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

Mathieu Lejay, Marie Alexis, Katell Quenea, Christelle Anquetil, François Bon

Elsevier Editorial System(tm) for Organic Geochemistry

Manuscript Draft

Manuscript Number: OG-4112R1

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

Article Type: Research Paper

Keywords: Fireplace; Experimental reference database; Organic signature; Nitrogen compounds; Prehistory

Corresponding Author: Mr. Mathieu Lejay, Ph.D

Corresponding Author's Institution:

First Author: Mathieu Lejay, Ph.D

Order of Authors: Mathieu Lejay, Ph.D; Marie A Alexis, Ph.D; Katell Quénéa, Ph.D; Christelle Anquetil; François Bon, Prof.

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 caracteristics. 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

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

Mathieu Lejay [1, 2, @]

Marie A. Alexis [3]

Katell Quénéa [3]

Christelle Anquetil [3]

François Bon [2]

[1] GéoArchÉon SARL, 30 rue de la Victoire, 55210 Viéville-sous-les-Côtes, France

[2] TRACES UMR 5608 : Équipe SMP3C, CNRS/Université de Toulouse Jean Jaurès, 5 allées Antonio Machado, 31058 Toulouse Cedex 9, France

[3] METIS UMR 7619 : CNRS/Université Pierre et Marie Curie, 75252 Paris cedex 5, France

[@] Corresponding author: lejay.m@gmail.com

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 caracteristics. 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.

Keywords

Fireplace; Experimental reference database; Organic signature; Nitrogen compounds; Prehistory

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. Introduction

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

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

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

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

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

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

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

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

2. Material and Methods

2.1. Context and Sampling

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

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

- Lighting and operation with wood for ~50min in order to form a bed of embers;

- Cooking of meat; in order to increase the organic signature of this type of use meat was placed directly on the bed of embers and was left to burn (Fig. 1a and b);

- No subsequent intervention, except for flipping the meat over;

- Mean total operation time: 125 min.

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

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

Table 1: Samples list and description.

Sample	**Description**
Control (same as in Lejay et al., 2016)	Yellow sandy loam with frequent calcitic features (*e.g.* hypocoatings, nodules)
YELLOW	Yellow sandy loam with frequent calcitic features (*e.g.* hypocoatings, nodules)
RED	Reddened sandy loam with frequent calcitic features
DARK_A	Darkened sandy loam; Sampled under a fat-derived char (*CHAR* 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)

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

2.2.Corg and N Contents

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

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

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

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

2.3.Solvent Extraction, Derivatization and GC-MS

The samples were extracted using an accelerated solvent extractor system (Dionex-ASE100). Between 5 and 7 g of sediments were placed in a 10 ml stainless steel cell topped with sterile and pre-extracted glass fiber. The extraction program ran at 60 °C, with a static time of 20 min, using dichloromethane and methanol (DCM/MeOH; 2:1, v/v). Each extract was concentrated using a rotary evaporator and dried under a gentle N_2 flow, dissolved in DCM in a 1.5 ml vial with a PTFE screw cap and stored at 4 °C until its analysis (adapted from Quénéa et al., 2012). Prior to their analysis,

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

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

2.4.Py(TMAH)-GC-MS

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

2.5. Analysis of Results

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

3. Results and Discussion

3.1. Experimental fireplaces

3.1.1. Field-based Remarks

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

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

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

3.1.2.Representativeness of the Experimental Design

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

3.2.Corg and N Contents

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

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

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

3.3.Molecular Content

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

3.3.1.Local Background

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

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

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

3.3.2.Fuel Signature

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

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

3.3.3.Meat Signature

3.3.3.1.Animal OM / Fat

The input of animal OM was significant in all altered samples, notably *RED, DARK_A* and *B*. The high contribution of $C_{18:1}$ unsaturated FA, of short (< C_{16}) saturated FA, and of short (C_5 to C_9) diacids detected with GC-MS could be attributed to the degradation of triacylglycerols forming animal fats (Dudd et al., 1998; Malainey et al., 1999; Evershed et al., 2002; Van Den Berg et al., 2002; Nieuwenhuyse et al., 2015; Lejay et al., 2016).

Numerous compounds in the solvent extract underline the thermal alteration of animal OM. Most of them are related to the alteration of FA via oxidation, dehydration and/or cyclization (lactones, ketones, aldehydes, methoxy-, epoxy- and hydroxy acids) and are probably related to the burning of meat fats (Nawar, 1969 and 1989; Evershed et al., 1995; Evershed et al., 2002; Simoneit, 2002). In *DARK_A* and *DARK_B* pyrolysates, short chain (C_{10} to C_{17}) *n*-alkane/*n*-alkene doublets which are also representative of such thermally altered fats were detected (Oudemans and Boon, 1991; Lejay et al., 2016). Some of these compounds (epoxy- and hydroxy- acids) might have originated from plant constituents like cutin and suberin (Kögel-Knabner, 2002); however, they were exclusively detected in samples affected by the experimental fire, which seems to exclude this hypothesis.

3.3.3.2.N-compounds

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

altered samples and to a lesser extent in the *YELLOW* sample, both in GC-MS (Fig. 3) and in py(TMAH)-GC-MS (Fig. 4). They correspond to amides and nitrile containing compounds (*DARK_A* and *CHAR*; Fig. 3A and B) and to heterocyclic and aromatic N-compounds (*RED, DARK_A, B* and *CHAR*; Fig. 3C and D, Fig. 4). In detail, C_{16} and C_{18} amides and nitriles were detected in *DARK_A* and mostly in *CHAR*. They are reported in several studies of aerosols related to the cooking of meat (Rogge et al., 1991; Schauer et al., 1999; Simoneit et al., 2003; Rono et al., 2017) and of by-products of Meat and Bone Meal (MBM) combustion (Ayllón et al., 2006; Cascarosa et al., 2011).

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

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

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

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

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

	Compound	**Main fragments (*m/z*)**	**MW**	**Class**	**Control**	**YELLOW**	**RED**	**DARK_A**	**DARK_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				

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

3.3.4.Application of Analytical Pyrolysis

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

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

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

4. Conclusions

4.1. Organic Signatures of a Meat-Cooking Fireplace

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

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

	Class	**YELLOW**	**RED**	**DARK_A**	**DARK_B**	**CHAR**	**Possible origin in this study**
r a	*Benzoic acid derivatives*		x	x	x	x	Vegetal OM from wood fuel

	Lignin derivatives		x			x	
	Benzenic derivatives			x	x		Thermally altered vegetal OM from wood fuel
	PAH				x		
	Glycerid derivatives	x	x	x			Animal OM from fat
	Lactones		x	x		x	Thermally altered animal OM from fat
	Ketones			x		x	
	Amides					x	Thermally altered animal OM from meat
	Nitriles			x		x	
	N heterocycles	x	x	x	x	x	
	N aromatics		x		x		
Bulk OM (PyTMAH-GC-MS)	*Lignin derivatives*		x	x	x	x	Vegetal OM from fuel
	Benzoic acids derivatives		x	x	x	x	
	PAH		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
	N heterocycles		x		x	x	
	N aromatics				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

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

4.2.Archaeological Potential

4.2.1.N-compounds in Archaeological Contexts

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

4.2.2.N Compound Preservation in Soil

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

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

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

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

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

Acknowledgements

Most of the analyses and data interpretation were conducted in the METIS laboratory (UMR7619 CNRS/Sorbonne Université) and especially its Geochemistry department. We are very grateful to its members for their

constant help and advice and particularly to Sylvie Derenne and Christelle Anquetil. We also thank Véronique Vaury (IEES Paris) for conducting the elementary analyses. The Régismont-le-Haut excavation project is made possible thanks to funding from the French Ministry for Culture and Communication (*Service Régional de l'Archéologie Occitanie*), the Departmental council of Hérault and the Partner University Fund in collaboration with the New York University (Aurignacian Genius project, 2011–2014). We warmly thank the excavation codirector, Romain Mensan, and volunteer excavators that helped us during the experimental sessions. Many thanks as well to the members of the scientific team studying the site for their input, and notably to Farid Sellami, Sandrine Costamagno and Isabelle Théry-Parisot who initiated experimental research on fireplaces at Régismont several years ago.

Many thanks to Erdem Idiz, editor-in-chief of Organic Geochemistry, for his consideration and time, and to two anonymous reviewers for their very helpful comments. The english version of this paper was greatly improved thanks to Elisa Caron Laviolette and Lars Anderson and a funding from TRACES laboratory (UMR5608 CNRS / Université de Toulouse 2).

Competing interests

The authors declare no competing interests.

References

Alexis, M., Rasse, D.P., Knicker, H., Anquetil, C., Rumpel, C., 2012. Evolution of soil organic matter after prescribed fire: A 20-year chronosequence. Geoderma 189–190, 98–107. doi:10.1016/j.geoderma.2012.05.003

601 Anderson, L.E., Lejay, M., Brugal, J.-P., Costamagno, S., Heckel, C., Ingreja de Araujo, M.,
602 Ménard, C., Pradeau, J.-. V., Salomon, H., Sellami, F., Barshay-Szmidt, C., Bon, F.,
603 Mensan, R., Bon, F., 2018. Insights into Aurignacian daily life and camp organization : the
604 open-air site of Régismont-le-Haut. Quaternary International.
605 doi:10.1016/j.quaint.2018.04.034

606 Audouze, F., 1987. Des modèles et des faits : les modèles de A. Leroi-Gourhan et de L.
607 Binford confrontés aux résultats récents. Bulletin de la Société préhistorique française 84,
608 343–352. doi:10.3406/bspf.1987.9848

609 Ayllón, M., Aznar, M., Sánchez, J.L., Gea, G., Arauzo, J., 2006. Influence of temperature
610 and heating rate on the fixed bed pyrolysis of meat and bone meal. Chemical Engineering
611 Journal 121, 85–96. doi:10.1016/j.cej.2006.04.013

612 Azemard, C., de Lumley, H., Khatib, S., Saos, T., Lebeau, D., Aychet, N., Lucas-Lamouroux,
613 C., 2013. Étude des matières organiques du sol d'occupation acheuléen de l'unité
614 archéostratigraphique UA 27 de la grotte du Lazaret. L'Anthropologie 117, 367–412.
615 doi:10.1016/j.anthro.2013.08.002

616 Belitz, H.D., Gross, W., Schieberle, P., 2009. Food Chemistry, 4th edition. ed. Springer-
617 Verlag, Berlin, Heidelberg. doi:10.1007/978-3-40-69934-7

618 Berruti, F.M., Ferrante, L., Briens, C.L., Berruti, F., 2012. Pyrolysis of cohesive meat and
619 bone meal in a bubbling fluidized bed with an intermittent solid slug feeder. Journal of
620 Analytical and Applied Pyrolysis 94, 153–162. doi:10.1016/j.jaap.2011.12.003

621 Beyries, S., Vaté, V., 2007. Les civilisations du renne d'hier et d'aujourd'hui. Approches
622 ethnohistoriques, archéologiques et anthropologiques. APDCA, Antibes.

623 Binford, L.R., 1978. Nunamiut Ethnoarchaeology. Academic Press, New York.

624 Binford, L.R., 1983. In Pursuit of the Past, Decoding the Archaeological Record. Thames &
625 Hudson, London.

626 Bodu, P., Julien, M., Valentin, B., Debout, G., 2006. Un dernier hiver à Pincevent: les
627 Magdaléniens du niveau IVO (Pincevent, La Grande Paroisse, Seine-et-Marne). Gallia
628 préhistoire 48, 1–180.

629 Bon, F., Mensan, R., Anderson, L.E., Lejay, M., Alexis, M., Brugal, J.-P., Costamagno, S.,
630 Heckel, C., Igreja, M., Pradeau, J., Quénéa, K., Salomon, H., Sellami, F., Szmidt, C., Théry-
631 Parisot, I., In press. Un campement résidentiel de plein air aurignacien, Régismont-le-
632 Haut (Hérault, France), in: Bodu, P., Montoya, C., Paris, C. (Eds.), 28e Congrès
633 Préhistorique de France Session N°2 "Palethnologie Du Paléolithique Supérieur Ancien: Où
634 En Sommes Nous ? ", Amiens, 30 Mai - 4 Juin 2016. Société préhistorique française.

635 Braadbaart, F., Poole, I., Huisman, H.D.J., van Os, B., 2012. Fuel, Fire and Heat: an
636 experimental approach to highlight the potential of studying ash and char remains from
637 archaeological contexts. Journal of Archaeological Science 39, 836–847.
638 doi:10.1016/j.jas.2011.10.009

639 Brockbals L., Habicht M., Hajdas I., Galassi F.M., Rühli F.J., Kraemer T. (2018)
640 Untargeted metabolomics-like screening approach for chemical characterization and
641 differentiation of canopic jar and mummy samples from Ancient Egypt using GC-high
642 resolution MS. Analyst 143, 4503-4512. doi: 10.1039/c8an01288a

643 Buonasera, T., 2005. Fatty acid analysis of prehistoric burned rocks: a case study from
644 central California. Journal of Archaeological Science 32, 957–965.
645 doi:10.1016/j.jas.2005.01.012

Buonasera, T., Herrera-Herrera, A.V., Mallol, C., 2019. Experimentally Derived Sedimentary, Molecular, and Isotopic Characteristics of Bone-Fueled Hearths. Journal of Archaeological Method and Theory 1–49. doi:10.1007/s10816-019-09411-3

Buonasera, T., Tremayne, A.H., Darwent, C.M., Eerkens, J.W., Mason, O.K., 2015. Lipid biomarkers and compound specific δ13C analysis indicate early development of a dual-economic system for the Arctic Small Tool tradition in northern Alaska. Journal of Archaeological Science 61, 129–138. doi:10.1016/j.jas.2015.05.011

Canti, M.G., Linford, N., 2000. The effects of fire on archaeological soils and sediments : temperature and colour relationships. Proceedings of the Prehistoric Society 66, 385–396. doi:10.1017/S0079497X00001869

Cascarosa, E., Fonts, I., Mesa, J.M., Sánchez, J.L., Arauzo, J., 2011. Characterization of the liquid and solid products obtained from the oxidative pyrolysis of meat and bone meal in a pilot-scale fluidised bed plant. Fuel Processing Technology 92, 1954–1962. doi:10.1016/j.fuproc.2011.05.015

Chaala, A., Roy, C., 2003. Recycling of meat and bone meal animal feed by vacuum pyrolysis. Environmental Science & Technology 37, 4517–4522. doi:10.1021/es026346m

Chiavari, G., Galletti, G.C., 1992. Pyrolysis—gas chromatography/mass spectrometry of amino acids. Journal of Analytical and Applied Pyrolysis 24, 123–137. doi:10.1016/0165-2370(92)85024-F

Choy, K., Potter, B.A., McKinney, H.J., Reuther, J.D., Wang, S.W., Wooller, M.J., 2016. Chemical profiling of ancient hearths reveals recurrent salmon use in Ice Age Beringia. Proceedings of the National Academy of Sciences 113, 9757–9762. doi:10.1073/pnas.1606219113

Coudret, P., Larrière, M., Valentin, B., 1989. Comparer les foyers : une entreprise difficile, in: Olive, M., Taborin, Y. (Eds.), Nature et Fonction Des Foyers Préhistorique, Nemours, 1987. A. P. R. A. I. F., pp. 37–45.

Cross, A.J., Sinha, R., 2004. Meat-related mutagens/carcinogens in the etiology of colorectal cancer. Environmental and Molecular Mutagenesis 44, 44–55. doi:10.1002/em.20030

Dallongeville, S., Garnier, N., Rolando, C., Tokarski, C., 2016. Proteins in Art, Archaeology, and Paleontology: From Detection to Identification. Chemical Reviews 116, 2–79. doi:10.1021/acs.chemrev.5b00037

De la Rosa, J.M., Faria, S.R., Varela, M.E., Knicker, H., González-Vila, F.J., González-Pérez, J.A., Keizer, J., 2012. Characterization of wildfire effects on soil organic matter using analytical pyrolysis. Geoderma 191, 24–30. doi:10.1016/j.geoderma.2012.01.032

Derenne, S., Quénéa, K., 2015. Analytical pyrolysis as a tool to probe soil organic matter. Journal of Analytical and Applied Pyrolysis 111, 108–120. doi:10.1016/j.jaap.2014.12.001

Dudd, S.N., Regert, M., Evershed, R.P., 1998. Assessing microbial lipid contributions during laboratory degradations of fats and oils and pure triacylglycerols absorbed in ceramic potsherds. Organic Geochemistry 29, 1345–1354. doi:10.1016/S0146-6380(98)00093-X

Dumarçay, G., Lucquin, A., March, R.J., 2008. Cooking and firing, on heated sandstone: an experimental approach by SEM, in: Longo, L., Skakun, N. (Eds.), Prehistoric Technology 40 Years Later : Functional Studies and the Russian Legacy. Verona (Italy), 20-23 April 2005, pp. 345–354.

690 Eglinton, G., Logan, G.A., Ambler, R.P., Boon, J.J., Perizonius, W.R.K., 1991. Molecular
691 Preservation [and Discussion]. Philosophical transactions of the Royal Society of London.
692 Series B, Biological sciences 333, 315–27; discussion 327-8. doi:10.1098/rstb.1991.0081

693 Evershed, R.P., Dudd, S.N., Charters, S., Mottram, H., Stott, A.W., Raven, A., Bergen,
694 P.F.V., Bland, H.A., Jones, M., Bada, J., 1999. Lipids as Carriers of Anthropogenic Signals
695 from Prehistory [and Discussion]. Philosophical transactions: Biological sciences 354, 19–
696 31. doi: 10.1098/rstb.1999.0357

697 Evershed, R.P., Dudd, S.N., Copley, M.S., Berstan, R., Stott, A.W., Mottram, H., Buckley, S.
698 a., Crossman, Z., 2002. Chemistry of Archaeological Animal Fats. Accounts of Chemical
699 Research 35, 660–668. doi:10.1021/ar000200f

700 Evershed, R.P., Stott, A.W., Raven, A., Dudd, S.N., Charters, S., Leyden, A., 1995.
701 Formation of long-chain ketones in ancient pottery vessels by pyrolysis of acyl lipids.
702 Tetrahedron Letters 36, 8875–8878. doi:10.1016/0040-4039(95)01844-8

703 Evershed, R.P., Tuross, N., 1996. Proteinaceous material from potsherds and associated
704 soils. Journal of Archaeological Science 429–436. doi:10.1006/jasc.1996.0038

705 Gallois, N., 2009. Etude des mécanismes de pyrolyse en présence d'hydroxyde de
706 tetramethylammonium de composés protéiques modèles Implication pour la détection de
707 matériel azoté dans la matière organique naturelle et l'identification de macromolécules
708 sources (Unpublished PhD dissertation). Université Pierre et Marie Curie - Paris VI.
709 URL:https://pastel.archives-
710 ouvertes.fr/file/index/docid/501159/filename/These_Nicolas_Gallois.pdf

711 Gallois, N., Templier, J., Derenne, S., 2007. Pyrolysis-gas chromatography-mass
712 spectrometry of the 20 protein amino acids in the presence of TMAH. Journal of Analytical
713 and Applied Pyrolysis 80, 216–230. doi:10.1016/j.jaap.2007.02.010

714 Goldberg, P., Miller, C.E., Mentzer, S.M., 2017. Recognizing Fire in the Paleolithic
715 Archaeological Record. Current Anthropology 58, S175–S190. doi:10.1086/692729

716 Goldberg, P., Miller, C.E., Schiegl, S., Ligouis, B., Berna, F., Conard, N.J., Wadley, L., 2009.
717 Bedding, hearths, and site maintenance in the Middle Stone Age of Sibudu Cave, KwaZulu-
718 Natal, South Africa. Archaeological and Anthropological Sciences 1, 95–122.
719 doi:10.1007/s12520-009-0008-1

720 Harris, D., Horwáth, W.R., van Kessel, C., 2001. Acid fumigation of soils to remove
721 carbonates prior to total organic carbon or CARBON-13 isotopic analysis. Soil Science
722 Society of America Journal 65, 1853–1853. doi:10.2136/sssaj2001.1853

723 Hérisson, D., Locht, J.L., Auguste, P., Tuffreau, A., 2013. Néandertal et le feu au
724 Paléolithique moyen ancien. Tour d'horizon des traces de son utilisation dans le Nord de la
725 France. L'Anthropologie 117, 541–578. doi:10.1016/j.anthro.2013.10.002

726 IUSS Working Group WRB, 2015. World reference base for soil resources 2014, update
727 2015. International soil classification system for naming soils and creating legends for soil
728 maps, World Soil Resources Reports No. 106. FAO, Rome.

729 Julien, M., Karlin, C., Bodu, P., 1987. Pincevent : où en est le modèle théorique
730 aujourd'hui ? Bulletin de la Société préhistorique française 84, 335–342.
731 doi:10.3406/bspf.1987.9847

732 Kaal, J., Brodowski, S., Baldock, J. a., Nierop, K.G.J., Cortizas, A.M., 2008a.
733 Characterisation of aged black carbon using pyrolysis-GC/MS, thermally assisted
734 hydrolysis and methylation (THM), direct and cross-polarisation 13C nuclear magnetic
735 resonance (DP/CP NMR) and the benzenepolycarboxylic acid (BPCA) method. Organic
736 Geochemistry 39, 1415–1426. doi:10.1016/j.orggeochem.2008.06.011

737 Kaal, J., Martínez Cortizas, A., Eckmeier, E., Costa Casais, M., Santos Estévez, M., Criado
738 Boado, F., 2008b. Holocene fire history of black colluvial soils revealed by pyrolysis-GC/MS:
739 a case study from Campo Lameiro (NW Spain). Journal of Archaeological Science 35, 2133–
740 2143. doi:10.1016/j.jas.2008.01.013

741 Kaal, J., Martínez Cortizas, A., Nierop, K.G.J., 2009. Characterisation of aged charcoal
742 using a coil probe pyrolysis-GC/MS method optimised for black carbon. Journal of
743 Analytical and Applied Pyrolysis 85, 408–416. doi:10.1016/j.jaap.2008.11.007

744 Kedrowski, B.L., Crass, B.A., Behm, J.A., Luetke, J.C., Nichols, A.L., Moreck, A.M.,
745 Holmes, C.E., 2009. GC/MS Analysis of Fatty Acids From Ancient Hearth Residues At the
746 Swan Point Archaeological Site. Archaeometry 51, 110–122. doi:10.1111/j.1475-
747 4754.2008.00384.x

748 Kiersch, K., Kruse, J., Eckhardt, K.-U., Fendt, A., Streibel, T., Zimmermann, R., Broll, G.,
749 Leinweber, P., 2012a. Impact of grassland burning on soil organic matter as revealed by a
750 synchrotron- and pyrolysis–mass spectrometry-based multi-methodological approach.
751 Organic Geochemistry 44, 8–20. doi:10.1016/j.orggeochem.2011.12.002

752 Kiersch, K., Kruse, J., Regier, T.Z., Leinweber, P., 2012b. Temperature resolved alteration
753 of soil organic matter composition during laboratory heating as revealed by C and N
754 XANES spectroscopy and Py-FIMS. Thermochimica Acta 537, 36–43.
755 doi:10.1016/j.tca.2012.02.034

756 Knicker, H., 2007. How does fire affect the nature and stability of soil organic nitrogen and
757 carbon? A review. Biogeochemistry 85, 91–118. doi:10.1007/s10533-007-9104-4

758 Knicker, H., 2010. "Black nitrogen" – an important fraction in determining the
759 recalcitrance of charcoal. Organic Geochemistry, Advances in Organic Geochemistry 2009
760 41, 947–950. doi:10.1016/j.orggeochem.2010.04.007

761 Knicker, H., González-Vila, F.J., Polvillo, O., González-Pérez, J.A., Almendros, G., 2005.
762 Fire-induced transformation of C- and N- forms in different organic soil fractions from a
763 Dystric Cambisol under a Mediterranean pine forest (Pinus pinaster). Soil Biology and
764 Biochemistry 37, 701–718. doi:10.1016/j.soilbio.2004.09.008

765 Kögel-Knabner, I., 2002. The macromolecular organic composition of plant and microbial
766 residues as inputs to soil organic matter. Soil Biology and Biochemistry 34, 139–162.
767 doi:10.1016/S0038-0717(01)00158-4

768 Kruse, J., Eckhardt, K.-U., Regier, T., Leinweber, P., 2011. TG–FTIR, LC/MS, XANES and
769 Py-FIMS to disclose the thermal decomposition pathways and aromatic N formation during
770 dipeptide pyrolysis in a soil matrix. Journal of Analytical and Applied Pyrolysis 90, 164–
771 173. doi:10.1016/j.jaap.2010.12.002

772 Leesch, D., Bullinger, J., Cattin, M., Müller, W., Plumettaz, N., 2010. Hearths and hearth-
773 related activities in magdalenian open-air sites: the case studies of champréveyres and
774 monruz (switzerland) and their relevance to an understanding of upper paleolithic site
775 structure, in: Poltowicz-Bobak, M., Bobak, D. (Eds.), The Magdalenian in Central Europe:
776 New Finds an Concepts. Collectio archaeologica ressoviensis, Tomus XI, Rzeszów, pp. 53–
777 69.

778 Leinweber, P., Kruse, J., Baum, C., Arcand, M., Knight, J.D., Farrell, R., Eckhardt, K.U.,
779 Kiersch, K., Jandl, G., 2013. Advances in Understanding Organic Nitrogen Chemistry in
780 Soils Using State-of-the-art Analytical Techniques, in: Sparks, D.L. (Ed.), Advances in
781 Agronomy. Elsevier, pp. 83–151. doi:10.1016/B978-0-12-407247-3.00002-0

782

Leinweber, P., Schulten, H.-R., 2000. Nonhydrolyzable forms of soil organic nitrogen: Extractability and composition. Journal of Plant Nutrition and Soil Science 163, 433–439. doi:10.1002/1522-2624(200008)163:4<433::AID-JPLN433>3.0.CO;2-F

Lejay, M., 2018. Approche archéologique et expérimentale des structures de combustion au Paléolithique supérieur ancien. Analyse multiscalaire (micromorphologie et géochimie organique) appliquée aux sites de Régismont-le-Haut et des Bossats (Unpublished PhD dissertation). Université de Toulouse 2, Toulouse, France. URL:https://hal.archives-ouvertes.fr/tel-01910569

Lejay, M., Alexis, M., Quénéa, K., Sellami, F., Bon, F., 2016. Organic signatures of fireplaces : experimental references for archaeological interpretations. Organic Geochemistry 99, 67–77. doi:10.1016/j.orggeochem.2016.06.002

Lucquin, A., 2007. Étude physico-chimique des méthodes de cuisson pré et protohistoriques (Unpublished PhD dissertation). Université Rennes 1. URL:http://www.theses.fr/2007REN1S101

Malainey, M.E., Przybylski, R., Sherriff, B.L., 1999. The Fatty Acid Composition of Native Food Plants and Animals of Western Canada. Journal of Archaeological Science 26, 83–94. doi:10.1006/jasc.1998.0305

Mallol, C., Henry, A., 2017. Ethnoarchaeology of Paleolithic Fire: Methodological Considerations. Current Anthropology 58, S000–S000. doi:10.1086/691422

Mallol, C., Hernández, C.M., Cabanes, D., Sistiaga Gutiérrez, A., Machado, J., Rodríguez, Á., Pérez, L., Galván, B., Sistiaga, A., Machado, J., Rodríguez, Á., Pérez, L., Galván, B., 2013. The black layer of Middle Palaeolithic combustion structures. Interpretation and archaeostratigraphic implications. Journal of Archaeological Science 40, 2515–2537. doi:10.1016/j.jas.2012.09.017

Mallol, C., Mentzer, S.M., Miller, C.E., 2017. 31-Combustion Features, in: Archaeological Soil and Sediment Micromorphology. Wiley & Sons, pp. 299–330.

March, R.J., 1995. Méthodes physiques et chimiques appliquées à l'étude des structures de combustion préhistoriques. L'approche par la chimie organique (Unpublished PhD dissertation). Université Paris 1, France. URL:http://www.academia.edu/1283524/M%C3%A9thodes_physiques_et_chimiques_appliqu%C3%A9es_%C3%A0_l%C3%A9tude_des_structures_de_combustion_pr%C3%A9historiques

March, R.J., 2013. Searching for the Functions of Fire Structures in Eynan (Mallaha) and their Formation Processes: a Geochemical Approach, in: Bar-Yosef, O., Valla, F. (Eds.), Natufian Foragers in the Levant. Terminal Pleistocene Social Changes in Western Asia. International Monographs in Prehistory 19, Ann-Arbor, pp. 139–145.

March, R.J., Baldessari, A., Gros, E.-G., 1989. Determinacion de compuestos organicos en estructuras de combustion arqueologicas, in: Olive, M., Taborin, Y. (Eds.), Nature et Fonction Des Foyers Préhistorique, Nemours, 1987. A. P. R. A. I. F., Nemours, France, pp. 47–58.

March, R.J., Dorta Perez, R., Sistiaga Gutiérrez, A., Galvan Santos, B., Hernadez Gomez, C.M., 2008. Tras el fuego de los neandertales. Química orgánica aplicada al estudio de las estructuras de combustión del yacimiento musteriense de El Salt (Alcoy, Alicante), in: Rovira Lllorens, S., Garci-Heras, M., Gener Moret, M., Montero Ruiz, I. (Eds.), ACTAS, VII Congreso Ibérico de Arqueometría. Madrid, 8-10 de Octubre 2007. pp. 28–41.

March, R.J., Lucquin, A., Joly, D., Ferreri, J.C., Muhieddine, M., 2014. Processes of Formation and Alteration of Archaeological Fire Structures: Complexity Viewed in the

Light of Experimental Approaches. Journal of Archaeological Method and Theory 21, 1–45. doi:10.1007/s10816-012-9134-7

March, R.J., Whallon, R., Morley, M.W., 2017. Chapter 18 - Studying Neanderthal Fire Structures from Crvena Stijena, in: Whallon, R. (Ed.), Crvena Stijena in Cultural and Ecological Context - Multidisciplinary Archaeological Research in Montenegro. National Museum of Montenegro Montenegrin Academy of Sciences and Arts, pp. 340–449.

Mentzer, S.M., 2014. Microarchaeological Approaches to the Identification and Interpretation of Combustion Features in Prehistoric Archaeological Sites. Journal of Archaeological Method and Theory 21, 616–668. doi:10.1007/s10816-012-9163-2

Mentzer, S.M., 2017. Hearths and combustion features, in: Gilbert, A.S. (Ed.), Encyclopedia of Geoarchaeology, Encyclopedia of Earth Sciences Series. Springer Netherlands, pp. 411–424.

Miller, C.E., Conard, N.J., Goldberg, P., Berna, F., 2010. Dumping, sweeping and trampling: experimental micromorphological analysis of anthropogenically modified combustion features. P@lethnologie 2010.2, 25–37.

Morrison R.I. (1969) Soil Lipids. in: Eglinton G., Murphy M.T.J. (Eds.) Organic Geochemistry. Springer, Berlin, Heidelberg, pp. 558-575. doi:10.1007/978-3-642-87734-6_29

Nawar, W.W., 1969. Thermal decomposition of lipids – a review. Journal of Agricultural and Food Chemistry 17, 18–21.

Nawar, W.W., 1989. Thermal decomposition of lipids An Overview, in: Parliment, T., et al. (Eds.), Thermal Generation of Aromas. ACS Symposium Series, pp. 94–104.

Nieuwenhuyse, O.P., Roffet-Salque, M., Evershed, R.P., Akkermans, P., Russell, A., 2015. Tracing pottery use and the emergence of secondary product exploitation through lipid residue analysis at Late Neolithic Tell Sabi Abyad (Syria). Journal of Archaeological Science 64, 54–66. doi:10.1016/j.jas.2015.10.002

Olive, M., 1997. Foyer domestique ou foyer annexe. Les modes d'occupation de l'espace des Magdaléniens d'Étiolles. Gallia préhistoire 39, 85–107.

Olive, M., Taborin, Y., 1989. Nature et fonction des foyers préhistoriques. Actes du Colloque International de Nemours 1987. A. P. R. A. I. F., Nemours, France.

Oudemans, T.F.M., Boon, J.J., 1991. Molecular archaeology: Analysis of charred (food) remains from prehistoric pottery by pyrolysis—gas chromatography/mass spectrometry. Journal of Analytical and Applied Pyrolysis 20, 197–227. doi:10.1016/0165-2370(91)80073-H

Pérez, L., Sanchis, A., Hernández, C.M., Galván, B., Sala, R., Mallol, C., 2017. Hearths and bones: An experimental study to explore temporality in archaeological contexts based on taphonomical changes in burnt bones. Journal of Archaeological Science: Reports 11, 287–309. doi:10.1016/j.jasrep.2016.11.036

Peters, K.E., Walters, C.C., Moldowan, J.M., 2005. The Biomarker guide, Second Edition. ed. Cambridge University Press.

Pollard, A.M., Batt, C.M., Stern, B., Young, S.M.M., 2007. Analytical Chemistry in Archaeology. Cambridge University Press, Cambridge. doi:10.1017/CBO9780511607431

Quénéa, K., Derenne, S., Largeau, C., Rumpel, C., Mariotti, A., 2005. Spectroscopic and pyrolytic features and abundance of the macromolecular refractory fraction in a sandy acid forest soil (Landes de Gascogne, France). Organic Geochemistry 36, 349–362. doi:10.1016/j.orggeochem.2004.10.005

Quénéa, K., Derenne, S., Largeau, C., Rumpel, C., Mariotti, A., Quenea, K., Derenne, S., Largeau, C., Rumpel, C., Mariotti, A., 2004. Variation in lipid relative abundance and composition among different particle size fractions of a forest soil. Organic Geochemistry 35, 1355–1370. doi:10.1016/j.orggeochem.2004.03.010

Quénéa, K., Mathieu, J., Derenne, S., 2012. Soil lipids from accelerated solvent extraction: Influence of temperature and solvent on extract composition. Organic Geochemistry 44, 45–52. doi:10.1016/j.orggeochem.2011.11.009

Regert, M., Alexandre, V., Thomas, N., Lattuati-Derieux, A., 2006. Molecular characterisation of birch bark tar by headspace solid-phase microextraction gas chromatography-mass spectrometry: a new way for identifying archaeological glues. Journal of chromatography. A 1101, 245–53. doi:10.1016/j.chroma.2005.09.070

Rogge, W.F., Hildemann, L.M., Mazurek, M. a, Cass, G.R., Simoneit, B.R.T., 1991. Sources of Fine Organic Aerosol .1. Charbroilers and Meat Cooking Operations. Environmental Science & Technology 25, 1112–1125. doi:10.1021/es00018a015

Rono, N., Kibet, J., Okanga, F.I., Limo, S.O., 2017. Molecular Products and Particulate Characterization of Emissions from High Temperature Cooking of Goat Meat. Eurasian Journal of Analytical Chemistry 12, 45–59. doi:10.12973/ejac.2017.00153a

Rottländer, R.C.A., 1983. Investigations chimiques sur les graisses en archéologie. Les nouvelles de l'archéologie 11, 38–42.

Rottländer, R.C.A., 1989. Chemische Untersuchungen an Sedimenten der Höhle Geissenklösterle Bei Blaubeuren. Fundberichte Aus Baden-Württemberg 14, 23–32.

Ryba, S.A., Burgess, R.M., 2002. Effects of sample preparation on the measurement of organic carbon, hydrogen, nitrogen, sulfur, and oxygen concentrations in marine sediments. Chemosphere 48, 139–147. doi:10.1016/S0045-6535(02)00027-9

Sandgathe, D.M., Berna, F., 2017. Fire and the Genus Homo. Current Anthropology Vol. 58, suppl.16, the Wenner-Gren Symposium Series, University of Chicago Press.

Sanjurjo-Sánchez, J., Kaal, J., Fenollós, J.L.M., 2018. Organic matter from bevelled rim bowls of the Middle Euphrates: Results from molecular characterization using pyrolysis-GC–MS. Microchemical Journal. doi:10.1016/j.microc.2018.05.001

Schauer, J.J., Kleeman, M.J., Cass, G.R., Simoneit, B.R.T., 1999. Measurement of emissions from air pollution sources. 1. C1 through C29 organic compounds from meat charbroiling. Environmental Science and Technology 33, 1566–1577. doi:10.1021/es980076j

Schauer, J.J., Kleeman, M.J., Cass, G.R., Simoneit, B.R.T., 2001. Measurement of Emissions from Air Pollution Sources. 3. C1−C29 Organic Compounds from Fireplace Combustion of Wood. Environmental Science & Technology 35, 1716–1728. doi:10.1021/es001331e

Schulten, H.-R., Schnitzer, M., 1997. The chemistry of soil organic nitrogen: a review. Biology and Fertility of Soils 26, 1–15. doi:10.1007/s003740050335

Shadkami, F., Helleur, R., 2010. Recent applications in analytical thermochemolysis. Journal of Analytical and Applied Pyrolysis 89, 2–16. doi:10.1016/j.jaap.2010.05.007

Sharma, R.K., Chan, W.G., Seeman, J.I., Hajaligol, M.R., 2003. Formation of low molecular weight heterocyclic and polycyclic aromatic compounds (PACs) in the pyrolysis of α-amino acids. Journal of Analytical and Applied Pyrolysis 66, 97–121. doi:10.1016/S0165-2370(02)00108-0

Sharma, R.K., Chan, W.G., Wang, J., Waymack, B.E., Wooten, J.B., Seeman, J.I., Hajaligol, M.R., 2004. On the role of peptides in the pyrolysis of amino acids. Journal of Analytical and Applied Pyrolysis 72, 153–163. doi:10.1016/j.jaap.2004.03.009

Simoneit, B.R.T., 2002. Biomass burning - A review of organic tracers for smoke from incomplete combustion. Applied Geochemistry 17, 129–162. doi:10.1016/S0883-2927(01)00061-0

Simoneit, B.R.T., 2005. A review of current applications of mass spectrometry for biomarker/molecular tracer elucidation. Mass spectrometry reviews 24, 719–65. doi:10.1002/mas.20036

Simoneit, B.R.T., Rogge, W.F., Lang, Q., Jaffé, R., 2000. Molecular characterization of smoke from campfire burning of pine wood (Pinus elliottii). Chemosphere - Global Change Science 2, 107–122. doi:10.1016/S1465-9972(99)00048-3

Simoneit, B.R.T., Rogge, W.F., Mazurek, M.A., Standley, L.J., Hildemann, L.M., Cass, G.R., 1993. Lignin pyrolysis products, lignans, and resin acids as specific tracers of plant classes in emissions from biomass combustion. Environmental Science and Technology 27, 2533–2541. doi:10.1021/es00048a034

Simoneit, B.R.T., Rushdi, A.I., Abas, M.R.B., Didyk, B.M., 2003. Alkyl amides and nitriles as novel tracers for biomass burning. Environmental Science and Technology 37, 16–21. doi:10.1021/es020811y

Sistiaga Gutiérrez, A., March, R.J., Hernadez Gomez, C.M., Galvan Santos, B., 2010. Aproximancion desde la quimica organica al estudio de los hogares del yacimiento del Paleolítico medio de El Salt (Alicante, España). Recerques del Museu d'Alcoi 20, 47–70.

Stadler, R.H., Lineback, D.R., 2009. Process-induced food toxicants: occurrence, formation, mitigation, and health risks. Hoboken, N.J, Wiley.

Théry-Parisot, I., 2002. Fuel Management (Bone and Wood) During the Lower Aurignacian in the Pataud Rock Shelter (Lower Palaeolithic, Les Eyzies de Tayac, Dordogne, France). Contribution of Experimentation. Journal of Archaeological Science 29, 1415–1421. doi:10.1006/jasc.2001.0781

Théry-Parisot, I., Chabal, L., Costamagno, S., 2010. Taphonomie de la combustion des résidus organiques et des structures de combustion en contexte archéologique, 27-29 mai 2008, Nice. P@lethnologie, Toulouse, France.

Tsuge, S., Matsubara, H., 1985. High-resolution pyrolysis-gas chromatography of proteins and related materials. Journal of Analytical and Applied Pyrolysis 8, 49–64. doi:10.1016/0165-2370(85)80014-0

van Bergen, P.F., Bull, I.D., Poulton, P.R., Evershed, R.P., 1997. Organic geochemical studies of soils from the Rothamsted Classical Experiments—I. Total lipid extracts, solvent insoluble residues and humic acids from Broadbalk Wilderness. Organic Geochemistry, Proceedings of the 17th International Meeting on Organic Geochemistry 26, 117–135. doi:10.1016/S0146-6380(96)00134-9

Van Den Berg, J.D.J., Van Den Berg, K.J., Boon, J.J., 2002. Identification of non-cross-linked compounds in methanolic extracts of cured and aged linseed oil-based paint films using gas chromatography-mass spectrometry. Journal of Chromatography A 950, 195–211. doi:10.1016/S0021-9673(02)00049-3

Walthert, L., Graf, U., Kammer, A., Luster, J., Pezzotta, D., Zimmermann, S., Hagedorn, F., 2010. Determination of organic and inorganic carbon, δ13C, and nitrogen in soils

containing carbonates after acid fumigation with HCl. Journal of Plant Nutrition and Soil Science 173, 207–216. doi:10.1002/jpln.200900158

Wandsnider, L., 1997. The roasted and the boiled: Food composition and heat treatment with special emphasis on pit-hearth cooking. Journal of Anthropological Archaeology 16, 1–48. doi:10.1006/jaar.1997.0303

Wang, J., Simoneit, B.R.T., Sheng, G., Chen, L., Xu, L., Wang, X., Wang, Y., Sun, L., 2017. The Potential of Alkyl Amides as Novel Biomarkers and Their Application to Paleocultural Deposits in China. Scientific Reports 7, 14667–14667. doi:10.1038/s41598-017-15371-z

Captions

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

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

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

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

Figure 1

Click here to download high resolution image

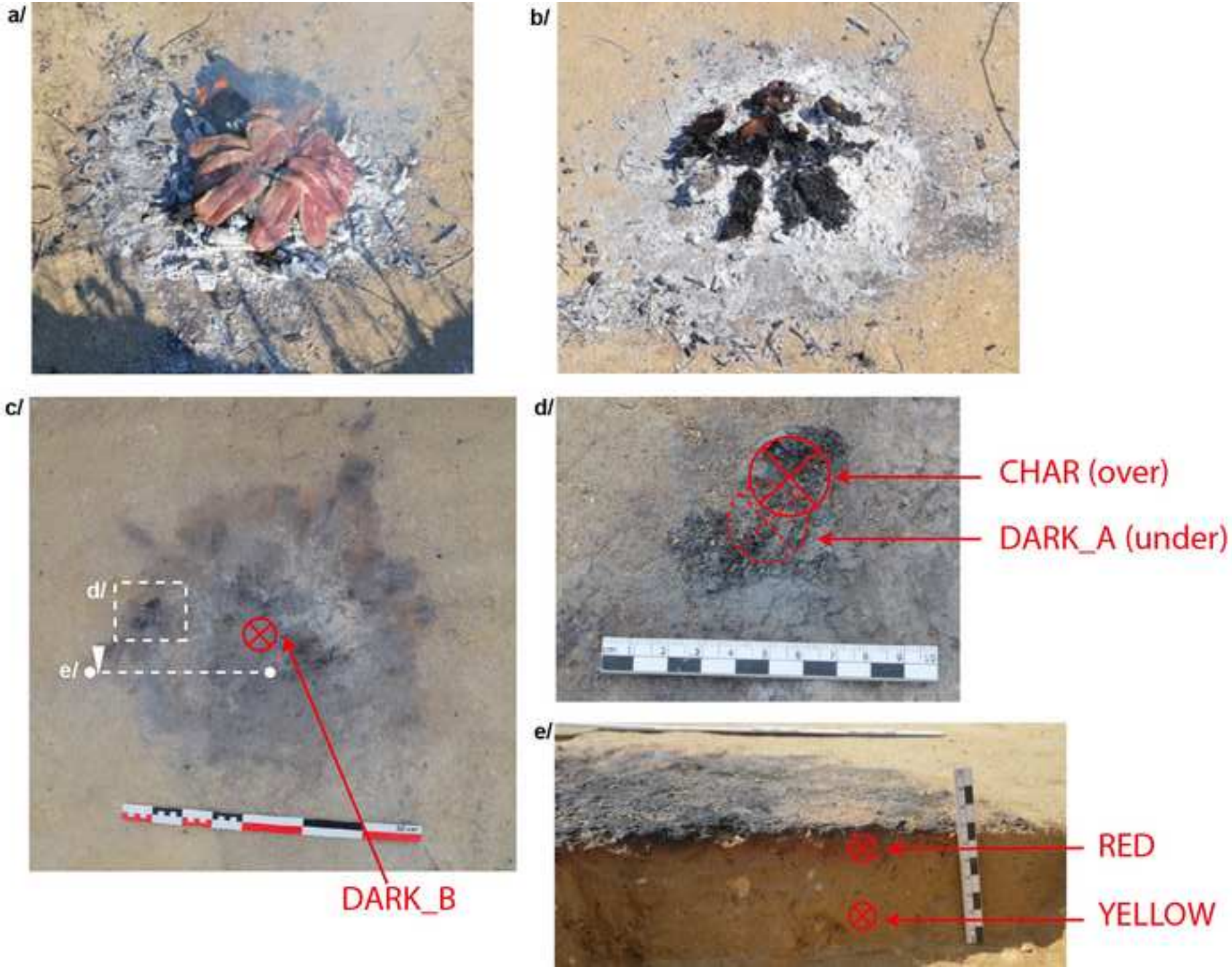


Figure 2

Click here to download high resolution image

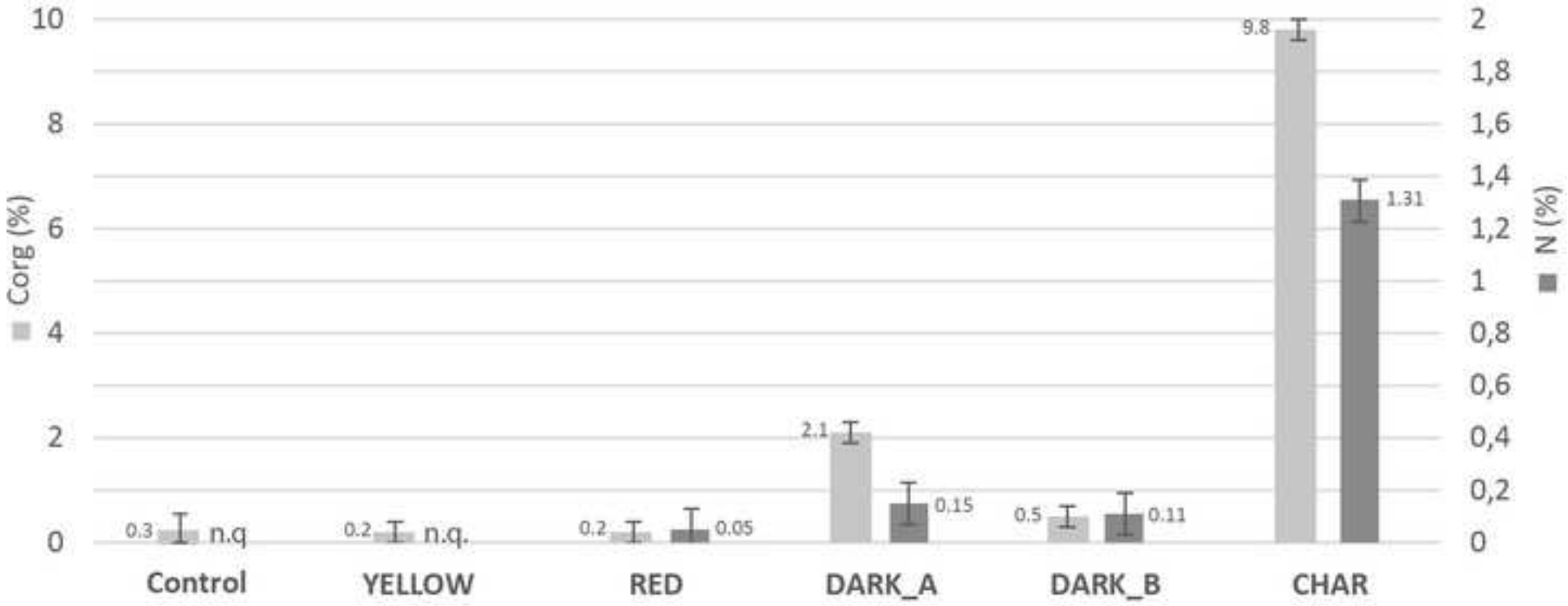
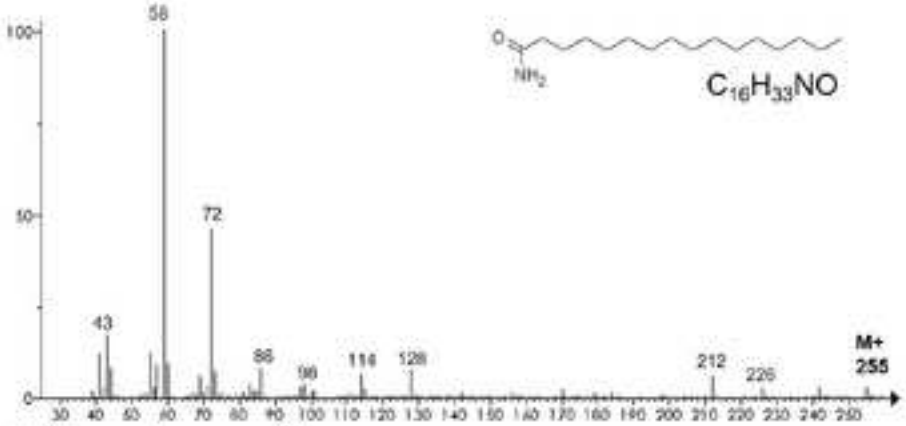
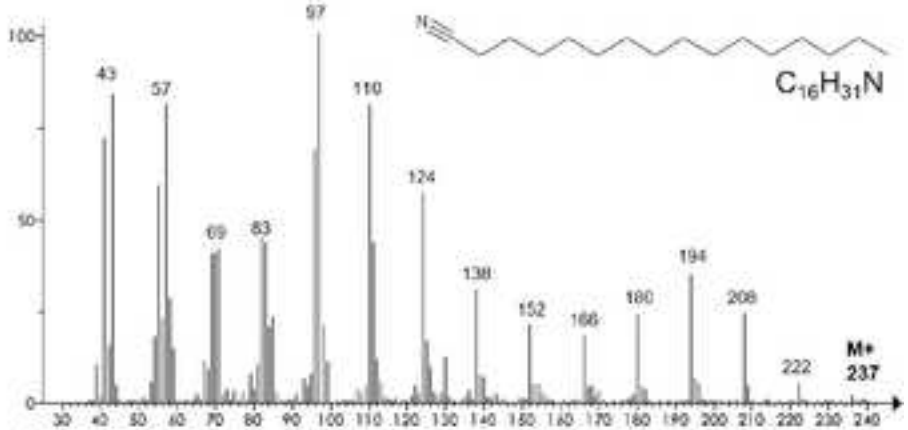


Figure 3

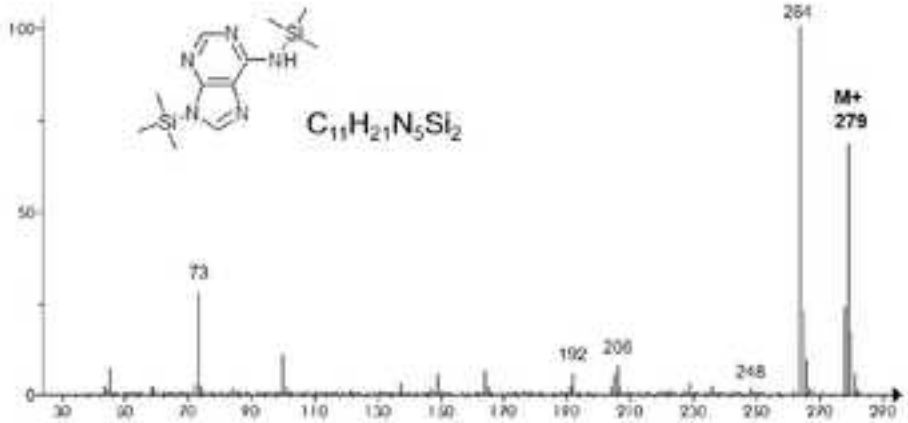
Click here to download high resolution image



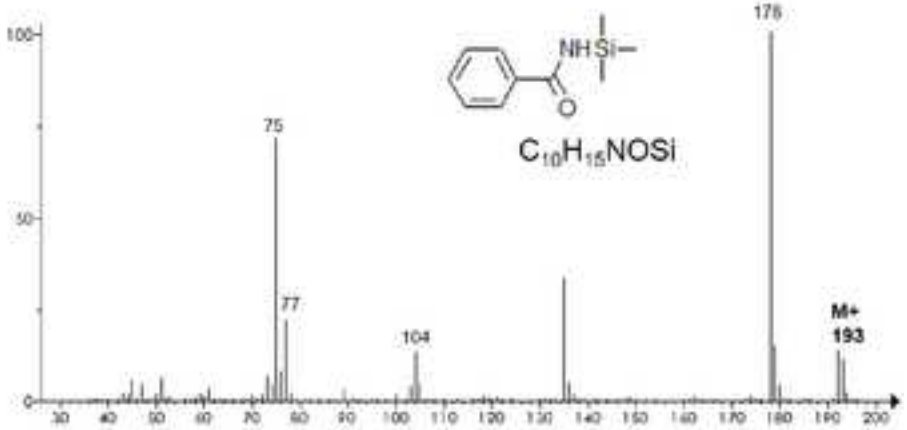
A/ Hexadecanamide



B/ Hexadecanenitrile



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

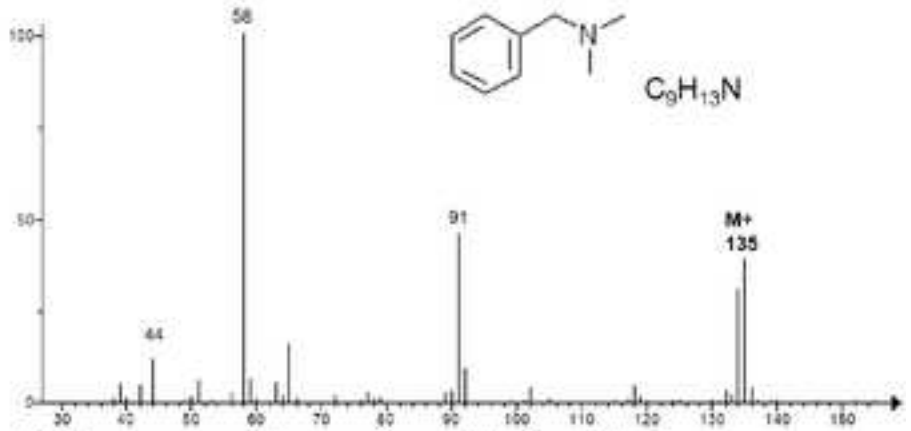


D/ Benzamide, N-(trimethylsilyl)-

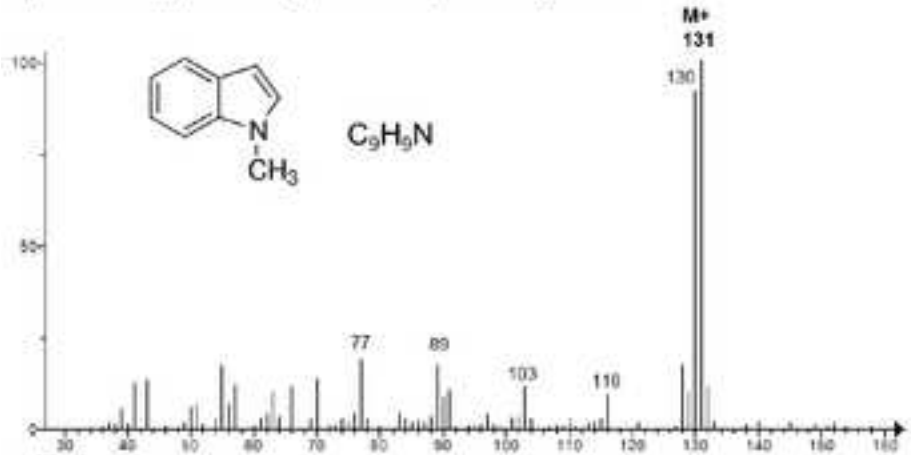
Figure 4

Click here to download high resolution image

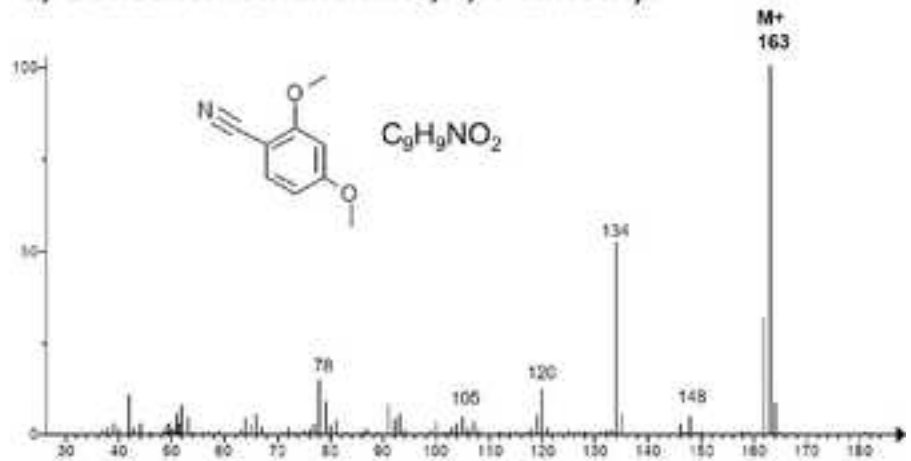
A/ Proline,2 methyl-5 oxo-, methyl ester



B/ Benzenemethanamine,N,N-dimethyl



C/ Methylindole



D/ Benzonitrile, 2,4-dimetoxy

*Response to Reviewers

Mathieu Lejay
GeoArchEon Sarl
UMR5608 TRACES
Université de Toulouse II
5 allée Machado
Toulouse 31058
France
Lejay.m@gmail.com

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

l. 115 - chemical signature of what?

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

l. 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.

l. 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.

l. 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

l. 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.

l. 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.

l. 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 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