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Neanderthal plant use and stone tool function investigated through non-pollen palynomorphs analyses and pollen washes in the Abri du Maras, South-East France.

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Abstract

This pioneering research performed at the Abri du Maras (Ardèche, South-East France) explores the potential of non-pollen palynomorphs (NPP) to provide new insights into the relationships between Neanderthals and plant resources. Remains of endophytic fungi found during the analysis are key indicators of the local presence of plants, which could have potentially been used by Neanderthal groups. The recording of high concentrations of *Chaetomium*-type (HdV-7A) in archaeological contexts from localized areas within the shelter suggests the local presence of decayed plants near an *in situ* fireplace. This could point to the use of grasses or other types of plants from aquatic or marshy areas such as reed and rushes for a surface preparation (bedding?) near the fireplace. Our research has also applied for the first time a combined study of archaeological sediments and pollen washes of artefacts following a microspatial approach in the study of Middle Palaeolithic archaeological contexts. One artefact (1079), identified as a sidescrapper, provided positive results, with the recording of high concentrations of *Diporotheca webbiae*-type (HdV-143). We could identify the host plant of the fungal signature documented on the tool surface as alder roots by comparing archaeobotanical results with NPP analyses in modern surface samples. Following this, we have proposed the use of this Middle Palaeolithic sidescrapper for processing this plant by Neanderthal groups. Marsh and riparian ecosystems were thus attractive for Neanderthals occupying the Abri du Maras. This study clearly shows that NPP must be considered as key taphonomical, environmental and cultural indicators in archaeobotanical analyses. It also underlines the contribution of combined studies of archaeological sediments and pollen washes of artefacts following a microspatial approach to a better understanding of Middle Palaeolithic lithic tools and plant use relationships.

Keywords

Non-pollen palynomorphs; *Diporotheca webbiae*; *Chaetomium*; Pollen washes; Stone tool; Plant use; Neanderthals

1. Introduction

Different studies performed in the last 15 years have demonstrated that the contribution of plants in the Neanderthal diet and culture has been undervalued. In this sense, Shipley and Kindscher (2016) report a large number of plants, which are involved in a wide range of activities or uses: diet (e.g. Lev et al., 2005), technical and fire-making activities (e.g. Hardy, B. et al., 2020; Mazza et al., 2006; Vallverdú et al., 2010; Vidal-Matutano et al., 2015), or medicinal uses (e.g. Hardy, K. et al., 2013). Varied subsistence strategies were already in place with early Neanderthals (Hardy and Moncel, 2011; Hardy, B. et al., 2013).

80 Nevertheless, plant remains are less likely to survive than animal remains in
81 archaeological sites especially in temperate environments where freeze-thaw and
82 moisture promote plant decay. Inferring the presence of plants and their uses by
83 Neanderthal groups requires approaches based on the analysis of a large set of
84 complementary macro- and microfossils. In addition to conventional indicators
85 (botanical macroremains, pollen and phytoliths), starch remains and residues
86 combined to lithic use-wear are nowadays frequently analysed (e.g. Hardy, B.,
87 2004; Venditti et al., 2019) as well as the exploration of new archaeological
88 materials (e.g. dental calculus, Power et al., 2018) or the development of new
89 tools like biomolecular markers (Sistiaga et al., 2014).

90 In the past decade, palynological investigations performed in lakes or mires
91 systematically associate Non-Pollen Palynomorphs (NPP) to the study of pollen in
92 order to obtain refined palaeoenvironmental reconstructions (e.g. Miras et al.,
93 2015; Luelmo-Lautenschlaeger et al., 2018). NPP correspond to different types of
94 resistance or dissemination elements produced by organisms belonging to the 5
95 kingdoms of life (van Geel, 1978). They mainly correspond to fungal spores and
96 phyto- and zooplankton remains. Nevertheless, systematic studies of NPP in
97 archaeological sites are very scarce and often concern protohistorical and
98 historical periods (e.g. Diot, 1991; Florenzano et al., 2019; Nicolas et al., 2012;
99 Revelles et al., 2016; van Geel et al., 2003). The even rarer NPP analyses carried
100 out in Palaeolithic sites (Medeanic and Sapozhnikov, 2008; Expósito et al., 2017;
101 Vinogradova and Burjachs Casas, 2018; Liu et al., 2018) demonstrate the
102 preservation of NPP in older archaeological contexts. The aim of the research
103 presented here and performed on materials from the Middle Palaeolithic site of
104 the Abri du Maras (Ardèche, France) is to explore the potential of these
105 microfossils to investigate plant use by Neanderthals. We will focus on the
106 analysis of endophytic fungi, which develop close relationships with plants as
107 they live within the host tissues and are liberated during host senescence (Abdel-
108 Azeem, 2020). Moreover, the cell walls of fungal spores are mainly composed of
109 chitin and glucans (Deacon, 2006), which makes them particularly resistant to
110 corrosion and oxidation - often more than pollen and peridiphyte spores (van
111 Geel, 1986). In consequence, endophyte fungal spores in archaeological layers
112 could thus provide direct evidence of the local presence of plant remains, which
113 may not have been otherwise preserved.

114 In addition to the study of fungal spores in the Abri du Maras, we present the
115 first application in a Palaeolithic site of the pollen washes technique. Geib and
116 Smith (2008) developed this technique in order to recover botanical microfossils
117 embedded in the porous surface of archaeological artefacts through a series of
118 chemical washes. The purposes are to investigate past plant usage and
119 consumption and to characterize the functionality of artefacts related to plant
120 processing, gathering and storage. Recent improvements of the technique (Miras
121 et al., 2018) allow a more thorough, deeper and precise washing method, which
122 permits the study of smoother and smaller lithic artefacts, such as Middle
123 Palaeolithic stone tools originating from the Abri du Maras in a level dated to 90
124 thousand years. In this paper, we aim to:

- 1) validate NPP as useful bioarchaeological microfossils, especially in Palaeolithic contexts;
- 2) explore the potential of the pollen washes technique to assess plant use and stone tool function for the Palaeolithic period;
- 3) gain an improved understanding of which plant resources Neanderthals used and for doing what;
- 4) obtain fresh insights on subsistence activities and behaviour of Neanderthal groups.

2. Site background

The Abri du Maras (44° 31' 20 "; 4° 56' 28"E) is located in a small valley less than 1 km from the Ardèche River, a tributary of the Rhône River, and close to the Rhône Valley. Its elevation is 170 m a.s.l. and 70 m above the Ardèche River (Fig. 1). Recent excavations (Moncel et al., 2014) revealed three discontinuous sedimentary layers, which result from successive infillings. The current research concerns layer 5, corresponding to a Neanderthal occupation, which took place under a large cave roof that collapsed over time. Based on ESR-U/Th dating (Richard, 2012), layer 5 is dated to the end of the MIS 5/beginning of MIS 4 at c. 90 thousand years. A rich assemblage of lithic remains and fireplaces found in the more than 52 m² of excavated surface suggest that the Abri du Maras was recurrently occupied during the deposition of layer 5.

Layer 5 corresponds to an organic brown level with a sandy-silty matrix, overlying the limestone substratum and covering in part the older layer 6 deposit (Puaud et al., 2015). Layer 5 is composed of two sub-units (upper level 5 and lower level 5, a more stony deposit). Layer 5 is truncated at the front of the site by erosion, and is covered by a sterile level, in discontinuity with disturbed sediments of the overlying layer 4.2 (Moncel et al., 2015). Layer 5 was probably deposited under a large shelter now completely collapsed, before incision during the Holocene. During the deposition of layer 5, the shelter covered both sides of the small valley, whereas now it only covers the right side of this valley. From a palaeoclimatic point of view, these geological data suggests temperate and humid conditions. This is indeed corroborated by ongoing faunal studies. Although reindeer dominates the faunal assemblage, there is an increasing presence of temperate species, which prefer forested conditions such as red deer, roe deer and wild boar (Daujeard et al., 2017). Taphonomical analysis in progress indicates that faunal assemblages were the result of Neanderthal's activities (Marin *in* Moncel, 2019).

2000 well-preserved artefacts have been recovered so far in layer 5 and their vertical distribution shows three main occupations phases (Moncel, 2019). The main density of these artefacts is located in the central and eastern part of the site, under the thick layer of limestone blocks, which originated from the main roof collapse. These blocks were removed during excavations performed between 2017 and 2019 (Fig. 1). Lithic studies are still in progress. Nevertheless, numerous small flint flakes and cores, entire pebbles of various stones and flake-

tools (including scrapers) compose the lithic Middle Palaeolithic assemblage (Moncel and Chacón Navarro *in* Moncel, 2019).

3. Material and methods

3.1. Studied artefacts, sampling and application of the pollen washes technique

Eight stone tools distributed throughout layer 5 were examined and processed using the pollen washes technique (Table 1, Fig. 2). These flints measure between 50 and 70 mm-long and are amongst the largest stone tools of the assemblage. Sediment samples from area immediately surrounding each artefact were collected for NPP analysis. This multiple sample strategy allows us to obtain a study of layer 5 at a high spatial resolution. No disturbances of the sediment (e.g. burrow, den) were noted during excavations. Layer 5 was well-preserved and protected over time by the overlying thick layer of blocks that completely covered the deposit. These blocks were removed just before the excavation and the collection of the stone tools. To reduce the possibility of modern contamination, the artefacts were immediately placed unwashed after excavation in a zip-style plastic bag until the realisation of the pollen washes. The latter took place in the University of Bretagne Sud (Geosciences Ocean, UMR 6538-CNRS, Vannes, France) following methods detailed in Miras et al., (2018). Sediment adhered to the artefact surface – called “sticking sediment” – was carefully collected (Fig. 2) and analysed separately. In order to retrieve biological microfossils embedded in the artefact surface’s pores and vesicles, stone tools were subsequently entirely washed using a high-pressure (between 3 and 4 bar) turbine spray gun machine (Fig. 2). This was filled with a cleaning solution based on hot distilled water and 5% dimethyl sulphoxide, a solvent and penetrant. A total of 24 samples comprising sediment samples from layer 5 in the vicinity of the artefacts, sticking sediments samples, and pollen washes samples were collected and correspond to the “archaeological sample assemblage” (see full description in Table 1).

Twelve modern control samples were also collected outside the shelter to monitor possible natural deposition of fungal spores in the shelter and to better identify fungal spore’s plant hosts. They correspond to the “modern control samples assemblage” (see detailed description in Table 2). Different contexts were selected for the modern control sample, including:

- mosses collected in the immediate vicinity of the shelter to monitor potential fungal aerial deposition/inputs to the site,
- soil surfaces in the immediate area surrounding the shelter to evaluate potential fungal contamination of the site by run-off processes,
- samples resulting both from a combination of soil samples and plant roots/rhizome and from exclusively roots/rhizome fragments to investigate possible plant host of the fungi evidenced in the shelter. Alder (*Alnus*) carrs (waterlogged wooded terrains populated with alder tress) were specifically sampled as *Diporotheca webbiae* D. Hawksworth., B. van Geel

et P. Wiltshire (NPP-type: HdV-143, van Geel et al., 1989; Hawksworth et al., 2016) were observed in fossil samples restricted to pollen zones interpreted as alder carr in previous research (Barthelmes et al., 2006; van der Wiel, 1982). Populations of *Thelypteris palustris* Schott. were also sampled as this fern is suspected to be the host of *Diporotheca webbiae* (Hawksworth et al., 2016). In the latter case, sampling was carried out in 2 of the 7 remaining populations of marsh fern situated in Ardèche.

The comparison between the archaeological and control samples allows the detection of microfossil enrichment, which could indicate potential anthropogenic botanical microfossils deposition related to plant use in specific archaeological microcontexts (Riera et al., 2018).

3.2. Non-pollen palynomorphs analysis

Modern control and archaeological samples were processed following a modified standard palynological procedure (Faegri and Iversen, 1989) at the GEOLAB UMR 6042 CNRS laboratory in Clermont-Ferrand, France). Samples were treated with hydrochloric and hydrofluoric acids and then sieved through a 0.20mm mesh and centrifuged. Samples were subsequently treated with heavy liquid separation using zinc chloride. Acetolysis was not conducted to avoid potential deterioration of fungal material, as demonstrated by recent experimental studies (Perrotti and van Asperen, 2019). A drop of safranin was added to stain the microfossils. *Lycopodium* tablets were added to allow the calculation of microfossils concentrations (Stockmarr, 1971). After processing, samples were suspended in glycerine jelly prior to being mounted on slides.

Fungal spore quantification was undertaken at the HNHP (UMR 7194 CNRS, Muséum national d'Histoire naturelle, Paris) using a light ZEISS microscope at $\times 600$ and $\times 1000$ magnification). Mean values of 350 *Lycopodium* spores were counted, which is sufficient to calculate significant concentrations of fungal spores (Etienne and Jouffroy-Bapicot, 2014). Fungal spores were identified using morphological keys and atlas (e.g., Cugny, 2011; van Geel, 1989). Results are expressed in number of elements.g⁻¹. Following Geib and Smith (2008), we also expressed the results of pollen washes samples as pollen concentrations per unit of artefact-surface area (denoted as elements.cm⁻²).

4. Results

4.1. General results

Concentration and preservation - Amongst the eight studied stone tools, only object n°1079 (square N6) gave significant positive results. This confirms the poor preservation of botanical microfossils in the Abri du Maras (Table 1). Concentrations of pollen and pteridophyte spores recovered from the archaeological samples are negligible in samples S13, S14 and S15 (median values around 60 elements.g⁻¹), and almost nil for the other samples originating from layer 5. This poor preservation can probably be explained by the sandy-silty composition of the deposit. The preponderance of coarse sediment may have

indeed enhanced oxidation of pollen and spores (Faegri and Iversen, 1989). A similar poor preservation was previously observed for the overlying layer 4 in the Abri du Maras (Moncel et al., 2015). By contrast, fungal spores have been more regularly observed in archaeological samples, ratifying their higher ability to be preserved than pollen in archaeological sediments (van Geel, 1986). Nevertheless, only object n°1079 (S14 and S15) and its surrounding sediment (S13) yielded statistically significant fungal assemblages with specific morphotypes (Table 1 and Fig. 3 and 4). This could be explained by a local accumulation of organic matter in this area. It is noteworthy that a fireplace was found very close (1 m distant) to the stone tool (square N7, x=37, y=72, z=414), which supports better organic preservation in this sector (Fig. 1). The record of 2 specific fungal morphotypes in this area seems to argue against a background fungal signature in the whole shelter, allowing us to associate the fungal features obtained on object 1079 and its nearby sediment sample to their microarchaeological context.

Pollen, spore and fungi preservation are generally good in the modern control samples except in those corresponding to roots or rhizomes fragments: S32, S34, S36. Despite low fungal concentration, the latter nevertheless provide interesting qualitative information (Table 2, Fig. 5 and 6). The other control samples are mainly characterized by high pollen concentration with mean and median values of 334136 and 53710 elements.g⁻¹, respectively. Undifferentiated fungal spores also show high abundances (mean and median concentrations of 42656 and 27055 elements.g⁻¹, respectively).

Diversity and prevalent morphotypes – 7 different morphotypes have been evidenced in the archaeological samples from object 1079 while 36 morphotypes characterized modern control samples. In the archaeological samples, NPP assemblages are largely dominated by fungi, and specifically by *Chaetomium*-type (HdV-7A) and *Diporotheca webbiae*-type (HdV-143). Some hyaline microfossils have also been punctually recorded at low values. HdV-984 is similar to zygospores of *Euastrum*-t (Carrión and van Geel, 1999), corresponding to the green algae *Euastrum* Ehrenberg ex Ralfs 1848, Desmidiaceae. HdV-989 is of unknown origin (Carrión and van Geel, 1999). In the control samples, fungi also largely prevail in the NPP assemblage. The number of fungal taxa is high and have different ecological affinities. The predominant taxa are first saprobic or parasitic fungi such as *Brachydesmiella*-type HdV-1005, *Glomus*-type HdV-207, *Microthyrium* Desm-type HdV-8B, *Gaeumannomyces*-type HdV-126, *Diporotheca webbiae*-type HdV-143, *Dictyosporium*-type TM-4093 and *Triposporium elegans*-type TM-M1. According to the classification of Perrotti and van Asperen (2019), other taxa observed are coprophilous fungi among which the most represented are *Sporormiella*-type (HdV-113), *Delitschia*-type (TM-23), *Sordaria*-type (HdV-55), *Arnium*-type (HdV-261) and *Cercophora*-type (HdV-112). Other abundant registered fungal taxa such as *Curvularia*-type HdV-1029, Phragmoconidies or TM-030 are more ubiquitous. Finally, low concentrations of algal spores such as *Spirogyra*-type HdV-315 and *Pseudoschizaea*-type, faunal remains such as Turbellaria, *Assulina*-type HdV-32, *Arcella*-type HdV-352 and *Macrobiotus hufelandi*-type, as well as and Bryophyta spores are also present.

304

305 **4.2. Spatial dimension: site vs off-site samples**

306 Through the application of the pollen washes technique, we obtained a
 307 microtransect sampling going from the sediment immediately surrounding the
 308 artefact (S13) until to the artefact itself (S14 and S15). This microtransect allows
 309 us to discriminate 2 different dominant taxa of the fungal spectrum between the
 310 stratigraphic unit and the tool (Fig. 3). A decreasing trend in *Chaetomium*-type
 311 (HdV-7A) concentrations is evidenced along this transect. High concentrations of
 312 *Chaetomium*-type characterized the stratigraphic unit (S13: c. 2000 elements.g⁻¹),
 313 while only rare occurrences were encountered in the sticking sediment (S14:
 314 43 elements.g⁻¹). The abundance of this fungus is not observed after the gun
 315 wash of the artefact (S15). *Chaetomium*-type appears thus to be directly related
 316 to the sediment of the archaeological layer 5. Conversely, values of *Diporotheca*
 317 *webbiae*-type (HdV-143) present an increasing trend from low values in the
 318 stratigraphic unit (S13: 341 elements.g⁻¹) to high concentrations in the tools
 319 samples (S14: 9361 elements.g⁻¹; S15: 2704 elements.g⁻¹). The area
 320 immediately surrounding a stone tool generally offers good preservation at the
 321 microscopic fragments, which can be associated with the artefact (Hardy, B. et
 322 al., 2020). This could explain the small amounts of *Diporotheca webbiae*-type
 323 recorded in the archaeological layer. Moreover, samples from artefact 1079
 324 register noticeable concentrations of aggregates of *Diporotheca webbiae*-type,
 325 with increasing values between the sticking sediment (S14: 280 elements.g⁻¹)
 326 and the artefact itself (S15: 375 elements.g⁻¹). The largest aggregates (between
 327 5 and 15 ascospores stuck together) come from the stone tool washes (S15). All
 328 this suggest that *Diporotheca webbiae*-type is directly related to the lithic object.
 329 Values expressed in concentration per unit of artefact-surface confirm that the
 330 stone tool n°1079 is strictly defined by high amounts of *Diporotheca webbiae*-
 331 type, both in ascospores – 283 and 56 elements.cm⁻² - and aggregates – around
 332 8 elements.cm⁻² in samples S14 and S15, respectively, Fig. 4). The object
 333 constitutes thus the archaeological microcontext of this fungus.

334 In the control samples (Fig. 5 and 6), *Chaetomium*-type and *Diporotheca*
 335 *webbiae*-type were only found at negligible values in respectively one of the 2
 336 sampling locations situated just outside the shelter (*Chaetomium*-type, S25: 62
 337 elements.g⁻¹, *Diporotheca webbiae*-type, S26: 24 elements.g⁻¹). NPP
 338 assemblages are moreover very different in the sediment samples inside (S13)
 339 and outside the archaeological site (S25, S26). In the control soil-surface
 340 samples, the composition of NPP is very diverse and mainly dominated by
 341 morphotypes which were not evidenced in the archaeological layer (S25
 342 dominant taxa- spores of Bryophyta: 17760, Pseudoschizaea: 1603,
 343 Phragmoconidies: 1480 elements.g⁻¹, *Microthyrium* Desm-type HdV-8B: 493;
 344 S26 dominant taxa- *Triposporium elegans*-type TM-M1: 1243, *Dictyosporium*-
 345 type TM-4093: 853, *Gaeumannomyces*-type HdV-126: 755, *Microthyrium* Desm-
 346 type HdV-8B: 585, *Assulina*-type HdV-32: 390, *Sphaeroides* cf. *fimicola*-type TM-
 347 20: 366, Pseudoschizaea: 317 elements.g⁻¹) or at lower frequencies (*Glomus*-
 348 type HdV-207- S13: 214, S14: 151, S15: 19, S25: 3761, S26: 731 elements.g⁻¹).
 349 Similar discrepancies are also evidenced between the archaeological samples

and the moss samples which are mainly characterized by taxa unregistered at the site: S27- Phragmoconidies: 7785, *Arcella*-type HdV-352: 4933; S28- Phragmoconidies: 89712, *Triposporium elegans*-type TM-M1: 906, *Cercophora*-type HdV-112: 906, Sordariaceae: 453 elements.g⁻¹. The lack of both *Chaetomium*-type and *Diporotheca webbiae*-type in the control samples rules out natural inputs of both fungal spores into the shelter due to run-off processes or by wind. Once again, sampling in the shelter was carried out immediately after the removal of the collapsed stone slabs covering layer 5 during the excavations performed between 2017 and 2019. All this strengthens the local presence of these 2 fungi inside the archaeological site.

It is noteworthy that substantial quantities of *Diporotheca webbiae*-type were observed in the samples concerning litter and roots from of an *Alnus glutinosa* (black alder) population located on a wet habitat of the Ardèche riverbank (S29 with 4406 elements.g⁻¹ and S30 with 9666 elements.g⁻¹). These concentrations are similar to the values obtained in the stone tool samples (S14 and S15). Moreover, sample S30 also contains some juvenile ascospores of *Diporotheca webbiae*-type (Fig. 7), described by Hawksworth et al. (2016). The absence of *Diporotheca webbiae*-type ascospores in samples S31 and S32, which also correspond to alder litter and roots may be explained by drier conditions of these locations in comparison to sample S29. This is supported by higher rates of *Brachydesmiella*-type HdV-1005 documented in S31 (7833 elements.g⁻¹) than in S29 (110 elements.g⁻¹). *Brachydesmiella*-type is indeed a saprobe fungus on dead and decaying leaves and wood, sometimes partially submerged (Gelorini et al., 2011). Secondly, this is coherent with higher rates of *Glomus*-type HdV-207 in S31 (2843 elements.g⁻¹) than in S29 (330 elements.g⁻¹). *Glomus*-type – probably *Glomus* cf. *fasciculatum* – is a mycorrhizal fungus in association with different plants, and especially Cyperaceae (sedges) (Pals et al., 1980), which prefer humid to seasonally flooded areas. All this suggests that *Diporotheca webbiae*-type is more common in more permanently submerged parasitized alder roots. Finally, only low concentrations of ascospores of *Diporotheca webbiae*-type were found in sample S35 (401 elements.g⁻¹), originating from populations of marsh fern (*Thelypteris palustris*). Zero or trace concentrations were evidenced in rhizome samples S34 and 36.

5. Discussion

5.1. Assessing Neanderthal plant-related activities in the Abri du Maras

Our data, combined with the few existing studies of Palaeolithic Non-Pollen-Palynomorphs (NPP) (Medeanic and Sapozhnikov, 2008; Expósito et al., 2017; Vinogradova and Burjachs Casas, 2018; Liu et al., 2018), validate their potential for identifying organic matter accumulations both in Pleistocene sediments and in Palaeolithic archaeological sites. The better preservation of NPP, mainly fungal spores, compared to pollen and fern spores, urges the systematic study of NPP in archaeological deposits. These pioneering studies explored the contribution of these microfossils to a better knowledge of the different processes (geological, biological, physical and chemical) that resulted in the archaeological deposits,

and successfully laid the groundwork for their use as environmental indicators. Our research is more focused on exploring the potential use of NPP, especially endophyte fungal spores, as a means of detecting and better understanding the presence of plants in Neanderthal sites and their possible uses. In this sense, the data discussed here aim thus to validate these palynomorphs as cultural indicators thanks to the microsampling method and the pollen washes technique.

High contents of *Chaetomium*-type (HdV-7A) were recorded in the square N6 of layer 5, in the immediate vicinity of stone tool n° 1079 (sample S13, Fig. 3 and 7). Chaetomiaceae G. Winter is a family of saprobic or parasitic fungi, growing on plant debris, dung, soils and different other substrates (Maharahchikumbura et al., 2016). *Chaetomium*-type is thus a cosmopolitan fungus, which can also be found in soils and in the air and that could be naturally transported into the shelter through airborne or erosive inputs. Nevertheless, the near absence of *Chaetomium*-type in the area immediately surrounding the Abri du Maras (samples S25 to S28, Fig. 5) and its confined observation at high values in a specific sector of layer 5 lead us to suggest that it indicates the local presence of decayed plant materials in that sector. Following this, *Chaetomium* species are mainly non-clavicipitaceous endophytes, found in the tissues of bryophytes, ferns, gymnosperms and angiosperms (Abdel-Azeem, 2020). They are cellulose decomposing fungi and occur wherever the substrate, most particularly plant remains like damp straw, is abundant (van Geel, 2001). The *Chaetomium*-type (HdV-7A) observed in the Abri du Maras is a lemon-shaped dark-brown spore with dimensions close to *Chaetomium globosum*-type. According to Chivers (1915), this is a very common species, which often occurs on dead stalks and leaves, especially from plants in damp wet places. In this sense, van Geel et al., (1989) and Sánchez Márquez et al. (2010) recorded many *Chaetomium*-type spores in samples from an initial sedge fen and leaves of *Holcus lanatus*, a grass that grows in humid soils in temperate zones. Moreover, *Chaetomium*-type seems to prefer decomposing aerial plant components and does not colonize roots of aquatic plant species (Fisher et al., 1991; Sánchez Márquez et al., 2010). This makes very unlikely that the observed *Chaetomium*-type is due to post-depositional developments from roots infiltrations. A local presence of decayed aerial plant materials possibly originating from wet places may explain this high amount of fungal spores in the archaeological sediments. An anthropogenic cause could also explain the localised presence of these spores in the shelter. In the Abri du Maras, *Chaetomium*-type spores were found nearby a fireplace *in situ* probably corresponding to the same occupation of the shelter (Moncel, 2019, Fig. 1). A close spatial co-occurrence between hearths and preparation of the living floor has been previously reported from some Middle Palaeolithic sites (Cabanès et al., 2010; Henry et al., 2004; Vallverdú et al., 2010; Wroth et al., 2019). It is thus probable that this area in the Abri du Maras had surface preparation such as bedding using grasses or other types of plants, possibly from nearby wet areas such as reed and rushes. It is noteworthy that analyses of residues carried out in the Abri du Maras show that Neanderthals exploited a wide range of plant species for diverse activities in the shelter, including the manufacture of string and cordage during MIS 3 (layer 4, Hardy, B. et al., 2013 and 2020). Some of the fibres discovered likely derive from reeds, rushes, or similar plants (Hardy, B. et al., 2013). Neanderthal groups from the

Abri du Maras may have had a special interest in plants from damp places between the end of MIS 5 and the beginning of the MIS 4.

Another possible hypothesis explaining these high amounts of *Chaetomium*-type is the local presence of dung. *Chaetomium* is not a strict coprophilous but rather a fimiculous fungi, for which dung is only one of the suitable substrates where they can develop (Abdel-Azeem, 2020). The proximity of dung to the hearth could indicate the use of animal dung as fuel but this practice remains uncertain for Neanderthal groups (Henry, 2007). Moreover, the presence of other coprophilous fungi may have been expected in S13 if dung was locally present in the area, which is not the case. All this makes unlikely that the presence of *Chaetomium*-type is related to dung.

5.2. New insights into the characterization of a potential plant-related Neanderthal stone tool

Neanderthals collected plants for different purposes, such as food, fuel, bedding and other constructive or technical activities (Riehl et al., 2015). The contribution of plants in Neanderthal behaviour and subsistence is a pressing question, which can be addressed by analysing stone tools. Botanical microfossils adhered and embedded into the artefact surface can indeed be sensitive indicators of plant past use. By analysing the pollen and – in the case of the Abri du Maras study, the non-pollen palynomorphs (NPP) - embedded in the porous surface of archaeological artefacts (microporosity in the case of flint tools), the pollen washes technique can contribute to identify lithic artefacts associated with plant processing (Miras et al., 2018). This initial application of the pollen washes technique on Middle Palaeolithic lithic materials gave only one positive result from eight objects, but did yield positive results even with the small sample size.

A striking NPP signature has been evidenced in the analysis of the object n°1079. This artefact is a backed flake with a scraper on the cutting edge opposite to the back. It is a small tool measuring 72 mm long, 66 mm wide and 27 mm thick (Fig. 8). The fact that high concentrations of *Diporotheca webbiae*-type (HdV-143, samples S14 and S15, Fig. 3 and 7) are almost exclusively found in the tool's surface sticking sediment (S14) and in the washes sample (S15) suggests direct exposure of the tool with this fungus. Moreover, the fact that substantial values of aggregates of this ascospores are documented in these two samples, together with both increased concentrations of clumps and larger clumps in S15, obtained after the gun wash of the tool surface, confirm that ascospores were embedded in the fine-grained structure of the tool. Ascospores of many fungi tend to stick together during their formation in the perithecia or when they are released from the fruit-body (van Asperen et al., 2016). Large aggregates are heavy and have a lower dispersal ability than single spores. Moreover, the near absence of *Diporotheca webbiae*-type in the modern control samples (S25 to S28, Fig. 5) allows us to dismiss a natural aerial or water source of these ascospores. Finally, artefacts were immediately collected and bagged from layer 5 just after the removal of the stone slabs, which rules out modern contamination. All these data imply that the stone tool was in direct contact with

the fruit body of *Diporotheca webbiae*-type. Diporothecaceae, a family of Ascomycetes described in 1995 by Mibey and Hawksworth (1995), are root colonizers or parasites of different vascular plants. As perithecia develop within and on the plant roots surface, only repeated and direct contact of the tool with the infected plant parts can explain the embedded presence of this fungus in the tool surface.

The ecology of *Diporotheca webbiae* (Hawksworth et al., 2016) is unfortunately poorly known, but this fungus especially occurs in mosses, soils and sediment from peat deposits or alder carrs (Barthelmes et al., 2006), where *Thelypteris palustris* is abundant. This led previous authors to consider this marsh fern as the plant host (van Geel et al., 1986). Control samples S29, 30, 33 and 35 (Fig. 6) clearly show that alder is a better candidate host plant. Samples recovered from *Alnus glutinosa* litter and roots show high concentrations of *Diporotheca webbiae*-type, which resemble those obtained by the stone tool wash (concentrations above several thousand elements.g⁻¹ in any case). Fungal assemblages also include immature ascospores of *Diporotheca webbiae*-type, which indicate the presence of perithecia on the infected roots (Fig. 7). It is noteworthy that Hillbrand et al. (2012) found a positive correlation between *Diporotheca webbiae*-type and *Alnus* macrofossils in the palaeoenvironmental sequence of Lake Nussbaumersee, in Switzerland. Conversely, low concentrations of the ascospore retrieved from fern samples (S33 and S35) tends to dismiss the marsh fern as the main plant host. Based on the results obtained in the modern control samples, the striking NPP feature in the tool samples is the fungal imprint of the contact between the stone tool and infected alder roots.

Before exploring several hypotheses to explain the contact of alder roots with the tool, modern contaminations need to be ruled out. The fact that alder is the host plant dismisses any possible modern contamination, as present-day dry environmental conditions do not allow any development of alder populations in the immediate vicinity of the shelter. A riparian or marsh vegetation including alder stands could have existed or developed in the surrounding of the site after the tool deposition. In this case, infected roots could have naturally enriched the artefact. Without being ignored, this assumption seems unlikely. If this was the case, high concentrations of *Diporotheca webbiae*-type may also be expected in the sediment sample from the microenvironmental context of the tool (S13). However, the bioarchaeological microsampling method followed with the pollen washes technique clearly shows that the archaeological sediment sampled in the vicinity of the stone tool (S13) only contains low values of *Diporotheca webbiae*-type which are between ten- and thirty-fold lower than those obtained in the tool's surface sticking sediment (S14). This low amount of *Diporotheca webbiae*-type in the immediate surrounding of the tool is consistent with the observation that the microenvironment of an artefact generally preserves microscopic elements related to it (Hardy, B. et al., 2020). A natural preferential accumulation of fungal spores near or beneath the tool as a result of taphonomic processes can also be dismissed as no significant increases of undifferentiated fungi are evidenced between the stratigraphic unit (S13) and the tool (S14 and 15) samples, and other fungi such as *Chaetomium*-type or *Glomus*-type

noticeably decline. Moreover, the multiple samples in layer 5 and the observed absence of *Diporotheca webbiae*-type in the sediment samples collected allows us to certify that no background signature of this NPP exists in this layer. The potential infiltration of infected alder roots in the proximity of the tool after the artefact was deposited due to the development of alder populations in the vicinity of the shelter, would have resulted in a more uniform dispersion of *Diporotheca webbiae*-type within layer 5. Results obtained from samples in direct contact with the artefact surface (S14 and 15) clearly associate high concentrations of *Diporotheca webbiae*-type with the tool, which suggests that the fungal signature may result from the use of the tool. Processing alder roots implies repeated and long-lasting contacts with the tool, which could explain the important recovery of Diporothecaceae in the embedded artefact surface (S14 and S15).

5.3. Uses of alder roots and attractiveness of damp environments

As documented in the ethnographic and historical literature, alder roots have been widely used by past societies, and could indeed have been a targeted plant resource by Neanderthal groups. Alder is a well-known medicinal plant and different parts of the plant were employed: leaves to treat fever as an alternative of using cinchona (Ratier, 1827), common alder (*Alnus glutinosa*) bark to treat swelling, inflammation and rheumatism (Sati et al., 2011) and roots. For instance, a mixture of common alder roots with fennel and burdock was traditionally recommended for the treatment of tremors (Buchoz, 1764), and roots of *Alnus nepalensis* are prescribed in the present-day to treat diarrhoea (Sati et al., 2011). A mixture of grey alder (*Alnus incana*) roots was used until recently in the northern Midwestern United States by the Chippewa women to facilitate difficult childbirth (Nesom, 2003). Alder roots are also rich in tannins extracted for dyeing. Technological purposes can also be possible as ethnographic studies show that Californian native groups (Mason, 1988) used red alder (*Alnus rubra* Bong. 1832) in baskets. Furthermore, roots of many trees, including alder and various conifers can be used for making cordage (Hurcombe, 2014). This is of particular interest as residue analyses in the Abri du Maras demonstrate that Neanderthal groups manufactured string and cordage (Hardy, B. et al., 2020) during MIS 3. Some artifacts exhibited fibers, which likely derive from reeds, rushes, or similar plants (Hardy, B. et al., 2013). These plants share similar habitats with alder, as birch (*Betula*) charcoals fragments documented in layer 5 (square M8). It is noteworthy that riverside wood is not frequently recorded among firewood remains when other more suitable and abundant woods like pine were available (Allué et al., 2017). If all these data are considered altogether, it appears that marsh and riparian ecosystems were attractive for Neanderthal groups occupying the shelter. Our results are in good agreement with those obtained by Hardy, B. et al., 2013, and confirm that Neanderthal groups of the Abri du Maras had precise knowledge of their surrounding environment and located appropriate plants for specific purposes. It is noteworthy to mention that alder roots developed nodules - a symbiotic relationship with nitrogen-fixing bacteria, mainly the genus *Frankia* (Pojar and Mackinnon, 1994). These nodules accumulate starch and soluble sugars (Wheeler

et al., 1983), which could enhance the potential interest of alder roots by Palaeolithic groups. In the Abric Romaní Middle Palaeolithic site in the Iberian Peninsula, a similar interest for riverside forests was evidenced (Burjachs et al., 2012). If we refer to anthracological studies in Middle Palaeolithic caves and shelters, it appears that firewood was recollected from nearby sources (Allué et al., 2017; Vidal Matutano et al., 2015). It could be thus hypothesized that riparian forest or marsh/damp environments were established not far from the shelter. This could also reveal the proximity of the Ardèche River. These interpretations are consistent with sporadic records in layer 5 (squares L9, M6, M10 and N6) of *Microtus cf. agrestis*, a rodent with a preference for fresh meadows and riparian forests (Desclaux in Moncel, dir., 2019). The existence of forested areas around the Abri du Maras is further suggested by the predominance of *Cervus elaphus* as well as by observations of *Sus scrofa* and *Dama dama* in the macrofaunal assemblages (J. Marin in Moncel, 2019), which agree with more temperate climatic conditions prevailing at the end of MIS 5 (Moncel et al., 2018).

Conclusion

In this pioneering research, we have explored for the first time the potential of non-pollen palynomorphs (NPP) to provide new insights into an improved understanding of the relationships between Neanderthals and plant resources. Though rarely studied in Palaeolithic sites, NPP, and particularly fungal spores, have better preservation than pollen and fern spores. This encourages to systematic NPP studies in archaeological deposits. Endophytic fungi, which develop close relationships with plants by living within the host tissues and can be liberated during host senescence, can serve as key indicators of the local presence of plants, which could potentially have been used by Neanderthal groups. This study clearly shows that NPP must be considered as key taphonomical, environmental and cultural indicators in archaeobotanical analyses. Our research has also applied for the first time the combined study of archaeological sediments and pollen washes of artefacts following a microspatial approach in the study of Middle Palaeolithic archaeological contexts.

The positive result obtained from tool 1079 of the Abri du Maras provides a significant contribution to our understanding of plant-related tool use. With the comparison of archaeobotanical results with NPP analyses in modern surface samples, we could determine the host plant of the fungal signature documented in the tool surface as alder roots and propose the use of the lithic tool for processing this plant by Neanderthal groups. Alder roots have multiple potential uses for both medicine and technology (cordage or basketry).

Plants are generally ignored or underrepresented at Palaeolithic sites due to a lack of preservation. Archaeobotanical analyses including the application of the pollen washes technique must be pursued in Middle Palaeolithic sites as the function of Neanderthal tools is always difficult to identify and to correlate to plant processing or use (e.g. soil surface preparation). By scrutinizing particular pollen, spore and NPP signatures, embedded in the porous or irregular surface of

archaeological artefacts, and consequently related to the tool use, this technique, in combination with the study of other microfossils (phytoliths, residues etc.), allows better assessing Neanderthal plant uses that is otherwise invisible archaeologically. Our research provided conclusive evidences that NPP can provide meaningful cultural information. Their systematic tracking and mapping on the different living floors will constitute the next step of their analysis in the Abri du Maras and will provide new information to our understanding of Neanderthal plant use and site spatial organization.

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Table 1. Description of the archaeological samples. Samples with statistically significant fungi concentrations are marked in grey.

year of excavation	number	square	x	y	z	sample code and description
2017	947	K6	94	50	382	S1 : Stratigraphic Unit S2 : Sticking Sediment S3: Stone Wash
2017	1015	K6	43	57	388	S4: Stratigraphic Unit S5: Sticking Sediment S6: Stone Wash
2017	1306	M6	89	72	401	S7: Stratigraphic Unit S8: Sticking Sediment S9: Stone Wash
2017	252	N6	46	92	378	S10: Stratigraphic unit S11: Sticking Sediment S12: Stone Wash
2017	1079	N6	21	67	408	S13: Stratigraphic Unit S14: Sticking Sediment S15: Stone Wash
2018	765	M10	87	27	541	S16: Stratigraphic Unit S17: Sticking Sediment S18: Stone Wash
2018	1918	L6	21	30	420	S19: Stratigraphic Unit S20: Sticking Sediment S21: Stone Wash
2018	1227	L6	38	35	381	S22: Stratigraphic Unit S23: Sticking Sediment S24: Stone Wash

Table 2. Description of the modern control samples. Samples with statistically significant fungi concentrations are marked in grey.

sample Code	sample description
S25	Soil surface sampled in the upper and eastern immediate surrounding of the shelter
S26	Soil surface sampled in the lower and eastern immediate surrounding of the shelter
S27	Mosses collected from a tree located in the immediate vicinity of the shelter
S28	Mosses collected from a rock located in the immediate vicinity of the shelter
S29	Combination of litter and roots of <i>Alnus glutinosa</i> (L.) Gaertn. The population of <i>Alnus glutinosa</i> is located on the Ardèche riverbank (ca 70m away from the shelter)
S30	Roots of <i>Alnus glutinosa</i> (L.) Gaertn. (same location as S29)
S31	Combination of litter and roots of <i>Alnus glutinosa</i> (L.) Gaertn. The population of <i>Alnus glutinosa</i> is located in the slope between the river and the shelter (ca 40 m away from the shelter)
S32	Roots of <i>Alnus glutinosa</i> (L.) Gaertn. (same location as S31)
S33	Combination of litter and rhizome of <i>Thelypteris palustris</i> (spot 1: Sagnet in Saint-Paul-le-Jeune, Ardèche)
S34	Rhizome of <i>Thelypteris palustris</i> (same location as S33)
S35	Combination of litter and rhizome of <i>Thelypteris palustris</i> (spot 2: Ribes, Ardèche)
S36	Rhizome of <i>Thelypteris palustris</i> (same location as S35)

Captions

Fig. 1. Situation and site background.

1: location of the Abri du Maras and other major Palaeolithic sites (CAD S. Puaud, in Moncel et al., 2018); 2: view of the present-day shelter; 3: stone slabs recovering the layer 5; 4: top of the layer after the removal of the stone slabs; 5: hearth. Credits: M.H. Moncel.

Fig. 2. Studied stone tools and process of the pollen washes technique.

1: unwashed studied stone tools; 2: washed studied stone tools; 3: removal of the sticking sediment; 4: gun wash of object n°1079; 5: both sides of object n°1079 after wash.

Fig. 3. Concentrations of non-pollen palynomorphs of the archaeological samples (results expressed in number of elements per gram).

Fig. 4. Concentrations of non-pollen palynomorphs of the archaeological samples (results expressed in number of elements per cm²).

Fig. 5. Concentrations of non-pollen palynomorphs of the control samples (results expressed in number of elements per gram): soil surfaces and mosses.

S25: Soil surface sampled in the upper and eastern immediate surrounding of the shelter; S26: Soil surface sampled in the lower and eastern immediate surrounding of the shelter; S27: Mosses collected from a tree located in the immediate vicinity of the shelter; S28: Mosses collected from a rock located in the immediate vicinity of the shelter.

Fig. 6. Concentrations of non-pollen palynomorphs of the control samples (results expressed in number of elements per gram): litters, roots and rhizomes.

S29: Combination of litter and roots of *Alnus glutinosa* (L.) Gaertn. The population of *Alnus glutinosa* is located on the Ardèche riverbank (ca 70m away from the shelter); S30: Roots of *Alnus glutinosa* (L.) Gaertn. (same location as S29); S31: Combination of litter and roots of *Alnus glutinosa* (L.) Gaertn. The population of *Alnus glutinosa* is located in the slope between the river and the shelter (ca 40 m away from the shelter); S33: Combination of litter and rhizome of *Thelypteris palustris* (spot 1: Sagnet in Saint-Paul-le-Jeune); S35: Combination of litter and rhizome of *Thelypteris palustris* (spot 2: Ribes).

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953 Fig. 7. Major non-pollen palynomorphs (NPP) evidenced in the Abri du Maras
954 (Saint-Marcel-d'Ardèche, Ardèche).

955 1: spore of *Chaetomium*-type HdV-71, sample S13; 2: ascospore of *Diporotheca*
956 *webbiae*-type HdV-143, sample S14; 3 and 4: aggregates of ascospores of
957 *Diporotheca webbiae*-type HdV-143, sample S15; 5: ascospore of *Diporotheca*
958 *webbiae*-type HdV-143, sample 29; 6: immature ascospore of *Diporotheca*
959 *webbiae*-type HdV-143, sample 30.

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961 Fig. 8. Sidescraper on a backed flake, n°1079, level 5 upper.

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963

964 Table 1. Description of the archaeological samples. Samples with statistically
965 significant fungi concentrations are marked in grey.

966 Table 2. Description of the modern control samples. Samples with statistically
967 significant fungi concentrations are marked in grey.

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