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Title: 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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54 - 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; Hérissou 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 (C_{org}) 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-extractible 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 (<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)

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 (Walther 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 N₂ 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; Hrisson 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* ($0.3 \pm 0.3\%$; see Lejay et al., 2016), is low, and similar Corg content was measured in *YELLOW* ($0.2 \pm 0.2\%$). 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* ($0.2 \pm 0.2\%$), indicating no significant input of carbon. The N content of this sample is measurable, although very low ($0.05 \pm 0.08\%$), 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 $2.1 \pm 0.2\%$, $0.5 \pm 0.2\%$ and $9.8 \pm 0.2\%$) and N contents ($0.15 \pm 0.08\%$, $0.11 \pm 0.08\%$ and $1.31 \pm 0.08\%$). 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 diacylglycerols) as well as terpenes and sterols (notably phytosterols) were detected which completed 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 (< C₁₆) saturated FA, and of short (C₅ to C₉) 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 (C₁₀ to C₁₇) *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, C₁₆ and C₁₈ 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	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, benzemethanamine 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

Characterization of N-compounds through analytical pyrolysis in soils and sediments is still a challenging domain of research (Derenne and Quénée, 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 Quénée, 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	YELL OW	RED	DA RK A	DA RK B	CH AR	Possible origin in this study
4	<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

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.

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Competing interests

The authors declare no competing interests.

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972

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
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Figure 2
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Figure 3
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A/ Hexadecanamide

B/ Hexadecanenitrile

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

D/ Benzamide, N-(trimethylsilyl)-

Figure 4
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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 °C/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](#)