content of the sediment, acid
186 fumigation was carried out prior to elemental analysis, following Harris et

187 al. (2001). After the samples were weighed in Ag foil capsules, they were
188 humidified (15 µl deionized water) and placed in a desiccator under vacuum,
189 with a beaker of HCl (12 M), for 6 hours. Lastly, the samples were air-dried
190 for 12 hours at 40 °C.

191 Several reports indicated that this operation could induce biases, such as an
192 increase (Ryba and Burgess, 2002) or a decrease (Walther et al., 2010) of N
193 content. Consequently, the results presented here regarding N content
194 should be considered as indicators of relative differences between each
195 samples rather than for their absolute values.

196 Corg and N contents were measured on fumigated samples using an
197 elemental analyzer (Vario PYRO cube; Elementar). To allow for
198 quantification of low C and N content, two Ag capsules of the same sample
199 were added for a single analysis. Tyrosine standards were conjointly
200 analyzed to assess reproducibility through standard deviation calculation.

201 2.3.Solvent Extraction, Derivatization and GC-MS

202 The samples were extracted using an accelerated solvent extractor system
203 (Dionex-ASE100). Between 5 and 7 g of sediments were placed in a 10 ml
204 stainless steel cell topped with sterile and pre-extracted glass fiber. The
205 extraction program ran at 60 °C, with a static time of 20 min, using
206 dichloromethane and methanol (DCM/MeOH; 2:1, v/v). Each extract was
207 concentrated using a rotary evaporator and dried under a gentle N₂ flow,
208 dissolved in DCM in a 1.5 ml vial with a PTFE screw cap and stored at 4 °C
209 until its analysis (adapted from Quénéa et al., 2012). Prior to their analysis,

210 the samples were derivatized using a Gerstel multipurpose autosampler
211 (MPS) operating for the Agilent GC-MS device. The derivatization of the
212 extracts was achieved by adding 10% (v/v) BSTFA [N, O-
213 bis(trimethylsilyl)trifluoroacetamide] and heating at 60 °C for 10 min to
214 provide trimethylsilyl (TMS) derivatives.

215 Each extract was then analyzed with an Agilent 6890 gas chromatograph
216 coupled with an Agilent 5973N mass spectrometer using electron ionization
217 at 70 eV. The GC instrument was equipped with a 30 m Restek 5 Sil MS
218 column (i.d. 0.25 mm, film thickness 0.5 µm). The carrier gas was He at a
219 constant flow of 1 ml/min. The samples were injected in splitless mode with
220 the injector at 300 °C. The oven temperature was programmed from 80 °C
221 (0.5 min) to 100 °C at 10 °C/min, then to 320 °C at 4 °C/min.

222 2.4.Py(TMAH)-GC-MS

223 Bulk OM from the upper subsample was characterized through Curie-Point
224 pyrolysis (Pilodist) coupled with a GC instrument (Thermo Trace – 30 m
225 RXI 5 Sil MS column, i.d. 0.25 mm, film thickness 0.5 µm with Integra
226 guard column) and a MS instrument (DSQ). The samples mixed with TMAH
227 (25% in MeOH) were loaded into a ferromagnetic tube and pyrolyzed for 9.9
228 s at 650 °C under a 1 ml/min flow of He (adapted from Quénéa et al., 2005).
229 The pyrolysis products were separated in the GC system, whose oven
230 temperature was held at 50 °C for 10 min, before an increase of 2 °C/min to
231 310 °C. The ion source of the mass spectrometer was at 220 °C, which
232 scanned from m/z 35 to 800. The total ion current (TIC) trace was recorded
233 and products of pyrolysis assigned.

234 2.5. Analysis of Results

235 The chromatograms from GC-MS were handled with Agilent GC/MSD
236 ChemStation software (G1701DA D.00.00.38) and the pyrochromatograms
237 from Py(TMAH)-GC-MS with Thermo Scientific Xcalibur software (version
238 1.4 SR1). Compound assignment was based on comparisons with published
239 data (references below in the text) and the NIST mass spectral library
240 (version 2.2).

241 3. Results and Discussion

242 3.1. Experimental fireplaces

243 3.1.1. Field-based Remarks

244 The heating of meat led to the leaking of greasy fluids during fireplace
245 operation. Most of these fluids were in contact with or fell on embers and
246 were therefore immediately burned. However, the observation of several
247 patches of fat-derived char on top of the underlying sediment (Fig. 1d)
248 indicated that this burning process was locally incomplete and that part of
249 these fluids did in fact reach the underlying sediments. In section (Fig. 1e),
250 the fireplace displayed two overlapping types of alterations related to its
251 use. The sediments were darkened to a depth of 11 mm and then reddened
252 to a depth of 21 mm. Beneath the altered components sediments displayed
253 the same physical aspect as the local soil (Lejay et al., 2016; Anderson et al.,
254 2018) and seemed unaffected by the experimental fireplace.

255 These different phenomena matched those observed during the burning of
256 experimental fires using bone as fuel (*e.g.* Kedrowski et al., 2009; Hérissou
257 et al., 2013; Lejay et al., 2016), although the leaking of greasy fluids and the

258 darkening of the underlying sediments appears to have been less important
259 in the present meat-cooking fireplace. The darkening of the top first
260 centimeters of sediment therefore constitutes a macroscopic trace of the
261 implication of animal products in experimental fireplaces, regardless of
262 whether said product was bone or meat.

263 3.1.2.Representativeness of the Experimental Design

264 The experimental protocol induced at least two potential biases which
265 should be kept in mind: 1/ in the absence of archaeological evidence
266 regarding cooking devices, the “cooking method” was chosen in order to
267 maximize the formation of meat carbonization/pyrolysis by-products
268 (roasting on coals is nonetheless a common cooking method among hunter-
269 gatherer groups; Wandsnider, 1997); 2/ the maintenance operations, the
270 immediate sampling after use, and the sieving procedure largely prevented
271 the integration of macroscopic combustion residues within the sediment (*e.g.*
272 via bioturbation; Théry-Parisot et al., 2010; Mallol et al., 2017). While the
273 organic signature might have been enhanced by the first potential bias, the
274 second likely had the opposite effect.

275 3.2.Corg and N Contents

276 The measurement of Corg and N contents (Fig. 2) indicates that these
277 visible transformations were associated with a change in the elemental
278 composition of the sediment. The local Corg content, as indicated by *Control*
279 ($0.3 \pm 0.3\%$; see Lejay et al., 2016), is low, and similar Corg content was
280 measured in *YELLOW* ($0.2 \pm 0.2\%$). The N content is too low to be quantified
281 in these two samples (*i.e.* $< 0.025\%$).

282 In the reddened sediments (*RED*), Corg is similar to that of *Control* and
283 *YELLOW* ($0.2 \pm 0.2\%$), indicating no significant input of carbon. The N
284 content of this sample is measurable, although very low ($0.05 \pm 0.08\%$), and
285 might be illustrative of a slight input of organic N. In contrast, the three
286 other samples (*Dark_A*, *B* and *CHAR*) are characterized by higher Corg
287 (respectively $2.1 \pm 0.2\%$, $0.5 \pm 0.2\%$ and $9.8 \pm 0.2\%$) and N contents ($0.15 \pm$
288 0.08% , $0.11 \pm 0.08\%$ and $1.31 \pm 0.08\%$). Thus, these samples were not only
289 visibly transformed by the experiment but also affected by a significant
290 input of C and of N related to the functioning of the fireplace. The *RED*
291 value indicates that the reddening of sediments resulted only from the
292 oxidation of mineral constituents and did not include significant Corg or N
293 input. High values from *CHAR*, compared with *Dark_A* and *B* values,
294 illustrated the organic nature of this residue. Finally, the differences
295 observed between two visibly undistinguishable samples (*Dark_A* and *B*)
296 had to be noticed as they reflect significant lateral variability of organic
297 matter enrichment in the underlying sediments during the operation of the
298 fireplace. Moreover, considering intra- and inter-site variability of all the
299 experimental fireplaces from the same site (Lejay et al., 2016), the increase
300 of Corg appears especially related to the darkened sediments whereas no
301 Corg enrichment was noticed in the reddened sediments. Significant spatial
302 variability illustrates the interest and necessity of the multiplication of
303 samples, both in planimetry and in stratigraphy. Regarding the N content,
304 only the meat-cooking fireplace produced measurable contents, indicating a
305 nitrogen input related to this particular use. Indeed, no N input could be

306 quantified in the samples from bone or bone/wood-fueled fireplaces
307 (unpublished data; see Lejay, 2018).

308 3.3.Molecular Content

309 The compounds and groups of compounds significant for this study are
310 presented and discussed in this part. A full presentation of GC-MS and
311 py(TMAH)-GC-MS results is provided in the Supplementary Materials:
312 Documents 1 and 2 and corresponding Tables 1 and 2.

313 3.3.1.Local Background

314 The local background was documented by the analysis of the *Control*
315 sample, presented in Lejay et al. (2016), after an additional concentration
316 step that allowed for the identification of the compounds initially present at
317 very low levels in the extract. It is dominated by aliphatic compounds and in
318 particular by C_{16:0} and C_{18:0} saturated fatty acids (FA). Discontinuous series
319 of FA, *n*-alkanes (mostly odd-number carbon chains) and *n*-alcohols (mostly
320 even-number carbon chains) were detected as well as C_{16:1} and C_{18:1}
321 unsaturated FA. Several glyceride derivatives (mono-acylglycerols and di-
322 acylglycerols) as well as terpenes and sterols (notably phytosterols) were
323 detected which completed this list of lipids common in soil OM (van Bergen et
324 al., 1997; Kögel-Knabner, 2002). With py(TMAH)-GC-MS detected
325 compounds are rare and correspond to benzene derivatives that may be
326 related to soil OM pyrolysis during the analysis (Kaal et al., 2008a).

327 The *Yellow* sample shares many of these same characteristics, and can
328 therefore, for the most part, be considered as unaffected by the experiment
329 and similar to *Control*. In detail, however, several nitrogen compounds were

330 detected both in GC-MS and py(TMAH)-GC-MS in this sample and might
331 reflect a limited, but still non-negligible, result of the functioning of the
332 fireplace (see below).

333 3.3.2. Fuel Signature

334 The use of wood as a fuel is illustrated by several compounds both in
335 extracts and in bulk OM. Most of them are related to benzoic derivatives,
336 phenol derivatives and more generally derivatives related to lignin
337 degradation (Simoneit et al., 1993; Quénéa et al., 2004; Simoneit, 2005;
338 Regert et al., 2006; Shadkami and Helleur, 2010; Lejay et al., 2016). As they
339 are absent from the *Control*, they can be attributed to the fuel used in the
340 present study. Yet, in a sedimentary context where soil may contain
341 significant contribution of plant OM, these compounds could not be
342 considered as diagnostic.

343 Benzene and benzenic derivatives as well as PAH were detected in
344 py(TMAH)-GC-MS analyses of all the altered samples (*RED*, *DARK_A*,
345 *DARK_B* and *CHAR*). These groups of compounds are common by-products
346 of wood combustion (Simoneit et al., 1993; Schauer et al., 2001; Knicker et
347 al., 2005; Kaal et al., 2008a et b, 2009; Alexis et al., 2012; De la Rosa et al.,
348 2012; Lejay et al., 2016). Although they might reflect the alteration of OM
349 through analytical pyrolysis (Kaal et al., 2008a), their abundance and
350 diversity in altered samples in comparison with the *Control* sample argue
351 for their direct relationship with the fuel used in the fireplace, as is the case
352 in several similar studies (Simoneit et al., 2000; March et al., 2014; Lejay et
353 al., 2016).

354 3.3.3. Meat Signature

355 3.3.3.1. Animal OM / Fat

356 The input of animal OM was significant in all altered samples, notably
357 *RED*, *DARK_A* and *B*. The high contribution of C_{18:1} unsaturated FA, of
358 short (< C₁₆) saturated FA, and of short (C₅ to C₉) diacids detected with GC-
359 MS could be attributed to the degradation of triacylglycerols forming animal
360 fats (Dudd et al., 1998; Malainey et al., 1999; Evershed et al., 2002; Van
361 Den Berg et al., 2002; Nieuwenhuyse et al., 2015; Lejay et al., 2016).

362 Numerous compounds in the solvent extract underline the thermal
363 alteration of animal OM. Most of them are related to the alteration of FA
364 via oxidation, dehydration and/or cyclization (lactones, ketones, aldehydes,
365 methoxy-, epoxy- and hydroxy acids) and are probably related to the
366 burning of meat fats (Nawar, 1969 and 1989; Evershed et al., 1995;
367 Evershed et al., 2002; Simoneit, 2002). In *DARK_A* and *DARK_B*
368 pyrolysates, short chain (C₁₀ to C₁₇) *n*-alkane/*n*-alkene doublets which are
369 also representative of such thermally altered fats were detected (Oudemans
370 and Boon, 1991; Lejay et al., 2016). Some of these compounds (epoxy- and
371 hydroxy- acids) might have originated from plant constituents like cutin and
372 suberin (Kögel-Knabner, 2002); however, they were exclusively detected in
373 samples affected by the experimental fire, which seems to exclude this
374 hypothesis.

375 3.3.3.2. N-compounds

376 Apart from these compounds, several N-compounds (Table 2, see also
377 Supplementary Materials: Tables 1 and 2) were detected in all visibly

378 altered samples and to a lesser extent in the *YELLOW* sample, both in GC-
379 MS (Fig. 3) and in py(TMAH)-GC-MS (Fig. 4). They correspond to amides
380 and nitrile containing compounds (*DARK_A* and *CHAR*; Fig. 3A and B) and
381 to heterocyclic and aromatic N-compounds (*RED*, *DARK_A*, *B* and *CHAR*;
382 Fig. 3C and D, Fig. 4). In detail, C₁₆ and C₁₈ amides and nitriles were
383 detected in *DARK_A* and mostly in *CHAR*. They are reported in several
384 studies of aerosols related to the cooking of meat (Rogge et al., 1991;
385 Schauer et al., 1999; Simoneit et al., 2003; Rono et al., 2017) and of by-
386 products of Meat and Bone Meal (MBM) combustion (Ayllón et al., 2006;
387 Cascarosa et al., 2011).

388 The detection of nitriles directly related to the operation of fireplaces was
389 only mentioned in Buonasera et al. (2019) study, which included the heating
390 of cow marrow, a bone-fueled fireplace and a wood-fueled fireplace. The
391 analytical protocol included a GC-MS analysis of total lipid extract (TLE)
392 and of several fractions with increasing polarities. The formation of nitriles
393 was reported in cow marrow heated at 300 and 350 °C (detected in their 3rd
394 fraction with ketones and esters) and disappeared over 400°C. The same
395 nitriles were also observed in the bone-fueled fireplace, both in 3rd fraction
396 and in TLE. This type of compound could not be identified in Lejay et al.
397 (2016), where relatively similar experiments and analysis were conducted.
398 However, Buonasera et al. (2019) mentioned that some muscle tissues
399 remained on the surface of some bone pieces, which could explain this
400 discrepancy. Moreover, the protocol used by Buonasera et al. (2019),
401 including fraction separation, may be more suitable to pinpoint sparse

402 compounds which may otherwise stay concealed in TLE. This distinction
403 could explain why nitriles are well pronounced in Buonasera et al. 3rd
404 fraction from marrow heating and bone-fueled fireplaces.

405 In the present study, heterocyclic and aromatic N-compounds were
406 identified in all the samples, and notably in the *CHAR* pyrolysate. The
407 identified N-heterocyclic compounds correspond to pyridine, pyrimidine and
408 indole derivatives. Similar compounds were also reported in relation to the
409 cooking of meat (Oudemans and Boon, 1991; Rono et al., 2017) and MBM
410 combustion (Chaala and Roy, 2003; Cascarosa et al., 2011; Berruti et al.,
411 2012). Aromatic N-compounds, such as benzamides, detected in the present
412 study, were mentioned in those studies as well. Surprisingly, compounds
413 frequently obtained through proteinaceous compound pyrolysis, like imide,
414 imine or diketopiperazine (Chiavari and Galletti, 1992; Sharma et al., 2004;
415 Kruse et al. 2011; Knicker, 2007), seem absent in this study. The
416 destruction of these compounds through cyclisation processes may be
417 responsible for their absence (Knicker, 2010) and could result from the
418 elevated temperature reached during the operation of the fireplace, as well
419 as from the analytical pyrolysis procedure. Cyclic N-compounds were
420 detected both in solvent extracts and in bulk OM, indicating that they were
421 already present in the samples and are not only related to analytical
422 pyrolysis. However, it is possible that some of them, detected by py(TMAH)-
423 GC-MS, result from the cyclisation of linear N-compounds during
424 thermochemolysis (Gallois et al., 2007).

425 By definition, solvent extraction, followed by GC-MS analysis, provides
 426 access to the “lipid” fraction (Morrison, 1969). Because of their relatively
 427 high polarity and the use of non-polar columns, most of the natural N-
 428 compounds are usually not detected using this protocol. In the present
 429 study, the high contribution of N-compounds to solvent extracts is
 430 noteworthy. This result might be explained by 1) the high contribution of N
 431 in the animal organic matter; 2) the thermal alteration which induced
 432 cyclization processes, decreasing the polarity of N-compounds and allowing
 433 for their detection. Nonetheless, the question of the presence of N-
 434 compounds, their formation, and their transformation in fire-related
 435 activities involving animal material remains a widely under-explored
 436 subject.

437 Table 2: Nitrogen compounds from solvent extracts (GC-MS) and bulk OM
 438 (py[TMAH]-GC-MS). The molecular weight (MW) and main mass fragments
 439 (m/z) of original compounds, TMS and methylated derivatives are reported
 440 (with decreasing contributions). Examples of N compounds, as indicated
 441 between brackets, are illustrated in figures 3 and 4.

	Compound	Main fragments (m/z)	MW	Class	Content	YELL OW	RED	DA R K_A	DA R K_B	CHAR
Solvent Extract (GC-	2-Piperidinecarboxylic acid, 1-(TMS)-, TMS	156, 73	273	N hetero		x			x	
	Pyrimidine, 2,4-bis[(TMS)oxy]-	241, 99, 256, 73, 147	256	N hetero		x			x	
	Pyridine / Pyridine, TMS	79, 52 / 152, 167	79 / 167	N hetero			x	x	x	x

	Piperidine	84, 85, 57	85	N hetero				x		
	Pyrimidine, 5-methyl-2,4-bis[(TMS)oxy]-	255, 270, 73, 113, 147	270	N hetero			x			
	Benzamide, N-(TMS)- (Fig. 3D)	178, 75, 77, 104, 193	193	N arom.			x		x	
	Adenine, 2TMS (Fig. 3C)	264, 279, 206, 192	279	N hetero					x	
	Hexadecanenitrile (Fig. 3B)	97, 43, 57, 110, 124	237	Nitrile				x		x
	Oleanitrile	41, 55, 43, 122, 69	263	Nitrile						x
	Hexadecanamide (Fig. 3A)	59, 72, 43, 212, 86, 114, 128	255	Amide						x
	9-Octadecenamide, (Z)-	59, 72, 55, 238, 281	281	Amide						x
	Octadecanamide	59, 72, 55, 238, 239, 283	283	Amide						x
	Erucylamide	59, 72, 83, 126, 240, 294, 337	337	Amide						x
Bulk OM (py[TMAH]-GC-MS)	2-(2-Hydroxyethylamino)pyrimidine	108, 139, 53	139	N hetero.						x
	2-Piperidinone, 1-methyl-	113, 44, 57	113	N hetero.						x
	3-pyridinecarboxylic acid, methylester	106, 137, 78	137	N hetero.					x	
	2(1H)-Pyridinone, 1-methyl-	109, 81, 80	109	N hetero.						x
	Methylindole (Fig. 4C)	131, 130, 77, 89, 103, 116	131	N hetero.						x
	4[1H]-Pyridone 3-hydroxy-1,2,6-trimethyl-	108, 138	153	N hetero.						x
	Proline, 2-methyl-5-oxo-, methyl ester (Fig. 4A)	98, 42, 41, 68, 157, 59	157	N hetero.						x
	Dimethylindole	145, 144	145	N hetero.						x
	Pyridine, 2-phenyl-	155, 154, 128	155	N hetero.					x	
	Trimethylindole	158, 159, 144	159	N hetero.			x			
	Piperidine, 1-(ethoxymethyl)-	98, 42	143	N hetero.						x
Benzonitrile, 2,4-dimethoxy-	163, 134, 162,	163	N arom.					x	x	

(Fig. 4D)	78, 120									
Phenol, 3-(dimethylamino)-	136, 121, 91, 150	136	N arom.			x	x	x		
Benzenemethanamine, N,N-dimethyl- (Fig. 4B)	58, 91, 135	135	N arom.			x	x	x	x	
Methanamine	140, 42, 85	140	Amine		x					
Ethyldiethanolamine	102, 58	133	Amine						x	
Hexadecanamide, N,N-dimethyl-	87, 100, 72, 283	283	Amide				x			x
9-Octadecenamide, N,N-dimethyl-	87, 100, 55, 72, 309	309	Amide				x			x
Octadecanamide, N,N-dimethyl-	87, 100, 72, 311	311	Amide				x			x
D-Alanine, N-ethoxycarbonyl-ethyl ester	116, 44, 144, 189	189	N hetero			x			x	
L-Alanine, N-L-alanyl-	44, 99, 142	160	N hetero		x					

442

443 From a general point of view, most of these compounds are non-specific and
444 could be by-products of analytical pyrolysis from initial molecules such as
445 indoles from tryptophan, benzenemethanamine and benzonitrile from
446 phenylalanine, or piperidines/piperidones from arginine (Tsuge and
447 Matsubara, 1985; Sharma et al. 2003 and 2004; Gallois et al., 2007; Gallois,
448 2009; Stadler and Lineback, 2009). Nevertheless, considering the context of
449 our experiment, involving protein-rich material, as well as the absence of
450 similar compounds in the *Control* sample and in samples from previous
451 experimental fires where bone was used as fuel (Lejay et al., 2016), these N-
452 compounds are thus proposed as diagnostic of the involvement of meat
453 during the operation of a fireplace.

3.3.4. Application of Analytical Pyrolysis

454
455 Characterization of N-compounds through analytical pyrolysis in soils and
456 sediments is still a challenging domain of research (Derenne and Quénéa,
457 2015), as it is admitted that most identified compounds may be produced by
458 the pyrolysis itself (Schulten and Schnitzer, 1998). As a result, analytical
459 pyrolysis is unlikely to detect non-heterocyclic nitrogen (Derenne and
460 Quénéa, 2015). More generally, pyrolysis may appear inappropriate to
461 identify combustion products since secondary and initial reactions may be
462 confounded. Nonetheless, in the present work, the objective was not
463 primarily to determine the nature of molecules initially present in fire-
464 affected samples, but to discriminate the organic signature of soils affected
465 by the combustion of different fuels. The pyrolysis seemed to be a useful tool
466 for detecting the effect of meat cooking on the soil organic signatures,
467 notably because expected molecular markers (cyclic and aromatic
468 compounds) are classically not detected in the lipid fraction.

469 Other analytical set-ups and the combination of several ones (see Leinweber
470 et al., 2013 and references therein for example) would probably lead to more
471 detailed results on this OM molecular composition. However, because of the
472 low chemical recalcitrance of black nitrogen (Knicker, 2010), the application
473 of chemical oxidation frequently used to isolate pyrogenic OM should
474 probably be avoided. The recent use of the untargeted metabolomics-like
475 screening approach (Brockbals et al., 2018) may also be helpful in
476 distinguishing the diagnostic compounds in the complex signature produced
477 by the operation of fireplaces. However, this would likely necessitate the

478 development of a larger experimental reference dataset, with an initial set-
479 up adapted to quantification.

480 4. Conclusions

481 4.1. Organic Signatures of a Meat-Cooking Fireplace

482 The results obtained from this experimental fireplace share common
483 characteristics with the previously reported experiments (Lejay et al., 2016).
484 Namely, the use of wood as fuel is recognizable from the high contribution of
485 lignin derivatives and the presence of wood combustion by-products (e.g.
486 benzenes and PAH), whereas the involvement of animal OM is indicated by
487 the darkening of the top layer of sediments, the increase of Corg and the
488 detection of animal fat degradation and thermal alteration by-products.
489 Without further evidence, important overlap would still exist with other
490 types of fireplace, notably those using a mix of wood and bone as fuel.

491 Table 3: Summary of diagnostic compounds in extracts (GC-MS) and from
492 the bulk soil OM composition (py[TMAH]-GC-MS), and their possible origins
493 in this study. In italics, compounds with expected long-term preservation
494 due to their recalcitrance (according to the literature, see Section 4.2, and
495 Lejay et al., 2016). In grey, compounds that appear specific to meat cooking
496 (see Section 4.2). See also Supplementary Materials: Tables 1 and 2 for a
497 detailed list of each class components.

	Class	YE LL OW	RE D	DA RK_ A	DA RK_ B	CH AR	Possible origin in this study
<i>h</i> <i>3</i>	<i>Benzoic acid derivatives</i>		x	x	x	x	Vegetal OM from wood fuel

	<i>Lignin derivatives</i>		x			x	
	<i>Benzenic derivatives</i>			x	x		Thermally altered vegetal OM from wood fuel
	<i>PAH</i>				x		
	Glycerid derivatives	x	x	x			Animal OM from fat
	Lactones		x	x		x	Thermally altered animal OM from fat
	<i>Ketones</i>			x		x	
	Amides					x	Thermally altered animal OM from meat
	<i>Nitriles</i>			x		x	
	<i>N heterocycles</i>	x	x	x	x	x	
	<i>N aromatics</i>		x		x		
Bulk OM (PyTMAH-GC-MS)	<i>Lignin derivatives</i>		x	x	x	x	Vegetal OM from fuel
	<i>Benzoic acids derivatives</i>		x	x	x	x	
	<i>PAH</i>		x	x	x		Thermally altered vegetal OM from fuel
	Aldehydes					x	Animal OM from fat
	Methoxy/epoxy/hydroxy fatty acids			x		x	
	Lactone			x		x	Thermally altered animal OM from fat
	Amides and amines	x		x		x	Thermally altered animal OM from meat
	<i>N heterocycles</i>		x		x	x	
	<i>N aromatics</i>				x	x	

498

499 However, the increase of N content and the detection of N-compounds are
500 specific to samples from the meat-cooking experiment. The presence of
501 nitrogen in this context can be explained by the high content of proteins,
502 and therefore of amino acids, in meat (Belitz et. al., 2009; Rono et al., 2017).
503 The detection of alkyl-amides, alkyl-nitriles, hetero-amines and nitrated
504 polycyclic aromatic hydrocarbons (nitro-PAHs) is common in studies
505 involving the cooking of meat, although those compounds have mostly been

506 considered for their presence in aerosols and their carcinogenic properties
507 (e.g. Simoneit et al., 2003; Cross and Sinha, 2004).

508 4.2. Archaeological Potential

509 4.2.1. N-compounds in Archaeological Contexts

510 To our knowledge, nitrogen compounds have seldom been reported in
511 studies regarding archaeological practices related to alimentation. Protein
512 markers (amide, pyrrole and indole derivatives) were detected both in
513 experimental and in archaeological samples analyzed by pyGC-MS
514 (potsherd residues dated from the Late Iron Age/Early Roman; Oudemans
515 and Boon 1991: p. 219). In a study by Wang et al. (2017), amides and nitriles
516 were detected and identified in the sedimentary record from a Chinese
517 Neolithic archaeological site (ca. 5 to 4 Ky cal. BP). Using stratigraphical
518 sampling, these authors were able to monitor these compounds from a
519 diachronic perspective and interpret them as potential markers for an
520 important climatic and/or cultural change, involving the burning or cooking
521 of biomass. Recently, a study by Sanjurjo-Sánchez et al. (2018) involving py-
522 GC-MS analysis of archaeological pottery samples (3rd millennia BC)
523 detected pyrrole and pyridine that were attributed to potential proteins
524 from vegetal or animal material.

525 4.2.2. N Compound Preservation in Soil

526 It has long been argued that N-compounds lack adequate potential for
527 preservation in soils at prehistoric timescales (Eglinton et al., 1991;
528 Evershed and Tuross, 1996; Peters et al., 2005; Pollard et al., 2007). As a
529 consequence, N-compounds and proteins have generally been disregarded.

530 However, previously mentioned studies (Oudemans and Boon, 1991; Wang
531 et al., 2017; Sanjurjo-Sánchez et al., 2018) as well as recent progress in the
532 field of paleoproteomic studies (Dallongueville et al., 2016) have highlighted
533 conservation of such compounds at the scale of millennia and therefore
534 encourage a reconsideration of their relevance to prehistoric archaeology.

535 Leinweber et al. (2013) have reviewed various studies mentioning the
536 stability potential of N-heterocyclics. While important turnover and loss
537 related to bacterial activity and leaching of hydrolyzable compounds must
538 be considered, they have described several processes of pedo-mineral
539 interactions leading to long-term preservation. In particular, they have
540 described binding processes of N-compounds to minerals (especially Fe
541 oxides), leading to their stabilization and significantly reducing their
542 disappearance through hydrolyze (see also Leinweber and Schulten 2000).
543 The impact of fire on N-compound preservation in soil should also be
544 mentioned. Knicker (2007, 2010) has highlighted the potential stability of
545 black nitrogen, and notably of heterocyclic/aromatic variants, in soil, despite
546 a relatively low chemical recalcitrance. She has also described the inclusion
547 of unaltered or slightly altered organic molecules in charcoal pieces and
548 carbonized OM that may create micro-environments that protect relatively
549 labile compounds. Other studies (see Leinweber et al., 2013 for a review)
550 have highlighted the transformation of the N fraction of soil OM (mostly
551 amides and proteins) to unsaturated or heterocyclic/aromatic N-compounds
552 following thermal alteration (Kiersch et al., 2012a and b), leading to an
553 increase of this OM recalcitrance.

554 To conclude, while it is true that the low chemical recalcitrance and the
555 polarity of N-compounds constrain their preservation, the soil mineral
556 matrix seems to be an environment where several physico-chemical
557 processes can combine to form relatively favorable reservoirs. In particular,
558 considering their chemical structures, a relative stability of nitriles, N-
559 heterocyclic and N-aromatic compounds formed during thermal alteration
560 can be expected in such environments. The analysis of samples from
561 different fireplaces (here and in Lejay et al., 2016) after several years of
562 evolution in the open air will potentially allow for further assessment
563 regarding the preservation of organic signatures over time.

564 Our results illustrate the potential of these compounds when discovered in
565 fireplaces. Namely, this experiment pinpoints a way to differentiate, at both
566 elemental and molecular scales, the operation of a fireplace with a mix of
567 wood and bones (vegetal and animal OM signatures; Lejay et al., 2016) from
568 the use of a wood-fueled fireplace to cook meat (vegetal and animal OM with
569 increase of N contribution). Given the difficulty archaeologists usually have
570 face when interpreting Paleolithic fireplaces from a functional perspective
571 (Olive and Taborin, 1989; Sandgathe and Berna, 2017), this distinction is of
572 great interest from a palethnographic perspective.

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595 Competing interests

596 The authors declare no competing interests.

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973 Captions

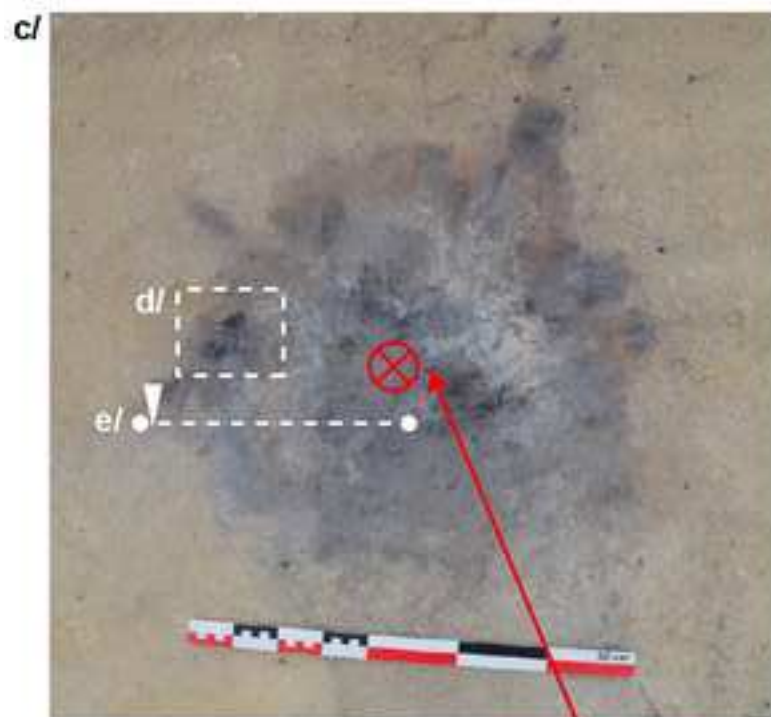
974 Figure 1: Operation and sampling of the experimental fireplace. a/ Adding of
975 700g of beef meat directly on embers. b/ Result of one use after the
976 extinction of the fireplace. c/ General view of the fireplace after raking out of
977 combustion residues. d/ Close-up on a fat-derived char formed on the surface
978 of sediments. e/ Section through the fireplace.

979 Figure 2: Corg and N contents measured by EA after HCl fumigation (1σ
980 margins of error; n.q.: non quantifiable).

981 Figure 3: Selection of representative N-compounds from solvent extracts
982 (GC-MS) and their associated mass spectrum.

983 Figure 4: Selection of representative N-compounds from bulk OM
984 (py[TMAH]-GC-MS) and their associated mass spectrum.

Figure 1
[Click here to download high resolution image](#)

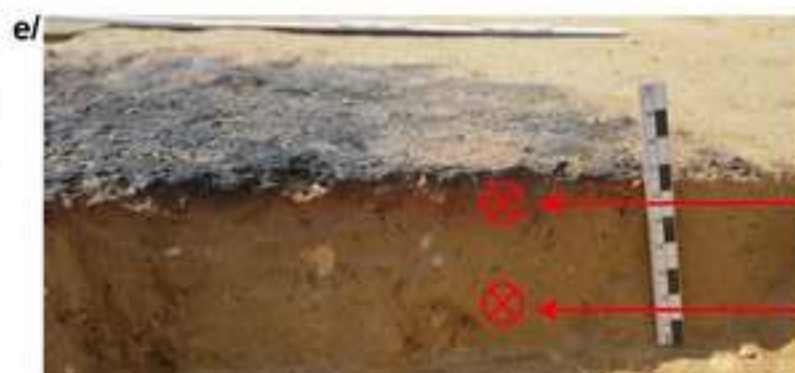


DARK_B



CHAR (over)

DARK_A (under)



RED

YELLOW

Figure 2
[Click here to download high resolution image](#)

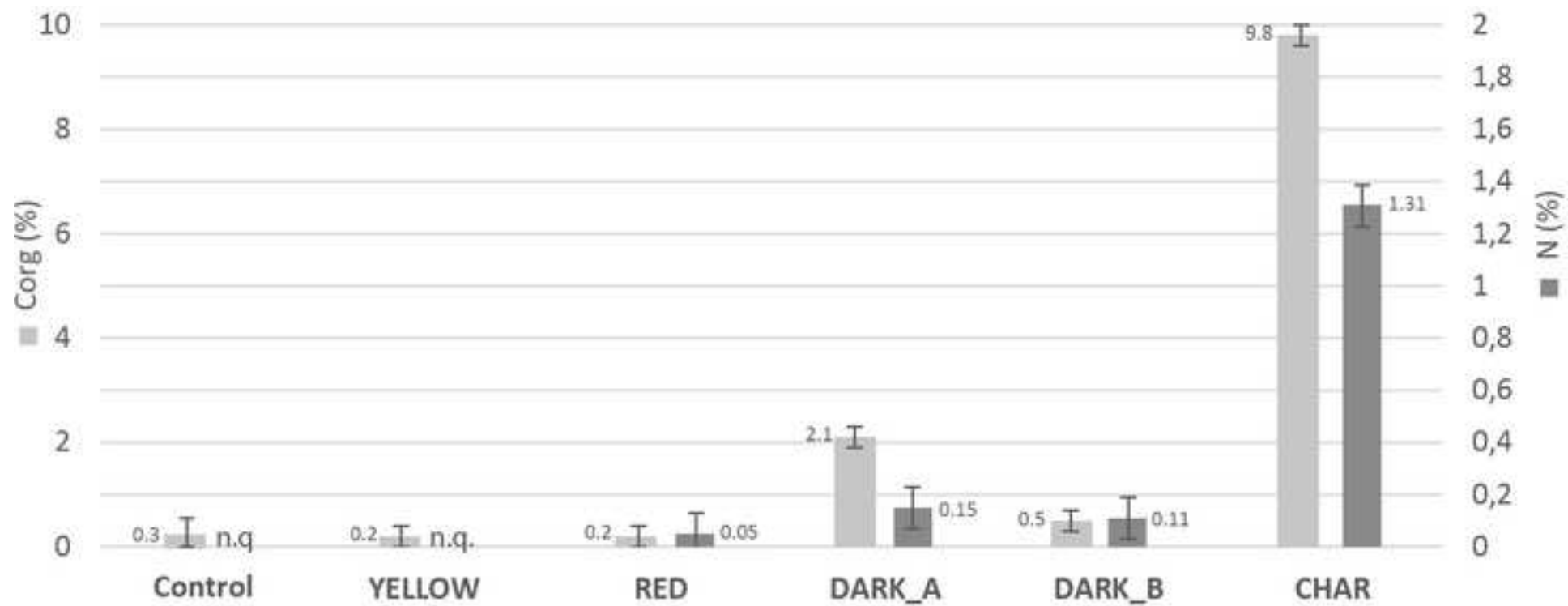
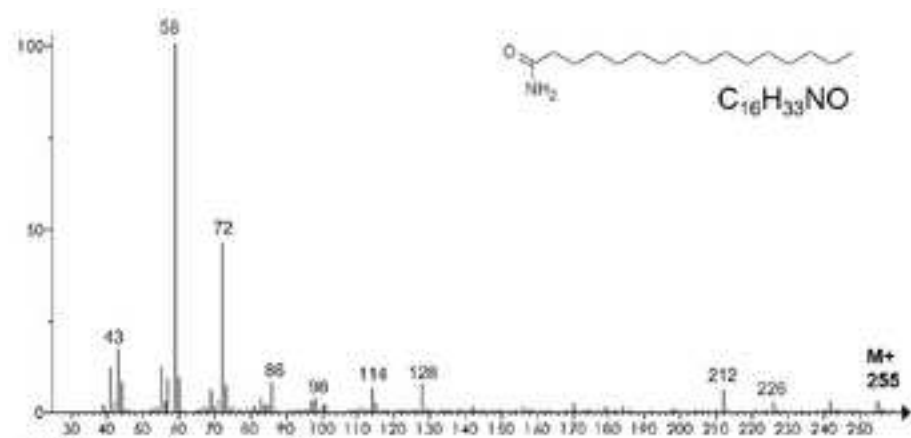
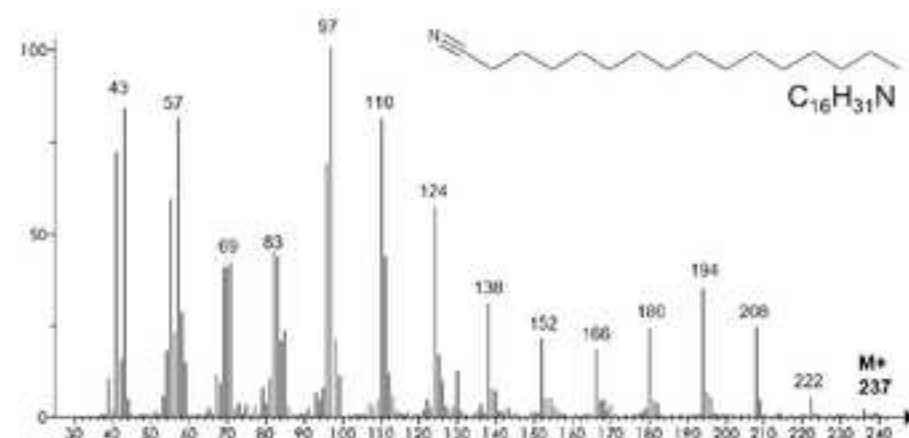


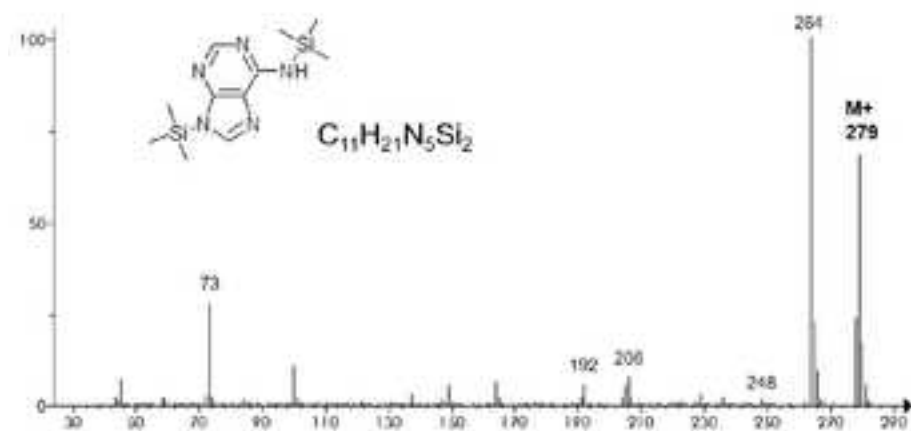
Figure 3
[Click here to download high resolution image](#)



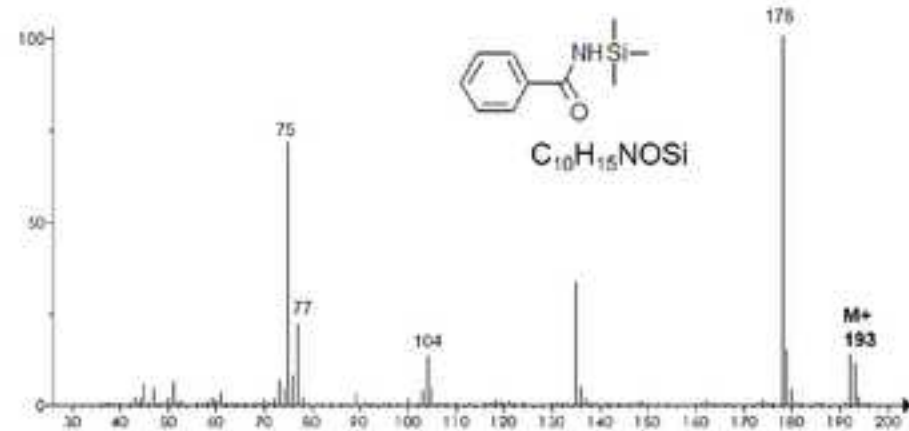
A/ Hexadecanamide



B/ Hexadecanenitrile

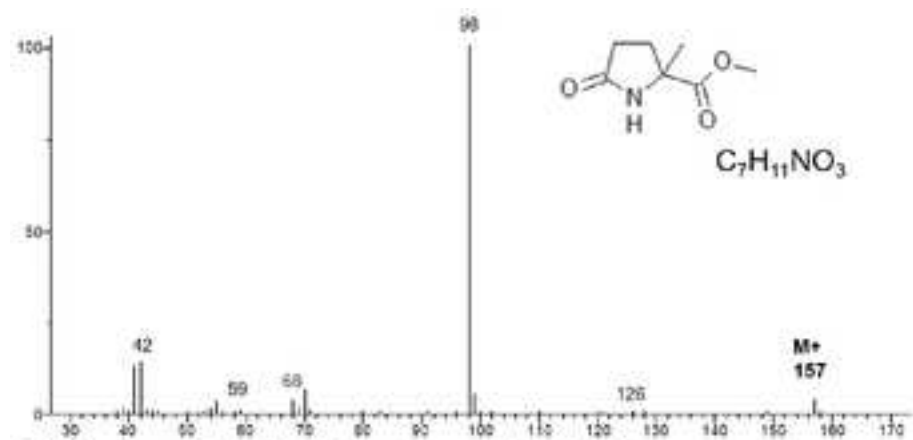


C/ Adenine, N,N' -bis(trimethylsilyl)-

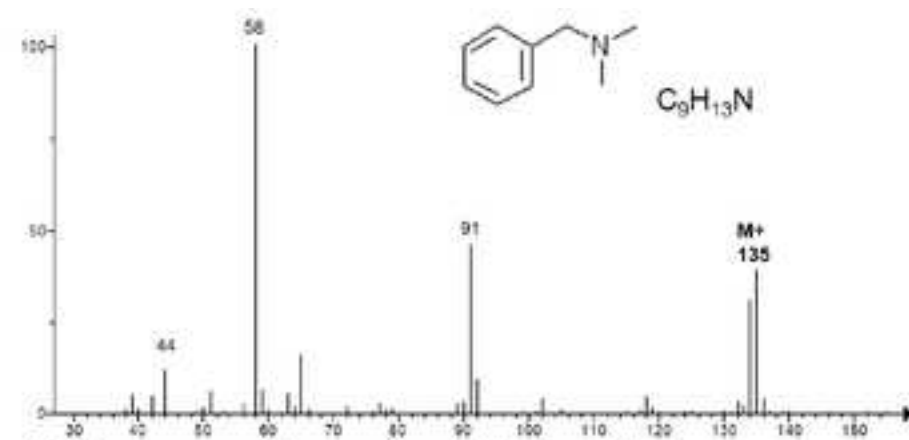


D/ Benzamide, N -(trimethylsilyl)-

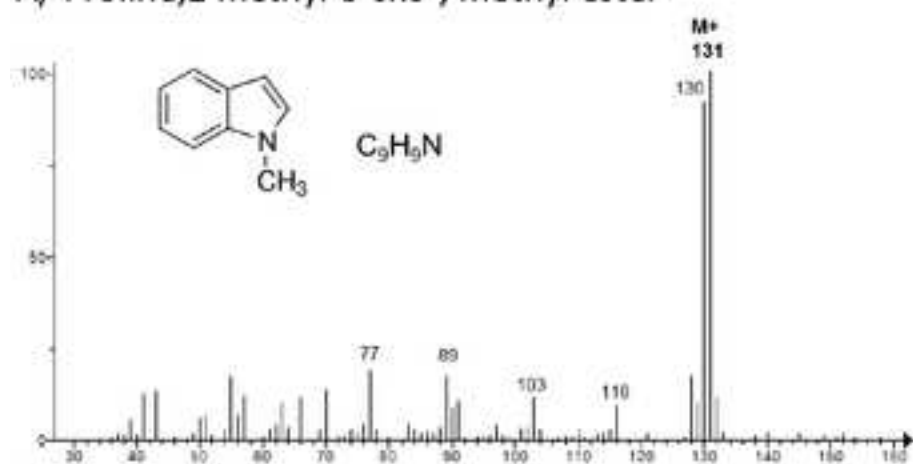
Figure 4
[Click here to download high resolution image](#)



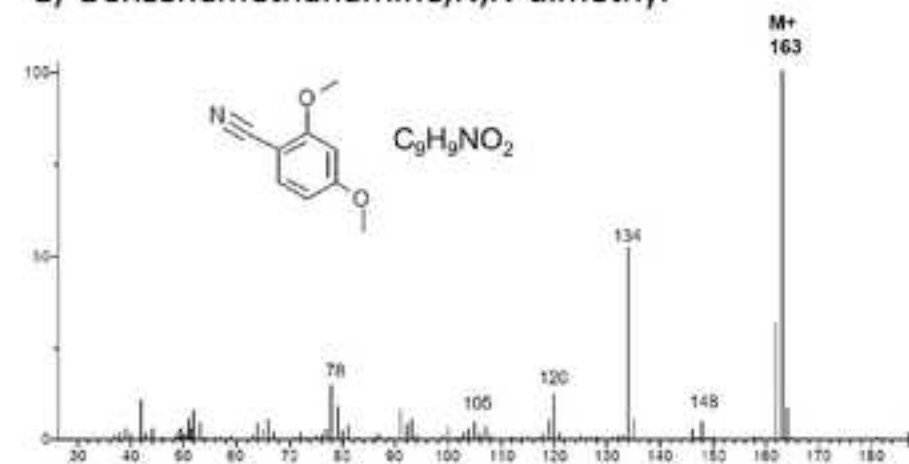
A/ Proline, 2-methyl-5-oxo-, methyl ester



B/ Benzenemethanamine, N,N-dimethyl



C/ Methylindole



D/ Benzonitrile, 2,4-dimethoxy

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Dear editor

Please find attached the revised manuscript entitled 'The Organic Signature of an Experimental Meat-cooking Fireplace: the Identification of Nitrogen Compounds and their Archaeological Potential' previously submitted for publication in *Organic Geochemistry* (OG 4112).

Many thanks to the reviewers for their time, correction and useful advice. We tried our best to take them into account. Please find below the answer to reviewers and in the attached document our revised manuscript.

I hope that the article will be acceptable for publication in your journal and look forward to hearing from you soon.

Yours sincerely,

Mathieu Lejay

Reviewer #1:

The manuscript presents the results of the analysis by Py-GC/MS and GC/MS of soil samples obtained from an experimental fireplace. The authors identify a suite of compounds in the fireplace used to cook/burn meat. The use of Py-GC-MS seem rather problematic in some instances as the compounds detected could have arisen for the pyrolysis of compounds during the experimental fire or from the analytical pyrolysis. This is however acknowledged by the authors.

>>As mentioned by the reviewer, the potential pyrolysis of some compounds during the analysis is inherent to the Curie point pyrolysis technique and can never totally be excluded. That is why, as many other authors, we mentioned it but decided to use it anyway. The reason is that this technique allow accessing a wide class of compounds that could not be extracted or identified by other techniques. Secondly, the objective of this work is more to identify a "specific organic signature" produced by the chosen techniques applied to experimental fires than to identify the initial compounds present in the samples. In the former and related article published in OG, some control samples were included, that allowed comparing the signature obtained with and without experimental fire, and the signal obtained with and without experimental burning of the fat from bone fire. Then, only the differences between fires and the controls are interpreted in this article. As a result we think all the cautions were taken to minimize the potential artefact related to this analytical technique and consequently our conclusions are rather prudent.

This paper is very similar to a previous paper published in OG from the same authors (Lejay et al. 2016, Organic Geochemistry 99, pp 67-77) where they study experimental fireplaces fuelled by bones and wood. The authors mentioned that N-compounds have been disregarded because "they have long been considered as lacking good preservation potential over archaeological timescales". However - although the authors report such N-compounds in the experimental fireplace, and simply highlight the compounds that could be recalcitrant through time.

This manuscript would thus greatly benefit from the analysis of soil samples from the same fireplace after few months / years (degradation experiment)

>> Indeed it is a potential perspective of this experimental project. Sampled fireplaces are still preserved on the experimental site and may eventually be re-sampled. Nonetheless it is not achieved yet and can't be integrated to the paper in a reasonable amount of time. We add mention of this in section 4.2.2

and/or from an archaeological fireplace where some compounds relating to meat burning are detected.

>> The application of the experimental referential is our ultimate goal. Some preliminary results from archaeological context (Aurignacian and Gravettian fireplaces), are available in M. Lejay PhD thesis (Lejay, 2018) and papers are under preparation. However it seems to us that the part of the work that concerns archaeological fireplaces would likely correspond to more "archaeological oriented" journals, whereas this first "experimental" part on organic signature is much more

adapted to OG. We extended our discussion about other archaeological and experimental papers.

Comments about specific aspects of the manuscript
I. 115 - chemical signature of what?

>>Modification was made in the text to clarify the objectives.

I. 215 - could the authors please check the oven temperature rate, which seems rather low (2 oC/min).

>> 2°C/min is the correct temperature rate applied in this study. The numerous compounds present in the sample generated highly complex chromatograms. This low temperature rate was thus necessary to distinguish and identify the compounds.

I. 249 - Nitrogen is undetectable in the control (YELLOW). What is the limit of detection of the EA method?

>>The quantification (not the detection) limit was about 120 µg for C and 15 µg for N, what give about < 0.2% for C and < 0,025% for N. It was added in the text.

I. 297 - would the authors be able to distinguish compounds related to wood used as fuel and lignin present in the soil due the degradation of OM?

>> In a broader perspective, there is of course potential overlapping between those two categories. In this study we believe that it was avoided thanks to the low vegetal OM content of the local soil (see Control Sample Section 3.3.1) and that compounds detected in fireplaces are genuinely related to fuel (see section 3.3.2)

Nonetheless, in our synthetic table these compound groups were classified as “vegetal OM” only and not specifically to fuel to avoid this confusion. We add an explanation to make it less misleading in section 3.3.2 and add in table 3 that origin proposition are made in the frame of this study

I. 365 - can the authors explicit what they mean by "the use of non-polar columns".

>> The Restek 5 Sil MS column that was used for the GC-MS analyses presented a relatively low polarity, what is classical for the lipid fraction analysis.

I. 445 - how are protein markers common in soil? Presence in control samples?

>> As mentioned in the « Local background », section 3.3.1, and in the Table 2 (reporting the N compounds in the different samples), no N compounds could be detected in the “control sample” of our study.

I. 459 - not clear. Mentions of what?

>> this section is now re-written

Reviewer #2:

This communication builds on previous work published in Organic Geochemistry, it seeks to identify a series of nitrogenous compounds that may be used as indicators of meat cooking in archaeological contexts. Whilst the manuscript itself needs some work, the experimental approach and methods used seem sound. The use of py-GCMS would appear to be somewhat confounding since it can produce the same compounds as an artefact of the technique. I am still not convinced that it is a useful addition to the study.

>>cf. answer to reviewer #1

Other references to analytical setups that could improve the N compound identification have been added in section 3.3.4

The authors go out of their way to provide evidence (from the literature) that their target 'meat indicating' compounds are recalcitrant enough to survive over archaeological timescales. Whilst admittedly, disregarded by the organic geochemistry community (who are more attuned to geological timescales) I think that such 'meat biomarkers' could survive given the right depositional environment. What is less clear (and left unmentioned by the authors) is the mobility of such compounds. Most are quite polar so there is a possibility of leaching although conversely, depending upon the depositional environment this may not be an issue due to lack of water and/or strong binding to the soil matrix.

>> We totally agree with this comment, and the importance of the sedimentary context was mentioned from lines 517 to 525. However the leaching process was not clearly quoted. This part of the discussion is now extended (see section 4.2.2)

The other thing that is lacking from this study is a more prescriptive framework for the identification of meat cooking. Table 3, which lists the 'diagnostic components' is very ambiguous. As somebody maybe interested in detecting the cooking of meat products in Antiquity I would find it very difficult to apply this table to another set of samples as non-specific classifiers such as 'ketone', 'PAH' and 'N amide' (as opposed to a non-N amide???) *>> corrected* don't give me the tools I need in order to achieve the sort of investigation this study purports to underpin. If I need to repeat this study in order to identify all of the most important biomarker compounds then what is the advance that it offers?

>>We decided to only integrate synthetic tables (such as Table 3 and 2) in the text but the complete data are provided as Supplementary Tables (1 and 2), with the compounds, their main fragments, their number on chromatograms, their potential origin and presence/absence in the different samples. This decision was made to achieve a clearer presentation of the result in the text since those tables are long and the paper already counted 4 figures and 3 tables. So, at this point, we clarified the Table 3, only mentioning the "diagnostic" compounds. However, if the editor agreed that the complete compound tables are needed in the text, we are ready to change our presentation (moving Supplementary tables 1 and 2 in the text)

Using an untargeted metabolomic-based approach would have helped to really target the key compounds responsible which would have been a useful and immediately applicable output as opposed to listing compounds classes which is not.

>> We agree that a more detailed study of such N compounds would be of great interest and despite not being expert of the untargeted metabolomics approach, we agree that it would offer new perspectives to this work. We extended our discussion

of it in section 3.3.4. Anyway it also seems to us that the different approaches are complementary and that the detailed composition of the organic signatures of the fireplaces already represent valuable dataset that can be useful for other studies and that deserves publication.

In summary, I would like to see this work published but the authors need to make their conclusions, specifically the molecular criteria for the identification of meat cooking, MUCH tighter and less ambiguous thereby providing something useful that future researchers can take-up and run with.

>> See above discussion about Supplementary Materials and modification of table 3

Specific

comments

1. The wording of the manuscript needs to be greatly improved throughout. I would suggest getting a native English speaker to proof-read it since, as it stands, the manuscript is littered with unusual constructs that jar against the overall flow of the narrative.

>> As suggested, the manuscript have been proof read by a professional translator

2. (lines 203-206) Insert the details about derivatisation into the correct point of the workflow just before details of the analysis.

3. (line 217) 'm/z' should be italicised. I would also remove 'uma', it is not needed.

4. (line 220-224) Which versions of the software and the NIST library?

5. (line 327) Italicise the 'n' before alkane and alkene

6. (line 374) See 3

>> Done

Supplementary Material doc 1

[Click here to download Supplementary Material: Lejay-et-al_Supplementary-Material_Document 1.pdf](#)

Supplementary Material doc 2

[Click here to download Supplementary Material: Lejay-et-al_Supplementary-Material_Document 2.pdf](#)

Supplementary Material tabl 1

[Click here to download Supplementary Material: Lejay-et-al_Supplementary-Material_Table 1.xlsx](#)

Supplementary Material tabl 2

[Click here to download Supplementary Material: Lejay-et-al_Supplementary-Material_Table 2.xlsx](#)