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Pastoral Geo-Ethnoarchaeology.
Micromorphology and biogeochemistry of
dung deposits in mobile societies.

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M.A. Natalia Égüez

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First referee: Prof. Dr. Cheryl Makarewicz

Second referee: Prof. Dr. Henny Piezonka

Additional referee: Dr. Carolina Mallol

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Declaration of scientific realization

This dissertation has been realized in accordance to the Rules of Good Scientific Practice of the German Research Foundation. Apart from the supervisors' guidance, the content and design of the present thesis is all the own work of the candidate Natalia Égüez. This thesis has not been submitted to any other institution apart from the one here concerned.

Natalia Égüez

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Summary

The main goal of this dissertation is to provide a robust, innovative analytical methodology for the accurate detection and characterisation, at the microscopic and molecular scale of analysis, of organic-rich anthropic sediments from contemporary nomadic pastoral sites, with special focus on dung deposits. This research provides new data for the archaeological investigation of mobile pastoral habitation sites, and contributes to current approaches in the archaeology of pastoralism.

Pastoral societies have been, since the origins of domestication, an exceptional example of adaptation and resilience. Nowadays, nomadic pastoralism and transhumant activities supports more than 200 million people globally in a range of different environmental settings: from humid high mountains to arid deserts.

The study of past pastoral societies through microscopic analysis in soils and sediments has been a recurrent topic of geoarchaeological research. Since the 1980s, methodological and technical advances in palaeobotany and geoarchaeology contributed to consolidate a robust analytical toolkit, including, among others, 1) soil micromorphology, 2) pollen analysis, 3) carbonized seed remains and 4) phytolith analysis. These techniques, used alone or in combination, have provided relevant information on past pastoral livestock. However, high-resolution analytical approaches are needed to fully characterise and understand domestic spaces and specific activities associated with animal husbandry, such as intensity of livestock penning, re-use, cleaning, breaking of dung deposits, corralling of different livestock species, and seasonality of foddering.

Organic matter (mainly composed of cellulose, proteins, chitin, lipids and lignin) mixed in the soils is a unique source of detailed information on the past. Its presence in archaeological contexts is many times underestimated because it is not visible to the eye, especially in high erosional environments. In recent years, advances in biomolecular analyses applied to archaeological research, such as lipid and stable isotope biogeochemistry, have provided new anthropic and animal biomarkers that offer promising insights on past subsistence strategies and mobility patterns. These analyses have been mostly applied to artefacts and biological remains (i.e. pottery, bones and teeth), although the experimentation of lipid and stable isotope biogeochemistry applied to organic-rich sediments remains seldom investigated. Only recently, studies focusing on herbivore faecal remains have shown the importance of excrements and their implication for identifying socio-economic activities in archaeology. Therefore, an accurate microstratigraphic and molecular examination of these deposits can provide us new insights into past activity areas, landscape land use, site formation processes, domestic use of fuel, manuring and stabling or foddering strategies.

Micromorphology of soil sediments, lipid analysis and compound specific stable isotopes analysis are useful scientific techniques that combined with archaeology and ethnography allows identification of herd penning and multi-resource economic strategies dynamics, that are crucial to understand living conditions and diet of humans and animals in Pre- and Protohistory. This research uses an innovative combination of state-of-the-art methods that will combine and statistically treat multiple proxy sources from sediments, animal dung and plants.

Through the presentation of four scientific articles, this thesis focuses on three different-case scenarios: a well-preserved Neolithic organic-rich cave deposit in the European Mediterranean region; a well-preserved organic-rich open-air ethnoarchaeological hyper-arid deposit in North Africa; and a well-preserved organic-rich open-air ethnoarchaeological hyper-continental deposit in Central Asia. These case studies represent an ideal setting to compare different taphonomical processes affecting the organic accumulations. The novel integration of soil micromorphology, bulk stable isotope analysis ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$), lipid analysis (*n*-alkanes) and $\delta^{13}\text{C}$ stable isotope analysis of *n*-alkanes in different anthropic deposits validates the proposed methodology in diverse ecological habitats and provides contextualised and quantifiable high-resolution data that goes well beyond current approaches in geoarchaeological research.

Zusammenfassung

Das Hauptziel dieser Dissertation ist die Etablierung und Bereitstellung einer robusten und innovativen analytischen Methode zur Analyse anthropogener Sedimente mit guter organischer Erhaltung. Der Fokus liegt dabei auf kontemporären Fundstellen, die von nomadischen und pastoralen Subsistenzstrategien geprägt sind, sowie auf der Analyse von Dungablagerungen. Methodisch erfolgt diese Untersuchung auf einer mikroskopischen und molekularen Analyseebene. Dieser Forschungsansatz leistet einen Beitrag zu aktuellen Ansätzen innerhalb der Archäologie des Pastoralismus und stellt der archäologischen Erforschung mobiler pastoral-genutzter Fundstellen neue Daten zur Verfügung.

Pastoral geprägte Gesellschaften sind seit den Ursprüngen der Domestikation ein herausragendes Beispiel unterschiedlicher Adaptions- und Resilienzstrategien. Auch heute bildet nomadischer Pastoralismus und Transhumanz die Lebensgrundlage von über 200 Millionen Menschen in global sehr unterschiedlichen Naturräumen, die von feuchten Bergregionen, bis ariden Wüsten reichen.

Die Untersuchung pastoraler Gesellschaften mithilfe mikroskopischer Analysen von Boden- und Sedimentproben ist ein wiederkehrender Ansatz innerhalb geoarchäologischer Forschungsansätze. Die seit den 1980ern zu verzeichnenden methodischen und technischen Fortschritte innerhalb der Paläobotanik und Geoarchäologie trugen dabei zur Konsolidierung eines robusten analytischen Toolkits bei. Dieses beinhaltet, neben anderen, folgende Methoden:

1) Bodenmikromorphologie, 2) Pollenanalyse, 3) Analyse verkohlter Saatgutreste, 4) Phytolithanalyse.

Diese Techniken, die allein oder in Kombination eingesetzt werden, haben relevante Informationen und Erkenntnisgewinne bezüglich pastoraler Viehhaltung geliefert. Dennoch sind hochauflösende analytische Ansätze erforderlich, um Aspekte der häuslichen Sphäre und spezifische Aktivitäten in Zusammenhang mit Tierhaltung vollständig zu charakterisieren und zu verstehen. Hierzu zählen Aspekte wie die Intensität der Tierhaltung, die Wiederverwendung, Reinigung und Auflösung von Dungablagerungen, die Zaunhaltung unterschiedlicher Tierarten, sowie die Saisonalität von Tierfutter.

Organisches Material (hauptsächlich bestehend aus Cellulose, Proteinen, Chitin, Lipiden und Lignin), das in den Böden vermischt ist, ist eine einzigartige Quelle für detaillierte Informationen über die Vergangenheit. Das Vorhandensein organischer Substanz in archäologischen Kontexten wird häufig unterschätzt, da sie mit dem bloßen Auge nicht erkennbar ist. Dies trifft vor allem auf Umgebungen zu, die durch starke Erosion geprägt sind. In den letzten Jahren haben methodische Weiterentwicklungen in der biomolekularen Archäologie, vor allem in den Bereichen der Lipid- und Stabilisotopen-Biogeochemie, neue Biomarker für Mensch und Tier hervorgebracht, die vielversprechende Einblicke in Subsistenzstrategien und Mobilitätsmuster der Vergangenheit bieten. Diese Analysen wurden hauptsächlich auf Artefakte und biologische Überreste (z.B. Keramik, Knochen und Zähne) angewendet. Versuchsdurchführungen unter Berücksichtigung der Lipid- und Stabilisotopen-Biogeochemie, die auf organisch-reiche Sedimente angewendet werden, wurden bisher jedoch selten durchgeführt. Erst kürzlich haben Studien, die sich auf Fäkalienreste von Herbivoren konzentrieren, die Bedeutung von Exkrementen und deren Implikationen für die Identifizierung sozioökonomischer Aktivitäten in der Archäologie gezeigt. Daher kann eine genaue mikrostratigraphische und molekulare Untersuchung

dieser Lagerstätten neue Erkenntnisse über frühere Aktivitätsbereiche, die Landschaftsnutzung, die Genese von Fundstellen, die häusliche Nutzung von Brennstoffen, Dünge- und Stallungsstrategien, oder auch Futterstrategien liefern.

Mikromorphologische Untersuchungen von Bodensedimenten, Lipidanalysen und komponentenspezifische Analysen von stabilen Isotopen sind nützliche wissenschaftliche Techniken, die in Kombination mit archäologischen und ethnografischen Daten die Identifizierung von Einzäunungen unterschiedlicher Tierarten ermöglicht. Auch die spezifische Dynamik von wirtschaftlichen Strategien, die auf multiplen Ressourcen beruhen, können so untersucht werden. Diese Faktoren sind für das Verständnis der Lebensbedingungen und der Ernährung von Mensch und Tier in der Vor- und Frühgeschichte entscheidend. Die vorliegende Forschungsarbeit verwendet eine innovative Kombination modernster Methoden, die multiple Proxyquellen aus Sedimenten, tierischem Mist und Pflanzen kombiniert und statistisch behandelt.

Durch die Präsentation von vier wissenschaftlichen Artikeln konzentriert sich diese Arbeit auf drei verschiedene Szenarien: eine gut erhaltene neolithische, organisch-reiche Höhlenablagerung im europäischen Mittelmeerraum; eine gut erhaltene, organisch-reiche, ethnoarchäologische, hyperaride Freiland-Lagerstätte in Nordafrika; und eine gut erhaltene, organisch-reiche, ethnoarchäologische, hyperkontinentale Freiland-Lagerstätte in Zentralasien. Diese drei Fallstudien repräsentieren ideale Bedingungen, um unterschiedliche taphonomische Prozesse miteinander zu vergleichen, die organische Akkumulationsprozesse beeinflussen. Durch die neuartige Integration von Mikromorphologie, die Analyse von stabilen Isotopen der Gesamtprobe ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$), die Lipidanalyse (*n*-Alkane) und die $\delta^{13}\text{C}$ -Analyse von *n*-Alkane in unterschiedlichen anthropogenen Ablagerungen konnte die vorgeschlagene Methode innerhalb diverser ökologischer Habitats validiert werden. Darüber hinaus werden kontextualisierte und quantifizierbare, hochauflösende Daten bereitgestellt, die weit über aktuelle Ansätze innerhalb der geoarchäologischen Forschung hinausgehen.

Chapter 1

1 General Introduction

The origin, spread and evolution of pastoralist societies is a fundamental anthropological questioning that is directly related to understanding complex processes associated with cultural change, human-environment interactions, social organisation and ecological adaptations. The present thesis aims to develop a methodology to inform on pastoralist archaeology clear-cut questions on animal husbandry that remain poorly understood, i.e. intensity of animal corralling, seasonality of plant diet and foddering strategies. This work presents an integrative analytical approach to characterise organic-rich sediments, specifically herbivorous dung deposits, from past and present mobile pastoral contexts at the microscopic and molecular scale of analysis.

1.1 Significance of the Study and Objectives

This section describes the key terms related to pastoralist's organic deposits that are used thoroughly in the research papers included in this dissertation and contextualise them within their broad scientific approach and background.

1.1.1 Pastoralism, Mobility and Geo-Ethnoarchaeology

Pastoralism is a fundamental food production strategy that dates back over the beginning of the Holocene (Chang and Koster 1986; Barfield, 1993; Little 2015). Nowadays, nomadic pastoralism and transhumant activities support more than 200 million people globally in a range of different environmental settings -from high mountains to desert (Behnke and Mortimore 2016). Furthermore, pastoral societies have helped the development of regional and trans-regional networks of interaction around the globe because of their capacity as an innovative social agent and architect of political complexity (Honeychurch and Makarewicz 2016).

The term "pastoralism" usually refers to a broad range of animal husbandry practices that may or may not include mobility and some amount of agriculture, as well as a significant variation in community size and social organisation (Cribb 1991; Khazanov 1984; Arbuckle and Hammer 2018). Specifically, archaeologists frequently use the term to refer to mobile communities that rely on herd animals for the majority of their subsistence and practice little or no agriculture, often being treated as a separate entity from settled communities (Chang 2015). This approach is problematic as pastoralism is a complex human lifeway that encompasses many forms and can be affected by many variables (Salzman 2004).

In order to exploit the pastureland in benefit of their animals, pastoralists are required to move sizable herds in a regional-scale, adapting to their local surrounding environment and resources, from which they are dependent: climate, topography, flora and water sources (Abdi, 2003). Mobility in pastoral societies is a critical factor that enables herders to provide quality food for their domesticates (e.g. cattle, yaks, goats, sheep, horses, camels) and, consequently, to successfully obtain human food resources – meat, fat, marrow, blood, and milk, and secondary products -milk by-products, dung for

fuel and fertiliser and textile fibres (Sherratt 1981). In addition, mobility facilitates social and political opportunities for networking and trade (Ventresca-Miller and Makarewicz 2018). Many scholars have tried to categorise pastoralists regarding their mobility patterns (see Cribb 1991 and Khazanov 1994), ranging from sedentary use of rich pasturelands (exceptional migration) to transhumance (seasonal migration) to seminomadic (permanent habitation sites) to nomadic pastoralism (frequent settlement movement) (Salzman 2004; Little 2015). Often, the resulting categories are strict compartments that neither take into account the cultural traits of pastoralists' communities nor the interactions with sedentary communities at regional and local levels. As a result, many pastoral populations do not fit into strict classifications. Moreover, the archaeology of pastoralism has been traditionally embedded in the idea that pastoral societies have ephemeral occupations in the landscape and therefore are archaeologically invisible (Smith 2005). Although recent survey techniques have shown that pastoralists do, in fact, leave identifiable marks on the landscape (Hammer 2014; Rosen 2016), the scarcity of substantial domestic structures, frequent displacements, and the light equipment of ancient pastoralists has traditionally compelled archaeologists worldwide to rely heavily on ethnographic sources for archaeological interpretations (Biagetti 2014).

During the last decade, long-standing critiques concerning the indiscriminate use of ethnographic analogy in pastoralism studies have been raised (see for example Makarewicz 2013; Potts 2014; González-Ruibal 2016). While it is true that direct analogies with current ethnographic models are an inappropriate proxy, these ethnographic models may be useful as far as they provide abstractions of laws of human behaviour. In this sense, ethnography could be used as an approximation to study past-day societies, always keeping in mind that the present is an amalgam of dynamic social, political and environmental circumstances. Untangling such complex mixtures is on what ethnoarchaeology can help with.

Ethnoarchaeology is the study of living, non-industrial societies and can be defined as a methodological approach aimed to understand the relationship of material culture to culture as a whole, both in the living context and the archaeological record (David and Kramer, 2001). In that perspective, it becomes relevant to emphasise the words of González-Ruibal (2003) when he states that "the purpose of ethnoarchaeology is twofold: to produce a less Western-centric archaeology and to inspire new ideas and interpretations of the archaeological record". Moreover, the author adds that whether for producing middle-range theory or simply finding inspiration in contemporary societies to do better archaeology, analogy remains essential in the definition of ethnoarchaeology. Following this discourse, the work presented in this thesis is encompassed under the theoretical framework of postcolonial ethnoarchaeology. And specifically, post-colonial geo-ethnoarchaeology. These two concepts are set out succinctly in the following lines.

Postcolonial ethnoarchaeology refers to the role of ethnoarchaeology as not just to better understand specific archaeological questions (such as the way garbage is managed, or fuel is used), but to find how these archaeological questions allow us to understand a specific community better (González-Ruibal 2003, 2006, 2016). Context and analogy, in this case, are given the same priority while in "classical ethnoarchaeology" (see Binford 1979) analogy is the most important priority. Placing the local context at the same level of interest as archaeology means that ethnoarchaeologists do not have

to choose between contributing to archaeology or to understanding a local context better and its socio-political specificities. To sum it up, ethnoarchaeology represents a privileged path for archaeological theory- and method-building and could serve as a purpose for the preservation of cultural and technical traditions in current societies (Mallol and Henry 2017).

Geo-ethnoarchaeology is a research strategy applying geological principles and methods in an ethnoarchaeological context in order to link human activities (i.e., within sites and human interaction with the environment) and the formation of archaeological sites and landscapes (Friesem 2016). Geo-ethnoarchaeology aims to study contemporary contexts to investigate both living and recently abandoned sites to directly link human behaviour with the formation of microscopic and chemical markers. Moreover, this approach allows following the post-depositional processes, which affect the formation and preservation of the archaeological record. This research strategy uses a broad range of analytical methods, ranging from field observations to laboratory-based analyses, studying the entire range of archaeological materials, from macroscopic to biochemical materials. Examples of analytical methods applied to sediments and materials in geo-ethnoarchaeological research are: soil micromorphology, mineralogy, carpology, charcoal, pollen and phytolith analyses, portable X-Ray Fluorescence spectroscopy and Fourier Transformed Infrared spectroscopy (Koulidou, 1998; Shahack-Gross et al., 2003; Wilson et al., 2008; Mallol et al., 2007; Tsartsidou et al., 2008; Milek, 2012; Elliot et al., 2015; Berna 2017; Friesem 2018; Gur-Arieh et al., 2018; Henry et al., 2018).

1.1.2 Dung deposits

Animal dung, and in particular herbivorous livestock dung, is a valuable archaeological material, as it embeds information regarding animal husbandry, agro-pastoralism and pastoralism, domestication and use of animals, exploitation of the environment, reconstruction of the paleoenvironment, domestic use of fuel, activity areas, site structure and finally archaeological site formation processes (Shahack-Gross, 2011; Friesem 2016).

Herbivorous dung accumulations are ubiquitous features in pastoral and agropastoral settlements since the Neolithic (Matthews et al., 1997; Macphail et al., 1997; Matthews 2005; Shahack-Gross et al., 2004) and this kind of deposits form by corralling different animal species (one or several) – usually, goat, sheep, cattle and horse, in a specific space over time. The spaces can be either natural shelters or built ones (with stone walls or dung walls for example) with differing degrees of roofing or not roofing at all (Shahack-Gross, 2017). Livestock is usually penned segregated by species, sex, and age in multi-structured corrals, especially through the low temperatures of the winter season (Makarewicz 2014)

The content of the deposit would reflect the corresponding different occupations (i.e. layered abundance of organic matter). As a result, dung deposits can be accumulated at different rates, have interruptions in accumulation, they can be unconsolidated or compressed and cemented, and may or may not have a distinct layered structure. Moreover, preservation of dung deposits may be different under various environmental conditions. In general, organic-rich dung deposits are well preserved in either waterlogged or ultra-arid conditions while organic-matter is degraded under variable moisture levels, extreme temperature fluctuations and aeolian erosion (Chase et al., 2015).

Dung is primarily an organic material. It is composed of undigested and partially digested food remains, dead intestinal epithelial cells and intestinal bacteria and parasites. The type of fodder and type of digestion affect the form, size and composition of dung (Brönnimann et al., 2017). Once deposited in the corral floor, the fresh organic dung becomes trampled by the animals, producing a laminar structure and getting mixed with the inorganic components that are also present in herbivore livestock dung as biologically formed minerals within the animals' intestinal system (dung spherulites), silica bodies from plant tissues (opal phytoliths) and dust mineral particles (silt and clay) (Shahack-Gross, 2017).

First studies on coprolites were conducted in the 1930s in order to characterise animal diets based on plant macrofossils (Harrington 1933; Lauder milk and Munz 1934). Since then, with the standardisation of palaeobotanical analyses in archaeological excavations, many research projects are now devoted to the examination of dung organic-rich deposits. Among the most common analysis for the identification of dung deposition in different contexts are pollen analysis (Carrión 2000; Kuzmicheva et al., 2013; Babenko et al., 2015), spherulite analysis – or calcium carbonate crystals that form in the intestines of herbivores (Canti 1997, 1998), phytolith analysis (Jones 1984; Portillo and Albert 2011; Marinova et al., 2013; Alonso-Eguiluz 2017) and micromorphology (Courty et al., 1991; Brochier et al., 1992; Goren 1999; Macphail et al., 2004; Mathews 2005; Boschian and Miracle 2007; Shahack-Gross 2008; Polo-Díaz 2009). All these methodologies, alone or in combination, have proved useful in elucidating the presence of livestock pens in settlements and have supplied information on the family taxonomic composition of herbivorous livestock diet. Regardless, our current state of knowledge of daily life, herding practices and mobility patterns of early pastoral societies is still very poor, especially in open-air sites where the degree of preservation of dung is climate-dependent. Analytical approaches that involve a more specific characterisation of maintenance activities such as replacement of flooring, re-use, cleaning, breaking of dung deposits, corralling of different livestock species or species taxonomic composition of livestock diet and foddering, remain relatively scarce, and indeed these are key questions for understanding human-domesticates economic relationships.

Some limitations of the previously mentioned analyses are summarised below:

Silica phytoliths are microscopic opaline ($\text{SiO}_2 \cdot n\text{H}_2\text{O}$) bodies found in many plant families and are especially prominent in the grass family. Largely knowable taxa persistently do not form silica bodies, and variation in phytolith production among different taxa of phytolith-producers and non-producers often leads to over- and underrepresentation of many plant species. Besides, phytolith type production varies within the same species and are relatively unstable in soils and sediments as they are prone to post-depositional alteration involving partial dissolution, especially in open-air site contexts (Piperno 2016).

Pollen grains are produced in varying amounts, shapes, and sizes by the male reproductive organs of all spermatophyte plants, but only those which are anemophilous (i.e. pollen distribution by wind) are recoverable through archaeological methods (taking sediment cores from marshes or lacustrine areas). Moreover, a deposit with pollen will usually contain a combination of local pollen from contemporary vegetation, regional pollen brought via wind, water, or soil erosion, and residual pollen accumulated over time (Evans and O'Connor, 1999). Moreover, pollen is often only identifiable to genus

level, so may not provide adequate specificity for any meaningful interpretation. For this reason, palynology is generally used by archaeologists to look at vegetation on a regional level rather than providing site-specific information (Bremond et al., 2004).

Micromorphology (the study of soils, sediments, and archaeological features and materials in thin section) is a technique that does not have enough resolution in organic deposits as the previous other mentioned techniques. Plant remains in most of organic-rich deposits often appear through the microscope lens only as a brownish amorphous mass with hardly any visible cell structures. Although in well-preserved deposits the anatomy of the plant remains can still be observed (Ismail-Meyer 2017), further interpretation other than taphonomical insights on the vegetal remains is difficult. Micromorphology in dung deposits should be considered the contextualising tool from where researchers could decide on what other molecular analytical tools shall be employed—and which shall not (Goldberg and Aldeias 2018)

1.1.3 Molecular approaches to dung deposits

The four articles in this thesis present self-contained studies on molecular analysis applied to pastoral deposits, but a few words below are written to describe the nature of lipid and stable isotope analysis and its application in dung deposits.

Carbon and nitrogen isotopic analyses of undigested plant material recovered from sediments have also been used to identify dung deposition associated with livestock corralling and the dietary intake of penned animals (Shahack-Gross et al., 2008). One major challenge, however, in the application of carbon and nitrogen analyses to archaeological sediments in which dung is thought to have been deposited, is decoupling natural process isotopic change in soil nitrogen and inorganic carbon pools from human-mediated inputs in the form of animal penning and dung deposition (Linseele et al., 2013). Bulk stable isotope analysis ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of archaeological dung contexts have been seldom explored in part due to the pre-existing inorganic carbon contained in the sediment and the nitrogen input (e.g. bacteria, insects, or mesofauna) that could affect the isotopic composition of bulk dung (Staddon 2004; Hobbie and Quimette 2009).

On the contrary, lipid *n*-alkanes analysis is one of the most reliable biomarkers for contemporary animal diet estimation (Duncan et al., 1999; Dove and Mayes 2005; Dungait et al., 2010). Alkanes are hydrocarbon molecules that are largely derived from epicuticular waxes of vascular higher plants (Eglinton and Hamilton 1967; Rieley et al., 1991). These molecules form linear chains (*n*-alkanes) each with a different number of carbon atom (C-number). Most plant species have a characteristic pattern of alkane concentrations in their cuticular wax, with different species producing varying quantities of alkanes depending on the season of growth. *n*-Alkanes are indigestible, relatively resistant to microbial degradation and easily extractable by chemical methods from plant tissues, excrements and sediments (Malossini et al., 1994; Duncan et al., 1999; Mayes and Dove 2006; Wershaw 2016; Ardenghi et al., 2017). In addition, due to their non-polar single covalent bonds and the lack of functional groups, *n*-alkanes may preserve an original isotope composition that can be stable over a geological timescale (Cranwell, 1981; Schimmelmann et al., 2006; Seki et al., 2012; Ardenghi et al., 2017). So far, *n*-alkanes have been successfully used to estimate diet composition in contemporary domestic animals (Duncan et al., 1999;

Dungait et al., 2010) and in wild herbivores (Bugalho et al., 2001; Hulbert et al., 2001). The distribution and stable isotopic composition ($\delta^{13}\text{C}$) of long-chain *n*-alkanes is commonly used as a means to reconstruct paleoenvironment at local and regional scales, in particular for reconstructing vegetation types. Therefore, the concentration pattern, distribution and carbon isotopic signatures of *n*-alkanes in dung deposits from pastoralist campsites offer excellent potential for the estimation of past diet composition and landscape reconstruction.

It is overall clear that research on mobile pastoralism requires a truly interdisciplinary effort that incorporates fieldwork, ecological and ethnographic studies, geoarchaeology and bioarchaeology. Thus, this thesis is conceived as a methodological exploration to test the utility of combining micromorphological analysis of dung remains with carbon isotope analyses of *n*-alkanes from organic matter in the past and modern dung deposits. Specifically, the aim is to investigate geophysical characteristics and biomolecular geochemical signatures of ancient mobile pastoralist dung corrals in known locations and under known climatic conditions. The novel inclusion of *n*-alkanes and compound-specific stable isotope analyses, together with soil micromorphological analysis, provide contextualised and quantifiable high-resolution data that goes well beyond current approaches in geoarchaeological research. Moreover, this approach narrows down the scale of analysis to the subregional and local environments to better understand pastoral household and its related husbandry strategies within its surrounding ecological settings in a trans-regional scale and highlights the potential of using a multi-proxy approach for the study of stabling deposits generating new comparative datasets. Within this context, three main research objectives were defined:

Objective 1: To accurately identify and characterise, at the micro-scale and molecular level, organic-rich anthropic sediments from past and contemporary nomadic pastoral habitation sites and stabling deposits.

Objective 2: To identify repetitive use of penning areas and define taphonomical processes and stratigraphic deposition events.

Objective 3: To understand the biogenic signatures that pastoralists leave in the sedimentary record by identifying molecular and isotopic fingerprints related to animal penning and husbandry practices, including seasonal foddering.

In order to achieve these objectives, this work focuses on three different-case scenarios that represents the main typologies of dung-rich deposits: a well-preserved Neolithic organic-rich cave deposit in the European Mediterranean region; a well-preserved organic-rich, open-air, ethnoarchaeological hyper-arid deposit in North Africa; and a well-preserved/poor-preserved organic-rich open-air ethnoarchaeological hyper-continental deposit in Central Asia. These case studies represent an ideal setting to study different taphonomical processes caused by environmental factors that affect the preservation of organic deposits and the dung itself, and at the same time, validate the proposed methodology in diverse ecological habitats.

1.2 Dissertation Outline

This dissertation consists of six chapters. In *chapter one*, a general introduction sets the theoretical framework for the articles and at the same time emphasises the methodological advances for the study of organic-rich pastoral deposits. Additionally, this section outlines the motivation and objectives of the research and describes the general structure of the thesis. Chapters two to five are the main body of the thesis and are presented as individual articles published in peer-reviewed scientific journals (2-4) and a manuscript prepared for submission (5).

Chapter two focuses on the importance of characterising archaeological organic-rich deposits at a microstratigraphic scale of observation. The combination of micromorphological analysis of sediment samples together with short-lived sample radiocarbon dating provided microcontextual evidence for synchronous domestic activity and pastoral sheep penning activities at Cueva de El Toro (Málaga, Spain). Recurrent burning episodes on the sheep/goat stabling deposits during the early Neolithic period is a practice that had not been previously documented in southern Spain for such early dates (5320 and 5170 BP). Although a powerful tool for contextualising the deposits, micromorphology alone was insufficient to discern among animal husbandry practices related to foddering and animal diet. Data from this chapter arises from an ongoing collaboration with the Department of Prehistory, Archeology, Anthropology and Ancient History of La Laguna University (Tenerife, Spain). The outcomes of this chapter were published as:

Égüez N., Mallol C., Martín-Socas D., Camalich D. 2016. Radiometric dates and micromorphological evidence for synchronous domestic activity and sheep penning in a Neolithic cave: Cueva de El Toro (Málaga, Antequera, Spain). *Archaeological and Anthropological Sciences* 8, 1: 107-123. DOI 10.1007/s12520-014-0217-0.

Chapter three is conceived as an ethnoarchaeological pilot test study to investigate resolution of lipid analysis and both bulk and compound-specific isotope analysis in organic-rich soils and sediments from arid and hyperarid environments, where biological material such as dung preserves very well. These techniques coupled together with micromorphology enabled detailed microstratigraphic characterisation of sheep/goat dung deposits from an ethnographic pastoral campsite located in SW Fazzan, at the border between Libya and Algeria. Information about pastoral habitat adaptations in this area, taphonomic processes of dung deposits, significant micro-features related to penning (trampling, bedding and presence of dung spherulites, druses, phytoliths, or micro-fragments of coprolites), diet, and seasonality of herd animals were identified. Data from this chapter entailed an ongoing collaboration with the CaSEs Complexity and Socio-Ecological Dynamics research group (Pompeu Fabra University, Barcelona, Spain). The research described in this chapter was published as:

Égüez, N., Zerboni, A., Biagetti, S. 2017. Microstratigraphic analysis on a modern central Saharan pastoral campsite. Ovicaprine pellets and stabling floors as ethnographic and archaeological referential data. *Quaternary International* 483: 180-193. DOI 10.1016/j.quaint.2017.12.016.

Chapter four highlights the importance of ethnoarchaeological studies in identifying specific biomarkers that convey information on pastoralist animal exploitation practices. The article presents a combined micromorphological and biomolecular investigation of dung deposits in pastoral nomadic winter campsites in Eastern Mongolia, an ecological setting where multitude of taphonomic processes affect the degradation of organic deposits. Coupled microstratigraphic analyses and compound-specific carbon stable isotope analysis of plant *n*-alkanes of dung deposits allowed the exploration of plant origin of graze ingested by livestock and evaluated potential biomolecular signatures associated with the intensity and duration of dung deposition. The $\delta^{13}\text{C}$ values of plant *n*-alkanes in dung deposits were unusually depleted when compared to carbon isotope values of plant *n*-alkanes in soil control samples from non-anthropogenic deposits in the same region. All data from this chapter were collected during survey and fieldwork in Mongolia within the Archaeological Stable Isotope Laboratory (ASIL) from Christian-Albrechts-Universität zu Kiel (CAU). The outcomes of this chapter were published as:

Égüez, N., Makarewicz, C. 2018. Carbon isotope ratios of plant *n*-alkanes and microstratigraphy analyses of dung accumulations in a pastoral nomadic winter campsite (Eastern Mongolia). *Ethnoarchaeology*. DOI: 10.1080/19442890.2018.1510614.

Chapter five presents the study conducted on compound-specific carbon stable isotope analysis of plant *n*-alkanes on caprine dung deposits located in two different phytogeographic zones, the forest-steppe and desert steppe in Eastern Mongolia. In addition, we performed analyses on stems, grains and flowers of five common forage grass and herb species as reference material for modern vegetation chemotaxonomy at a regional scale. Plants from the forest-steppe and desert steppe exhibited similar mean *n*-alkane $\delta^{13}\text{C}$ values but significantly wider carbon isotopic variation in desert steppe flora reflecting larger variations in temperature and rainfall. Dung deposits yielded *n*-alkane profiles dominated by C_{29} and C_{31} , in correspondence with profiles yielded by stems and flowers, and a mean *n*-alkane carbon isotopic value that greatly differs from that of a non-anthropogenic control soil sample and from that of fresh plants. Moreover, ACL values in the dung deposit samples reflect an open-range diet in grassland environments and a more restricted diet in forests. In addition, CPI environmental differences between forest and grassland samples were detected. This paper contributes to the notion that *n*-alkanes profiles and their isotopic values vary geographically depending on the environment and adds the viability in applying biomarker studies in pastoralist archaeological contexts.

Égüez, N., Mallol, C., Makarewicz, C. 2019. *n*-Alkanes and their carbon isotopes ($\delta^{13}\text{C}$) reveal seasonal foddering and long-term corralling of pastoralist livestock in eastern Mongolia. *Anthropological and Archaeological Sciences*.

Finally, *chapter six* presents a summary of the main conclusions from chapters two to five. This chapter also includes ongoing work and future research challenges.

1.3 Academic Framework

The present study was conducted as a PhD project fully funded by the Graduate School “Human Development in Landscapes” at the Christian-Albrechts-Universität zu Kiel (CAU). The main research laboratories involved for processing and analysis of the samples were:

- The Archaeological Stable Isotope Laboratory (ASIL), part of the Institute for Prehistoric and Historic Archaeology at CAU. This laboratory focuses primarily on investigating questions relating to ancient human activity and paleoenvironment through isotopic analyses of biogenic tissues including bone, teeth, hair, plant remains, and silica bodies. It also works in close conjunction with the aDNA Laboratory and Zooarchaeology Laboratory to develop projects and multi-disciplinary approaches to understanding ancient human behaviour.
- The Archaeological Micromorphology and Biomarkers Laboratory (AMBI Lab), a unique laboratory specifically designed for joint soil micromorphology and lipid analysis at the La Laguna University (Tenerife, Spain). The AMBI Lab is fully integrated within the IUBO, the main institute of the La Laguna University devoted to bio-organic research.

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Chapter 2

2 Radiometric dates and micromorphological evidence for synchronous domestic activity and sheep penning in a Neolithic cave: Cueva de El Toro (Málaga, Antequera, Spain)

Abstract

We present radiocarbon dates and preliminary micromorphological information from the Neolithic cave site of Cueva de El Toro (Antequera, Málaga, Spain). This site has yielded a rich early and late Neolithic archaeological record. The late Neolithic assemblage reflects specialized handcraft activity including in situ ceramic manufacture, textile production and food processing along with sheep and goat penning, suggesting the cave occupants and their domestic animals shared the same living space. Until now, dating of the stratigraphic sequence was incomplete and the function of the combustion activities carried out at the cave remained unclear.

New absolute dates from the main late Neolithic domestic activity area, corresponding to the most intense Neolithic occupation of the cave, allow us to place the entire sequence between 5320 and 5170 BP (or 4250-3950 to 2σ Cal BC). Micromorphological results show that many combustion features from this site represent recurrently burnt sheep/goat stabling deposits all along the sequence, corroborating human-goat/sheep co-habitation. This practice had not been previously documented in southern Spain for such early dates. Our results exemplify the importance of characterizing archaeological deposits at a microstratigraphic scale of observation.

2.1 Introduction

Archaeological investigations across the south of the Iberian Peninsula, particularly the southeast, have revealed complex and diversified Neolithic population dynamics (Martín-Socas et al., 1998; Camalich Massieu and Martín Socas, 2013; Martín Socas and Camalich Massieu, 2014). There is significant evidence of Neolithic occupation of this region dating to around the second half of the VII millennium BP at several sites. This evidence takes the form of seasonal settlements associated to highly mobile communities of unknown size. For sites located in the Málaga region, at the lower Guadalteba River valley and the Ronda Depression, a scenario featuring the existence of small hamlets along fertile soils of valleys and dry stream beds has been proposed (Aguayo et al., 1987: 61 and 513; Aguayo et al., 1987: 64; Morgado and Martínez 2005). Slightly east, in the Antequera region, socio-economic and settlement dynamics cannot be compared to other regions nor framed within the mentioned scenarios, as most of the evidence comes from cave sites. One exception is the open-air site of Piedras Blancas I, located on the northern slope of Peña de los Enamorados (García Sanjuán and Wheatley 2009: 137-139). For the rest, cave sites located within the Torcal Range, such as Sima del Tambor, the Marinaleda-La Cuerda complex and Picardía Cave (Sanchidrián and García 1987; Perdiguero 1981; Martín Socas et al., 2004) reveal evidence of Neolithic occupation. Thus, settlement dynamics in this region was possibly characterized by human groups inhabiting caves. However, no scenarios have yet been set forth. This is

partly due to a lack of archaeological evidence from the earlier stages of the Neolithic, which are crucial to understand the evolution of settlement dynamics at a regional scale. So far, the only evidence comes from cave sites with Cardial impressed pottery such as Cueva de las Goteras, in Sierra de La Camorra, Mollina (Navarrete 1977: 383-385).

In this context, Cueva de El Toro (Antequera, Málaga, Spain) (Fig. 2-1) is a rich, deeply stratified Neolithic cave site with organic remains in very good preservation states. Previous radiocarbon dates frame the sequence between the middle of the 7th millennium BP and the beginning of the 5th millennium BP (Martín Socas et al., 2004).



Fig. 2-1. Geographical location of Cueva de El Toro Neolithic site.

Excavation of part of the Neolithic deposit throughout the 1980s revealed the existence of two main chrono-cultural phases: Phase IV, marked by successive combustion episodes interpreted as residues from hide smoking activities (Martín-Socas et al., 2004), and Phase III, a rich deposit with remains of diverse domestic activities, from food processing to pottery and textile manufacture (Martín-Socas et al., 2013). The Phase III deposit also yielded an assemblage of stone-lined, circular combustion structures near the original (now sealed) cave entrance (Fig. 2-2A). Field observations including the spatial distribution of the stones associated with some of these hearths suggested restructuring and possible recurrent use. In addition, a series of laterally extensive, partially overlapping combustion features was documented towards the interior of the cave (Fig. 2-2B and 2-2C). These combustion features of unknown formation, contained charred cereal seeds mixed in with charred sheep/goat coprolites (see Fig. 2-2A).

The richness, good preservation states and undetermined nature of the Cueva de El Toro combustion features motivated microstratigraphic investigation of sediment samples and new radiocarbon dating of short life samples from Stratigraphic Unit IIIB, corresponding to Phase III - the richest part of the sequence. These analyses can provide contextual evidence to advance our understanding of socio-economic and settlement dynamics in caves of the Antequera region during the Neolithic.

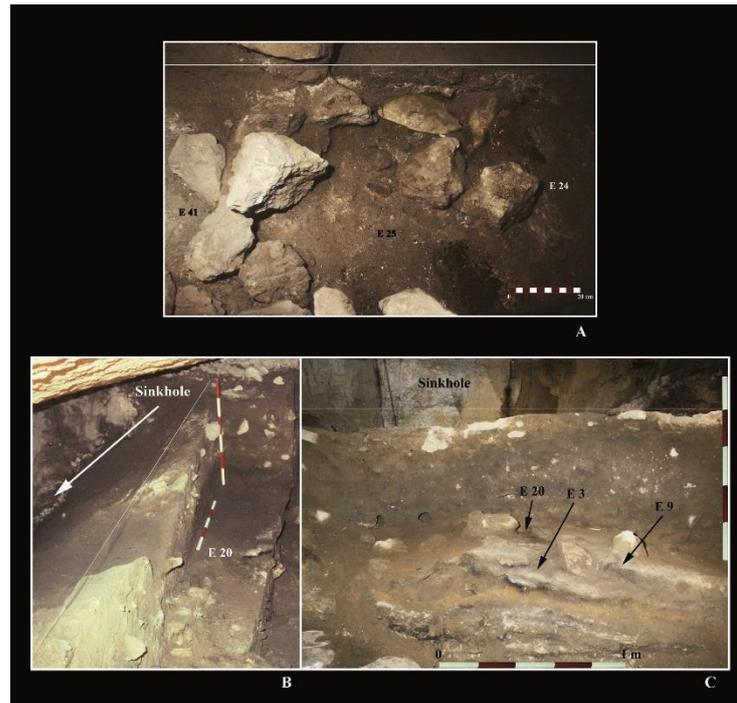


Fig. 2-2. A) Field view of stone lined combustion structure E25. The dark brown area at the lower right is a concentration of mixed charred cereal seeds and sheep/goat coprolites. Two similar structures (E24 and E41) were found adjacent to it. B) Field photograph taken during excavation of a one-meter wide section along the South profile. Note the proximity of this section to the sinkhole. Excavation yielded an assemblage of stacked combustion features (base of E20 shown here). C) Partially overlapping combustion features E3, E9 and E20 during excavation (plan view).

Micromorphological analysis of archaeological sediment allows for identification of microscopic particles and structures in their original microstratigraphic context (Courty et al., 1989) and has been a very effective method to approach the nature of archaeological combustion features (Mentzer 2012), as well as to identify ovicaprine dung (Brochier et al. 1992, Goren 1999, Shahack-Gross 2008) and stabling deposits in caves and rockshelters (Brochier 1991, Courty et al. 1991, Boschian and Miracle 2007, Angelucci et al., 2009, Polo-Díaz 2009, Polo-Díaz and Fernández Eraso 2010, Shahack-Gross 2011).

2.2 Site background

Cueva de El Toro (36° 57' 26" N; 4° 32' 10" W) is a cave site located in the Sierra del Torcal, Antequera, Málaga, at an elevation of 1190 meters above sea level (Fig. 2-3). Sierra del Torcal is a wide karstic mountain range separating Mediterranean Andalusia and the Sub-betic System of the Iberian peninsula. The morphogenesis of this region is characterized by limestones and diastolic systems that have conditioned the flow directions of the karst. Numerous sinkholes have been identified, some of which have yielded evidence of human habitation (Martín-Socas et al., 1998).

At present, the cave shows an inner structure with large fallen blocks and a >30 meter-deep open sinkhole near the original cave entrance, which is now sealed (Fig. 2-4). Most of the large blocks predate the Neolithic deposit.



Fig. 2-3. A) General view of the cave surroundings showing the distinctive Sierra del Torcal limestone formation. B) View of the cave interior. Note slabs detached from the ceiling. This roofspall is related to a major tectonic event that occurred at the time of formation of the Neolithic deposit. The latter was deformed by the weight of the slabs. Photograph by D. Whitley.

The original entrance to the cave was sealed at the time of formation of the Neolithic deposit by the tilting of a large ceiling slab. The tilting occurred during a major earthquake recorded at different sites throughout the region and known to have affected the entire Penibetic range, triggering subsidence of numerous cavities. This seismic episode has been recently dated at the site of El Aguadero, Periana, Málaga to the end of the 6th millennium BP (Beta-222473: 5110 ± 70 BP or $4045-3713$ 2σ cal BC; García Sanjuán, 2014). Besides sealing the original entrance to the cave, the tilting of the large slab, which occupied practically the entire space of the central gallery, faulted and deformed the underlying sedimentary fill (a Neolithic deposit) southwards (towards the sinkhole) (see Fig. 2-3).

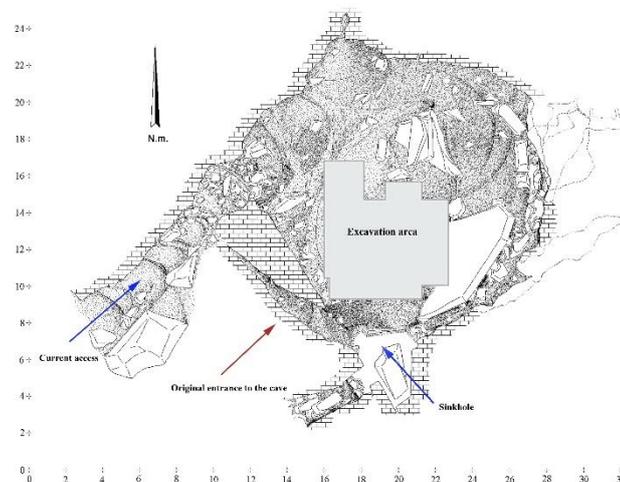


Fig. 2-4. Plan drawing of the major Cueva del Toro galleries showing the location of the excavated area

Two of us (D.M. and M.D.C.) carried out five excavation seasons at Cueva de El Toro (1977, 1980, 1981, 1985 and 1988), exposing approximately 16 m² at the central gallery of the cave near the original entrance. A 2.40 meter-deep stratified sequence was exposed and divided into a series of archaeological units following a Harris Matrix (Harris, 1979). These units were grouped into four chrono-cultural phases, from base to top (Figs. 2-5 and 2-6):

- Phase IV, early Neolithic, dated to 6150-6110 BP (5210-4950 2σ cal BC) (Martín Socas and Camalich Massieu, 2014). The deposit is about 35 cm thick, composed of clayey sediment containing charred inclusions and ashy lenses. The archaeological assemblage includes lithic, bone and pottery remains. The composition and spatial distribution of the ceramic remains indicate in situ ceramic production and use wear analyses of the bone and lithic implements indicate a predominance of butchery and meat processing activities, along with bone, wood and hide work (Martín Socas and Camalich Massieu, 2014).

- A sterile deposit, interpreted as a period of site abandonment. Based on dates obtained from sediments directly on top and underneath, this sterile deposit is estimated to have formed in around 600 years. It is quite thick (10-40 cm) and composed of gritty reddish clay. The origin of this clay remains unknown.

- Phase III, late Neolithic, subdivided into Phases IIIB (base) and IIIA (top) based on differences in the spatial patterning of archaeological remains. Hypothetically, the observed differences could be related to the major structural changes in the cave after the tectonic event mentioned above (Clavero et al., 2009; Clavero 2010), including sealing of the main entrance. The IIIB deposit is around 40 cm thick and exhibits lateral variation in texture and color.

- Phase II dating to the end of the 5th millenium BP (Martín-Socas et al., 2004) is characterized by a decrease in human impact at the central gallery, with fewer material remains and combustion features. The presence of owl pellets in the Phase II sediment suggests that humans occupied the cave less frequently during this period. However, evidence for geogenic surface erosion in the central gallery and postdepositional anthropogenic reworking of the Phase II deposit (up to the present) indicates that a portion of the original archaeological record might be missing.

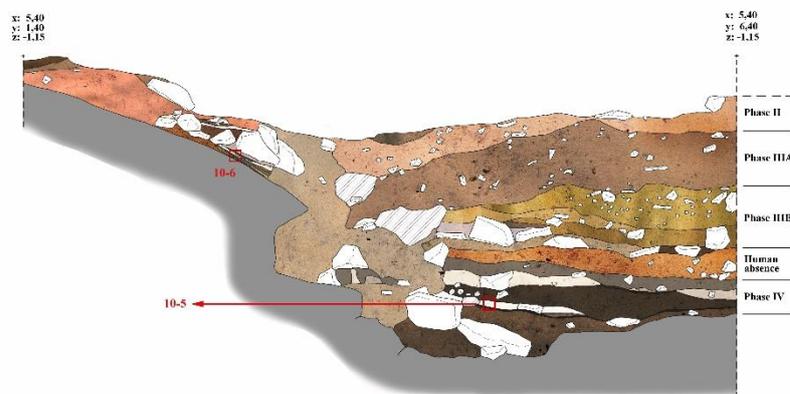


Fig. 2-5. South profile and location of micromorphological samples (red boxes).

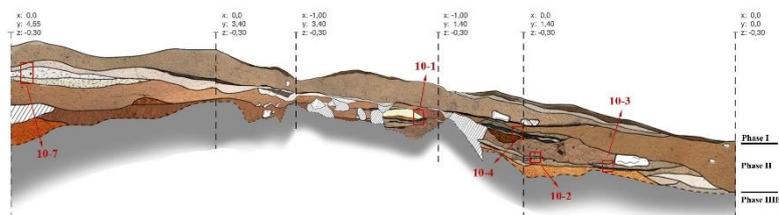


Fig. 2-6. North profile and location of micromorphological samples (red boxes). Note the absence of Phase IIIA sediment in this part of the cave. The sedimentary units associated with Phase II rest directly atop those from Phase IIIB with an erosional contact.

2.3 Materials and methods

Eight samples of short life organic material from Phase IIIB (7 carbonized seeds and 1 bone) were selected for radiocarbon dating, which was carried out by Beta Analytics (USA) following their standard protocol.

For micromorphology, 9 thin sections were prepared from 7 undisturbed sediment blocks collected from NE and S profiles of the inside of the cave related to Phases III and IV (Figs. 2-5, 2-6 and 2-7).

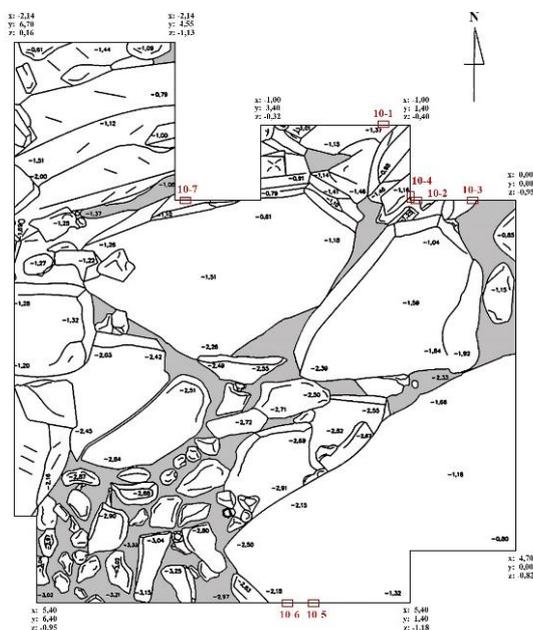


Fig. 2-7. General plan drawing of the current exposed surface (at the base of SU IV) indicating the location of the micromorphological samples (red boxes).

The blocks were impregnated with a mix of unpromoted polyester resin, styrene and a catalyzer (methyl ethyl ketone peroxide) in a ratio of 7:3:0.30. The thin sections were manufactured by Spectrum Petrographics Inc. (Vancouver, USA). Microscopic analysis was performed on a polarizing microscope (Olympus BX41) at 100, 200 and 400 magnifications under plane polarized light (PPL), crossed polarised light (XPL) and oblique incident light (OIL). Micromorphological descriptions were carried out following

guidelines from Bullock et al., (1985), Courty et al., (1989) and Stoops (2003). A sample of stable floor deposit from the upper part of the sequence dating to the Middle Ages was collected for reference. For complementary geochemical information, microprobe Scanning Electron Microscopy (SEM-EDAX) was applied to selected thin sections at the Centre for Scientific and Technological Studies of the University of Barcelona (CCiT-UB).

2.4 Results

New absolute dates obtained for the eight samples from Phase IIIb are presented in Table 2-1. Figure 2-8 shows a synthetic diagram of the stratigraphic sequence with representative thin section scans from each phase and their chronological context based on the new dates. Our micromorphological analysis yielded identification of 12 microfacies (Table 2-2). These are defined by groundmass, microstructure, organic and inorganic composition and pedofeatures.

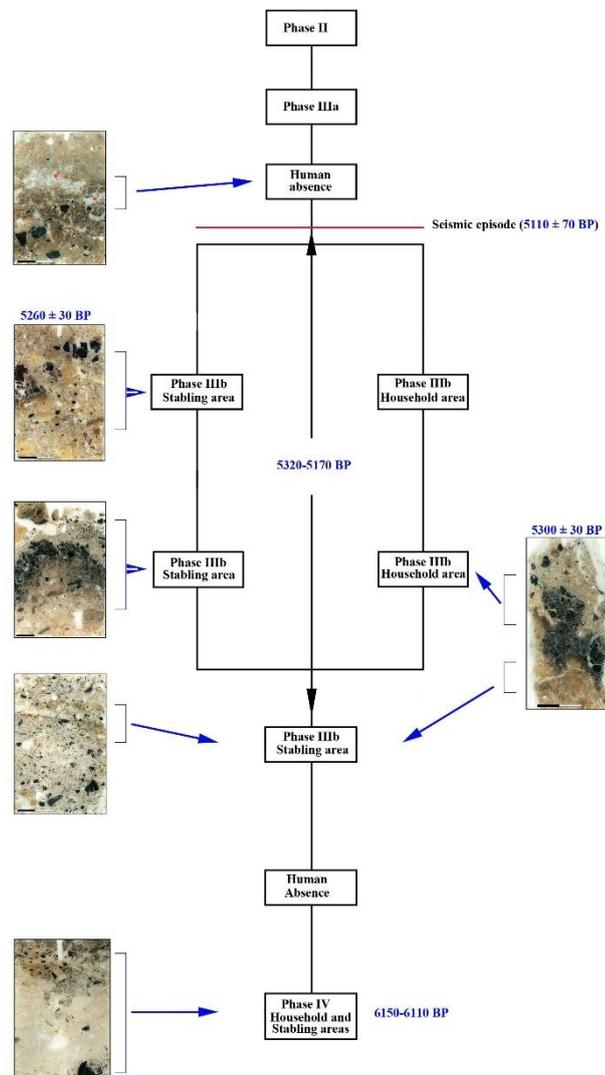


Fig. 2-8. Synthetic diagram of the stratigraphic sequence with representative thin section scans from each phase and their chronological context based on the new dates. Timing of the major tectonic event mentioned in the text is also indicated. *Charred seed date and reference number (Table 1). In these cases, dating and micromorphological samples were collected from the same place.

Cultural Period	Archaeol. Phase	Sample	Combustion Structure	Lab Number	BP	2σ cal BP	1σ cal BC	2σ cal BC
Late Neolithic	IIIB	Seed: <i>H. vulgare nudum</i>	E3	Beta-343182	5320 ± 30	6200-5990	4230-4050	4250-4040
Late Neolithic	IIIB	Seed: <i>H. vulgare nudum</i>	Ext. E25	Beta-347631	5300 ± 30	6180-5950	4230-4050	4240-4000
Late Neolithic	IIIB	Seed: <i>Quercus, sp.</i>	E41	Beta-343180	5290 ± 30	6180-5950	4230-4040	4230-4000
Late Neolithic	IIIB	Seed: <i>V. faba minor</i>	E17	Beta-347633	5280 ± 30	6180-5940	4220-4040	4230-3990
Late Neolithic	IIIB	Seed: <i>H. vulgare nudum</i>	E9	Beta-341130	5270 ± 30	6180-5940	4220-4000	4230-3990
Late Neolithic	IIIB	Seed: <i>Quercus, sp.</i>	Stabling	Beta-343179	5260 ± 30	6180-5930	4220-4000	4230-3980
Late Neolithic	IIIB	Seed: <i>H. vulgare nudum</i>	E25	Beta-343181	5240 ± 30	6170-5920	4050-3990	4220-3970
Late Neolithic	IIIB	Seed: <i>Quercus, sp.</i>	E20	Beta-343183	5210 ± 30	6000-5910	4040-3970	4050-3960
Late Neolithic	IIIB	Bone: <i>Ovis/Capra</i>		Beta-336259	5170 ± 30	5990-5900	3980-3960	4040-3950

Table 2-1. Table showing the results of radiocarbon dating for the late Neolithic.

MF	Description	Sample
1a	Loose, calcitic wood ash, containing abundant burnt heterometric spherulite-rich coprolite fragments (many of them rounded), rare coarse sand-sized angular brown burnt bone fragments and common subangular charcoal fragments with visible tissue, ranging from very fine to coarse sand.	10-1b 10-2a 10-2b 10-3 10-4 10-6
1b	No bone was identified. Very few subrounded grains of rubified clay with quartz inclusions.	10-1a 10-7
2	Calcitic wood ash microaggregates with browned and charred woody vegetation, few coarse sand-sized burnt coprolite fragments, coarse sand-sized angular brown and calcined burnt bone fragments. Large (several mm in size) subangular charcoal fragments with abundant scattered spherulites.	10-6
3a	Massive, spongy fibrous, spherulite-rich dung with browned and charred burnt plant material, and very few subrounded grains of rubified clay with quartz inclusions. There are very few subangular charred and calcined bone fragments and angular bioclastic limestone fragments.	10-2a 10-2b 10-4 10-6
3b	In situ broken fibrous dung (mm sized angular fragments) in a calcitic wood ash matrix deposit. Few mm sized angular charcoal fragments. Rare subangular bone fragments.	10-1b 10-2a 10-2b 10-4
3c	Rubified coprolites fragments, crumbly matrix with angular charred vegetal remains with visible tissue. Common charcoal fragments (3 mm. the largest).	10-1b 10-2a 10-2b
4	Massive compacted calcitic wood ash matrix with rare subrounded grains of rubified clay with quartz inclusions. Vegetal tissue with scattered dung inclusions. Few subangular calcined bone fragments.	10-1a 10-1b 10-7
5	Vughy calcitic wood ash, with very few subrounded grains of rubified clay with quartz inclusions. Few mm sized angular charcoal, few subangular spherulite-rich dung grains. Phenocryst calcite fragments (2 mm.).	10-3 10-5
6	Discontinuous massive clay with quartz and limestone fragment. It shows strongly fissured (planes, zig-zag, curved voids) desiccation marks.	10-2b 10-3
7	Loose calcitic wood ash with abundant charcoal dust, discontinuous.	10-5
8	Massive wood ash with medium-sized vughs and vesicles. Abundant mm-sized charcoal fragments with visible tissue including leaves. Calcined and diagenetic altered bone fragment.	10-5
9	Broken fibrous burnt dung with gypsum crusts. This gypsum has horizontal fissures. PPL: colourless; XPL: White/Grey.	10-1a 10-1b 10-4

Table 2-2. Result of the microfacies identified in micromorphological study of the thin sections.

The microfacies are representative of each occupation phase as follows.

2.4.1 Phase IV (1 thin section)

Sediment from the base of Phase IV (microfacies 5 and 7) shows a matrix of vughy calcitic wood ash mixed with few subrounded grains of rubified clay with quartz inclusions (possibly ceramic fragments), along with angular charcoal fragments and subangular spherulite-rich dung grains. These features are indicative of a burnt stabling floor deposit. Overlying this burnt floor with a sharp contact, the top of Phase IV (microfacies 8) comprises massive sediment composed of compacted wood ash with spherulite-rich rounded coprolite fragments, mm-sized charcoal fragments with visible tissue including leaves, as well as calcined bone fragments and phenocryst calcite fragments. The feature could be interpreted as a fireplace laid above the previous stabling floor deposit (Fig. 2-9).

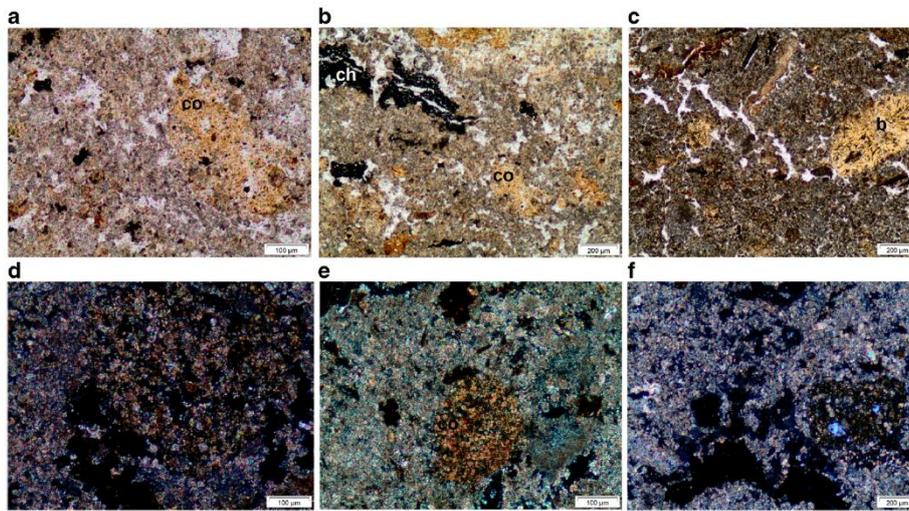


Fig. 2-9. Microphotographs showing microfacies 5, 7 and 8. Note the subangular spherulite-rich dung grains (a), the charcoal fragments with visible tissue including leaves (b) and the microstructure of calcitic wood ash with diagenetic altered bone (d). All images in PPL.

2.4.2 Phase III (5 thin sections)

Our samples represent mostly Phase IIIB, as we only obtained one thin section from the base of Phase IIIA, see Fig 8). The corresponding microfacies (1a, 1b, 2, 3a, 3b, 3c, 4, 5, 6 and 9) consist of loose, calcitic wood ash containing spongy, fibrous, spherulite-rich dung fragments and minor proportions of other particles including browned and black charred woody plant material, charcoal fragments, and very few subrounded grains of rubefied clay with quartz inclusions (possibly ceramic fragments) (Fig. 2-10). A single charred acorn was identified in microfacies 1a (Fig. 2-11). This microstratigraphic sequence is interpreted as a succession of burnt stabling floors. The dung fraction is prominent in all the microfacies. Dung spherulites, which are biomineralised calcium carbonate particles associated with herbivore excrement (Canti 1997), are ubiquitous. They are mostly found within coprolite fragments, although in some cases they also appear scattered in the groundmass (microfacies 2 and 4) (Fig. 2-12). Another noteworthy feature observed in the IIIB sequence is horizontal bedding of ashy sediment and its components (Fig. 2-13), together with in situ broken dung fragments (microfacies 3b, Fig. 2-14).

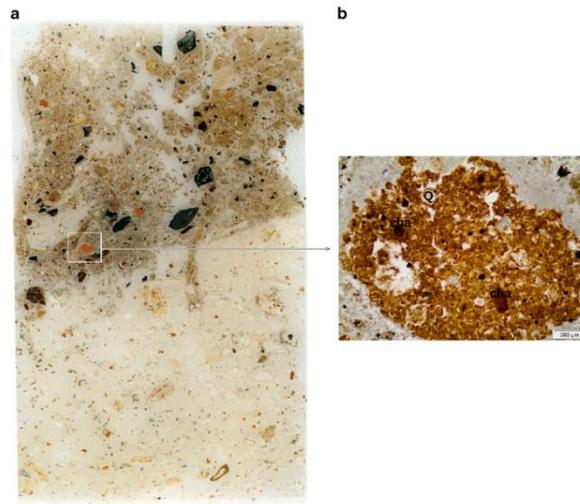


Fig. 2-10. Thin section scan from TORO 10-1b (a) with detail of subrounded grain of rubefied clay with quartz inclusions (b). Note the sharp contact between layers. Images shown in PPL.

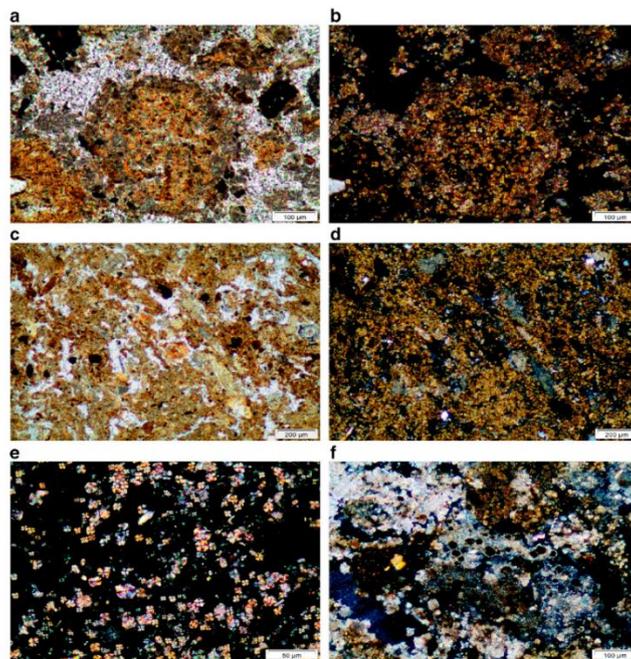


Fig. 2-11. Microphotograph of charred seed fragment mixed in massive calcitic wood ash. Image in PPL.

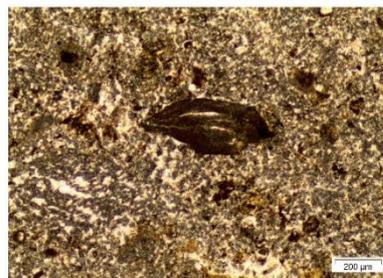


Fig. 2-12. Microphotographs of spherulites within coprolite fragments in PPL (a) and XPL (b); Some microfacies show broken rich-spherulite dung in the groundmass as observed in (c) PPL and (d) XPL. Microphotograph (e) corresponds to detail of spherulites at 400 magnifications in XPL and (f) illustrates burnt spherulites mixed in calcitic wood ash.

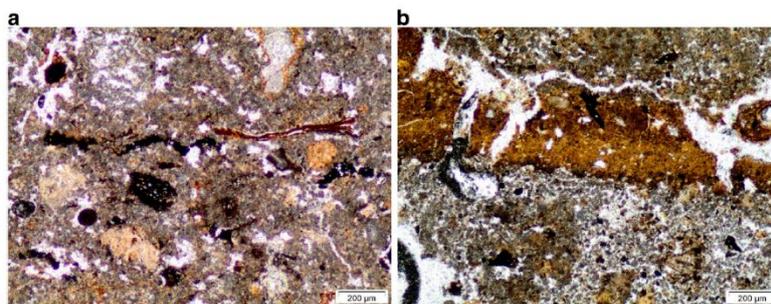


Fig. 2-13. Sample TORO 10-3 show horizontal disposition of in situ ash and its components. The microphotographs corresponds to microfacies 1a (a) and 6 (b). Note the sharp contact between combustión events. Images shown in PPL.

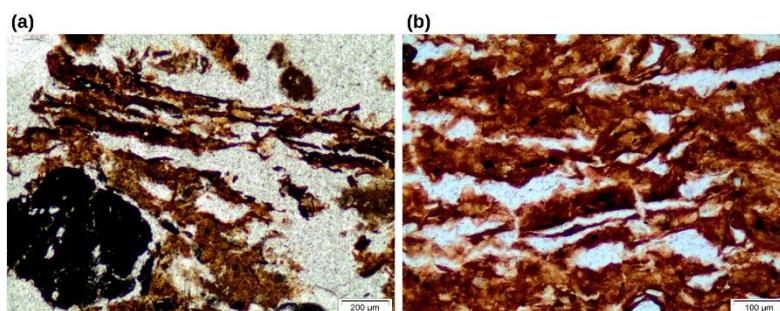


Fig. 2-14. Microphotographs showing in situ broken dung (a). In (b) detail of this fibrous burnt dung at 200 magnifications. Images in PPL.

A massive, 1cm-thick crust was observed at the contact between Phases IIIB and IIIA, between two stabling floor events. It is colourless in PPL and shows low birefringence (grayish white) in XPL (Fig. 2-15). SEM observation of the crust showed subangular clasts with low surface relief arranged into a mosaic framework (Fig. 2-16) and Energy Dispersive X-ray Analysis (EDAX) yielded a composition of 24.47% calcium and 17.61% sulfur compatible with the mineral gypsum (Table 2-3) (Fernández-Lozano 1997, Shahack-Gross 2004, Jackson and Rossetti 2006). This feature usually occurs after a slow phase of widespread recrystallization from the re-precipitation of the calcite present in the matrix of the stabling depositional surface (Mees and Stoops 2003, Jackson and Rossetti 2006).

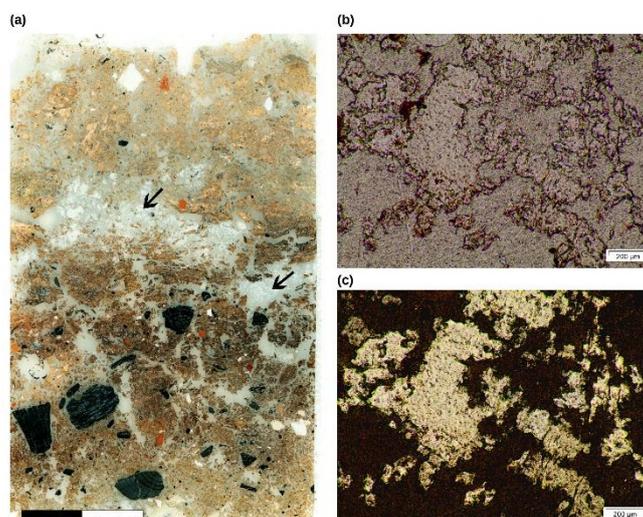


Fig. 2-15. Thin section scan from TORO 10-4 (a) with detail of gypsuim crust in PPL (b) and XPL (c).

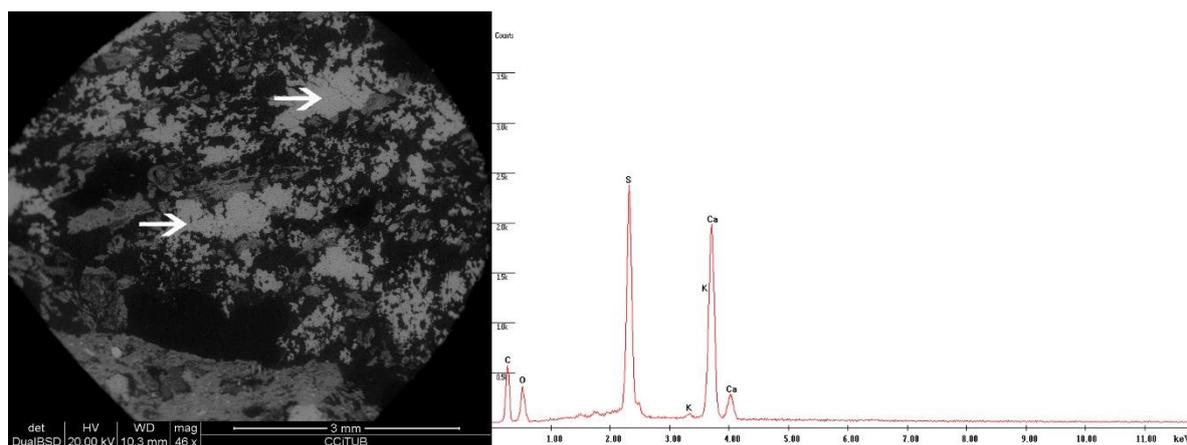


Fig. 2-16. SEM-EDAX analysis performed on sample TORO 10-4 showing the whitish silified crust and the resulting spectrum.

EDAX ZAF quantification (standardless)
Element normalized

Elem	Wt%	At%	K-Ratio	Z	A	F
C	37.91	56.92	0.0735	1.0351	0.1873	1.0003
O	19.29	21.74	0.0254	1.0177	0.1295	1.0001
Si	0.29	0.19	0.0024	0.9754	0.8191	1.0137
P	0.42	0.24	0.0036	0.9396	0.8989	1.0247
S	17.61	9.90	0.1626	0.9601	0.9500	1.0123
Ca	24.47	11.01	0.2231	0.9468	0.9628	1.0000
Total	100.00	100.00				

Table 2-3. Detailed table with the chemical components yielded by the SEM-EDAX analysis.

2.4.3 Control Sample (Medieval Sediment, one thin section)

The base of this sample relates to microfacies 1c and 4, showing a matrix of loose calcitic wood ash with abundant spherulite-rich broken dung fragments. At the top, the calcitic wood ash is compacted, with some grains of rubified clay with quartz inclusions and no presence of spherulites. This sequence, consisting of charred dung at the bottom (1cm thick) and pure ash on top (6cm thick), are interpreted as periodic combustion episodes for the maintenance of penning areas. These would be carried out to get rid of waste material and to reduce the volume of residues on the floor prior to moving the animals back into the cave (Polo-Díaz 2013).

2.5 Discussion

The new absolute dates provide a timeframe of 150 years (5320-5170 BP) or roughly five generations for Phase IIIB, a period of intense human occupation in the cave. Moreover, these dates indicate that the tectonic event that sealed the main cave entrance (dated at 5110 ± 70 BP) occurred at the end of Phase IIIB. This suggests that the changes observed in the Phase IIIA archaeological material record and its distribution (indicative of decreased human impact) might be related to the effects of the earthquake (Martín-Socas and Camalich Massieu 2014). Finally, the new Phase IIIB dates correlate well with the current regional chronological sequence, which interestingly, shows a population gap of 600 years

lasting until the middle of the 6th millennium BP (Martín-Socas y Camalich Massieu 2014). This would imply that the intense and diversified human activity and human/goat cohabitation documented in Phase IIIA possibly represents a newly arrived population after a prolonged period of regional depopulation. The new dates also provide evidence to support the cohabitation of humans and goats in the cave given the chronometric match between charred seed samples collected from sediment identified micromorphologically as a stabling deposit (Beta 343179) and charred seeds from sediment around combustion structure E25 (Beta 347631).

Regarding our micromorphological results, these must be taken with caution, as we were not able to obtain samples from different areas of the cave for neither Phase IV nor Phase III and we were not able to obtain samples from Phase II. Thus, our results remain as preliminary and only provide information for the part of the cave that was mainly used for penning. Nevertheless, we have been able to obtain some clues about the activities that were carried out in the cave during the relatively short time period represented by Phases IV and III and these correlate well with the archaeological evidence.

For Phase IV, archaeological evidence indicates low intensity occupation of the cave as well as the possible presence of hide smoking activity, particularly meat fileting as indicated by use wear analyses of the lithics (Rodríguez Rodríguez, 2004; Martín Socas et al., 2004). Our microstratigraphic observations show that this zone was used first for ovicaprine penning and subsequently for domestic activity, with no signs of abandonment between the two. The evidence for domestic activity is limited to combustion areas representing indistinct simple hearths, which may or may have not been used for smoking meat.

Phase IIIB represents a period of intense occupation with diverse domestic activities carried out at the central gallery. Unfortunately, except one sample (10-6) representing evidence for a combustion structure built on top of a stabling floor, the excavated area from which the remains of domestic activity were recovered was not available for micromorphological sampling. Nevertheless, the intensity of cave occupation during Phase IIIB is manifested by the common presence of microscopic fragments of charred seeds and ceramic fragments, as well as the possibly uninterrupted succession of burning events (a minimum of four in sample 10-2, Fig. 2-14) leading to the formation of "layer cake" deposits typical of ovicaprine penning contexts (e.g., Brochier 2002).

These deposits form by accumulation of fresh dung during the penning of animals, its subsequent drying, ramaging and mixing with the substrate, and anthropogenic burning of the stable floor. This sequence of events results in the following stratigraphic sequence: 1) a white ash layer generated by burning of the dung (5cm thick), 2) a black layer (2-3cm thick) representing charred dung and other (charred) organic particles present on the stable floor, and 3) a brown layer constituting unaffected or mildly rubified stable floor (2cm). Our observations corroborate the notion that black layers in archaeological contexts often represent the burnt substrate, not fuel (Mallol et al., 2013). Further quantitative analysis of the ash component (e.g., Gur-Arieh et al., 2013) will provide more details on fuel management, such as the nature of ash (dung/wood ratios) and estimates of the amount of fuel it represents.

Regarding the horizontal bedding of materials contained in the ash layers, a feature that was observed in all the microfacies, it is interpreted as evidence of trampling (Courty et al., 1991). This was specially marked in samples from Phase IIIB, where we observed well expressed horizontal bedding and

compaction of organic particles and subangular and angular bone fragments (Fig. 2-15) and suggests some degree of crushing derived from human or animal trampling (Akeret and Rentzel 2001; Miller et al., 2008). The archaeological evidence associated with this microfacies indicates domestic activity (particularly pottery and textile manufacture and food processing) and we identified a number of charred acorn seeds in the thin sections. This would suggest the stable floor was trampled by both animals and humans. Thus far, we cannot discern between both possibilities.

In contrast to Phase IIIB, Phase IIIA represents a period of decrease in occupation intensity of the cave as revealed by the archaeological record. We only observed sediment from the very base of this unit (sample 10-4), which exhibits a calcitic wood ash deposit with in situ broken fibrous dung fragments (microfacies 3b). Overall, this microfabric resembles to what was observed in samples from Phase IIIB (bases of thin sections 10-1a, 10-2a and 10-2b).

Our micromorphological observations for the contact between Phases IIIB and A suggests a rather long abandonment period after an episode of stabling activity, as shown by the presence of gypsum crusts (sample 10-4). The presence of gypsum has been previously associated to stabling deposits (Karkanas and Goldberg 2010). Isolated gypsum scatters were also identified at the very top of layer IIIB (Fig. 2-15). Further radiometric dating of this phase will contribute significant information on organic matter diagenesis by establishing the amount of time involved in the formation of the gypsum crust. Hypothetically, this abandonment period corresponds to the time in which the cave occupants left after a major earthquake transformed the structure of the gallery and sealed its entrance. Interestingly, this period also coincides with the earliest megalithic monument building in the region (García Sanjuán y Lozano Rodríguez 2014).

2.6 Conclusions

Preliminary microstratigraphic investigation of sediment samples and short life sample radiocarbon dating of Phase IIIB have provided micro-contextual evidence for synchronous domestic activity and sheep penning at Cueva del Toro, throughout the early Neolithic period in the north-western Mediterranean region. Our results also show that within a single zone of the cave, domestic and stabling activities alternated periodically. Further geoarchaeological investigation comprising a large sample size for the identification of site formation processes is necessary to test our hypotheses.

Our microstratigraphic approach coupled with short-life radiocarbon dates provides a temporal framework and its context at a scale suitable for approaching behavioral change, which is necessary to understand the regional evolution of Neolithic societies. First, we have provided empirical evidence to set forth a model of Neolithic settlement dynamics involving human groups that lived in caves and shared their living space with goat/sheep. This interpretation is supported by field observations suggesting the presence of recurrently used combustion structures (E3, E9 and E20) whose absolute dates match those of one of the penning layers. This contrasts with the traditional view of the function of caves during the Neolithic as loci for exclusive goat/sheep penning. Finally, our new Phase IIIB dates are in good agreement not only with the end of the regional 600 year archaeological gap documented throughout the region, suggesting the arrival of a new population. This issue should be further investigated as new radiometric dates are available in neighboring sites, but also with the dates for a

major tectonic event that possibly influenced the way the cave was occupied. This influence is strongly supported by the decreased human impact documented in the archaeological record of Phase IIIA.

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Chapter 3

3 Microstratigraphic analysis on a modern central Saharan pastoral campsite. Ovicaprine pellets and stabling floors as ethnographic and archaeological referential data

Abstract

Here we present an accurate microstratigraphic characterization of sheep/goat dung deposits from an ethnographic pastoral campsite located in SW Fazzan, at the border between Libya and Algeria. Research for referential data on current livestock contexts is essential for correctly interpreting archaeological records documented in ancient livestock spaces. Studies on herbivore faecal remains have played a key role in identifying socio-economic activities (e.g. domestic use of fuel, manuring, stabling or foddering strategies). Soil micromorphology, stable isotopes analysis ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, bulk and compound specific), and lipid analysis (*n*-alkane fraction) have been applied on soil samples and dung pellets in order to obtain reference biosignatures for *Caprinae* stabling deposits in arid and hyperarid environments, where the biological material as dung preserves very well. Information about pastoral habitat adaptations in this area, taphonomic processes of dung deposits, significant micro-features related to penning (trampling, bedding and presence of dung spherulites, druses, phytoliths, and/or microfragments of coprolites), diet, and seasonality of herd animals is herein shown.

3.1 Introduction

The archaeology of pastoralism has traditionally clashed with the problematic visibility of campsites. The paucity of substantial structures, frequent displacements, and light equipment of ancient pastoralists have challenged archaeologists worldwide for decades. Since the 1970s, archaeologists have been looking at the ethnographic present to find clues to recognize and interpret pastoral archaeological sites. Ethnoarchaeological and geoarchaeological research have both played a key role in the understanding of archaeological deposits generated by mobile societies (e.g., David, 1971; Robbins, 1973; Robertshaw, 1978; Hole, 1979; Smith, 1980; Cribb, 1991; Avni, 1992; Banning and Kohler-Rollefson, 1992; Bradley, 1992; Palmer et al., 2007; Saidel, 2009; Biagetti, 2014a). In addition, during the last decade new research has focused on analyses of micro-remains in arid contexts – phytoliths, pollen, organic matter, and soil constituents (Cremaschi and Zerboni 2009, 2011; Elliot et al., 2015; Friesem et al., 2011; Garcia-Granero et al., 2016; Gur-Arieh et al. 2013; Portillo et al., 2012, 2014; Shahack-Gross et al., 2008, 2011; Shillito et al., 2011; Cremaschi et al., 2014 to cite just a few) –, indicating that the study of current pastoral societies at a micro-scale can provide a better understanding of the use of space and the management of natural resources and therefore, contribute to correctly interpreting the archaeological record.

Although livestock dung at archaeological sites has been studied since the 1980s (e.g. Chang and Koster 1986) and research concerning animal dung is increasing (Elliot et al., 2015; Berna, 2017), building on studies conducted in modern ecosystems and under known conditions, biological and geological

taphonomic processes can be tracked, and complexities of isotopic variations in plant-soil systems can be also better understood. In this paper, we present an interdisciplinary approach for a controlled study of modern livestock dung in arid lands, in order to allow us to produce a detailed characterization of mobile pastoralist use of space as well as animal and natural resources management. The aim of this study is to serve as a reference for the interpretation of archaeological dung deposits and provide direct insights on ancient land-use and natural resources exploitation via a combined set of micro-analyses (Table 3-1).

Method	Potential information achieved from archaeological samples
Soil Micromorphology	Clearance and low impact activities (e.g., herding, cultivation, manuring, burning); identification of structures and occupation surfaces (e.g., trampling, trackways, ditches, hearths).
Soil Bulk Stable Isotope Analysis (C, N)	Composition of regional vegetation and past vegetation changes (C3, CAM, C4 plants). Diet, trophic level (herbivore, omnivore, carnivore).
<i>n</i> -alkanes Compound Specific Stable Isotope Analysis	Identification of fossil fuel contamination and burning, dietary practices, composition of regional vegetation and past vegetation changes (C3, CAM, and C4 plants). Isotopic fractionation behavior.
Lipid analysis (<i>n</i> -alkanes)	Biogeochemical transformations, organic matter input (aquatic, subaquatic, terrestrial plants), taphonomical processes (degradation/preservation).

Table 3-1. Relevance of the different methods applied in the paper showing the potential information achieved when applied to archaeological samples.

3.2 Overview of micromorphological analyses of dung

Soil micromorphology permits the identification of microscopic constituents and structures in their original microstratigraphic context (Courty et al. 1989). The analysis of archaeological sediment with this method has proven rather effective to identify ovicaprine dung (Brochier et al., 1992; Goren 1999; Shahack-Gross et al., 2008) and organic matter from stabling deposits in caves and rockshelters (Wattez et al., 1990; Brochier 1991, 1992; Boschian and Miracle 2007; Angelucci et al., 2009; Polo Díaz 2009; Polo Díaz et al., 2013, 2016; Shahack-Gross 2011; Nicosia and Polo Díaz 2012; Cremaschi et al., 2014; Égüez et al., 2016; Alonso Eguiluz et al., 2017) helping to explain the socio-economic features of pastoral groups and their interplay with environmental factors at a local and regional level, giving clues about seasonality, trampling, tillage or fuel management among others.

Isotope Ratio Mass Spectrometry (IRMS) is a specialized tool used to give information about the geographic, chemical, and biological origins of substances (Muccio and Jackson, 2008). IRMS is increasingly being used in archaeology especially for establishing dietary and migration patterns by measuring carbon, nitrogen, oxygen, and strontium isotopic abundances (e.g. Makarewicz, 2014; Henton et al., 2017), but also for palaeoenvironmental investigations analysing carbon, oxygen, and hydrogen isotopic abundances (e.g. Ziegler, 1989; Bowen et al., 2005; Finucane, 2007; Harrison et al., 2007). Stable isotope analysis ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) in archaeological bulk dung remains have been little explored (Linseele et al., 2013), but preliminary results demonstrated how this type of analysis is useful

to identify land management, livestock enclosures, and manured grasslands, and to distinguish between different dietary intake pathways (C3 and C4 plants) (Simpson et al., 1999; Shahack-Gross et al., 2008).

Higher resolution is achieved when performing a separation prior to isotope ratio analysis with Gas Chromatography (GC-IRMS) to study isotopic abundances of specific compounds as alkanes, ketones, or fatty acids. Long-chain *n*-alkanes are mostly derived from epicuticular waxes of vascular higher plant leaves (Eglinton and Hamilton, 1967; Rieley et al., 1991) and these molecules are resistant to biodegradation during burial and they exist almost everywhere in soils, sediments, atmospheric dust, and fossil fuels. This new line of research is currently being developed in archaeology (e.g., Dunne et al., 2016) and has been broadly applied in ecology and environmental sciences research since the 60s (e.g., Sachse et al., 2004, 2006, 2015; Hoffmann et al., 2013; Jambrina et al., 2016).

Chemical analysis of organic residues in archaeological soils (through GC-MS), particularly lipid biomarker techniques, have been applied for the detection and characterization of faecal organic matter since the late 90s (e.g., Evershed and Bethell, 1996; Bull et al., 2001; Sistiaga 2014). The dung biosignatures left by the occupations of nomadic pastoral communities in drylands are good and unequivocal indicators for animal husbandry in a site, and often represent the sole material evidence of 'light' and seasonal occupations. In the present study, we have employed all the above-mentioned methods, to demonstrate the usefulness of a combined micromorphological and bio-chemical approach to investigate dung deposits in arid environments.

3.3 Research area

The ethnoarchaeological study was conducted in the south-western corner of Libya, in the Fazzan region (Fig. 3-1), thanks to a research permit licensed to Sapienza University of Rome directed by Savino di Lernia. A mid-term (2003–2011) ethnoarchaeological project (Biagetti and Chalcraft, 2012; Biagetti, 2014a; Biagetti, 2015; Biagetti et al., 2016) focused on the study of the adaptation of current pastoralists in the Tadrart Acacus massif, is the main source of data used in the present paper.

The Tadrart Acacus is a sandstone massif, north-south oriented, of c. 100 x 50 km in size. Its main physiographic features are the west-east oriented valleys or wadis (dried river beds that can occasionally reactivate in case of rain), and a series of structural and climatic terraces, covered by a desert pavement (Zerboni et al., 2015).

Due to ancestral solutional processes, the flanks of the wadis are dotted by many caves and rock shelter, being attended by humans since the Upper Pleistocene (Cremaschi and Zerboni, 2011). The climate of the area is currently hyperarid, with very low and uneven rainfall, ranging from 0 to 20 mm per year. A proper rainy season cannot be envisaged, and years without rainfall are not uncommon. In spite of the lack of precipitation, the Tadrart Acacus massif features a patchy, permanent plant cover (Mercuri 2008), in the form of trees (*Acacia sp.*, *Tamarix* and more rarely, *Ficus*), shrubs (*Panicum turgidum*, *Zilla spinosa*), and grass (mostly *Aristida pungens*).

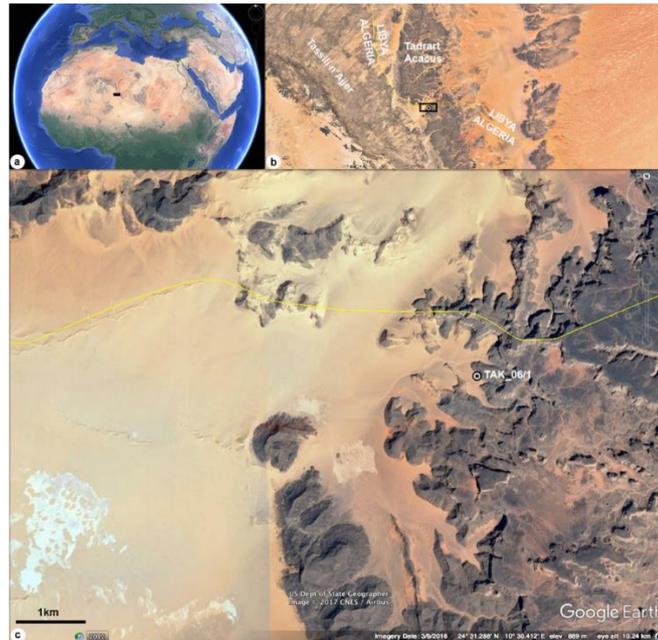


Fig. 3-1. The study area. a) Black rectangle indicates the localization of the study area; b) the study area and its setting; c) the campsite TAK_06/1. Images from GoogleEarth.

3.4 Pastoral habitat adaptations

The Tadrart Acacus is home to a small community of pastoral Tuareg, the Kel Tadrart. Less than 60 persons have been recorded living along the wadis of the Tadrart Acacus massif, roaming the dried river valleys with herds of sheep and goats, along with few camels and donkeys. No other inhabitants dwell this area. Mainly living upon their herds, the Kel Tadrart are (and have been) involved in a range of other activities, as wageworkers, soldiers, and tour guides, although pastoralism has always played a major role (Biagetti, 2014a). Flexibility is the hallmark of the Kel Tadrart pastoralism, and no fixed movements nor displacements, nor predetermined routes of transhumance been recorded. Rather, given the erratic nature of rainfall, the Kel Tadrart generally practice an opportunistic pattern of movements, leading their herds where grass occurred after precipitation. Therefore, every family has a 'main' settlement, where the domestic unit tends to spend most of the year. Every day, the Kel Tadrart flocks are conducted to grazing areas set no longer than ten km from those main settlements. In order to reduce the pressure on the pastures nearby, the Kel Tadrart can move for a variable time span to other locales. In the study area, along with main settlements, a single transhumance site, a number of 'alternative' and 'short-term' campsites have been recorded and studied (Fig. 3-2) (Biagetti, 2014b, 2014c).

Besides the campsites ALO_07/2, located outside of the Tadrart Acacus, that hosts a regular transhumance from the settlers of the nearby ALO_07/1, no regular patterns of year-round mobility have been recorded. Some households use 'alternative' settlements, and others have some short-term camps that allow the exploitation of specific zones. Yet, while the inventory of the Kel Tadrart 'main settlements' (Fig. 3-2) is exhaustive, only selected areas of the Tadrart Acacus were surveyed searching for secondary campsites (Biagetti, 2014c), and other alternative or short-term campsites were chosen and subjected to in-depth recording.

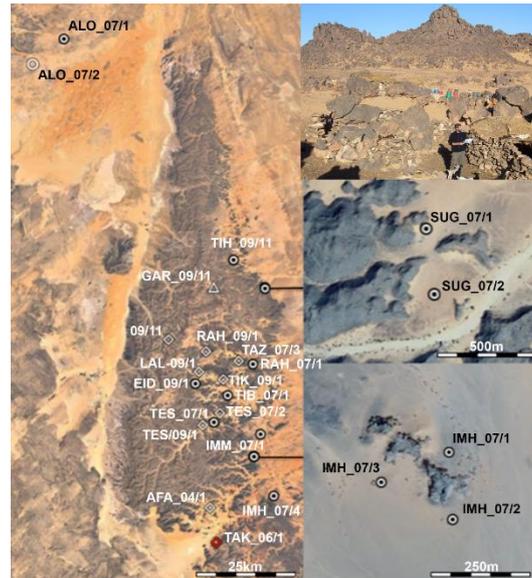


Fig. 3-2. The Kel Tadrart settlements. Key: empty circle: transhumance campsite; dotted circle: main settlement; triangle: alternative campsite; white diamond: short-term campsite; red diamond: campsite TAK_06/1; upper right: photo of campsite ALO_07/1 (courtesy and © The Archaeological Mission in the Sahara, Sapienza University of Rome).

Set along a wadi and close to the rocky flanks at the edges of the wadis, the layout of Kel Tadrart campsites is characterised by a common blueprint (Biagetti, 2014d; Biagetti et al., 2016). Domestic structures include sleeping huts, a kitchen, often a guest room (diwan) and smaller activity areas. Small stone pens for kid goats and sheep occur at all Kel Tadrart settlements, while larger corrals are generally absent (Biagetti, 2014d). Rarely, barbed wire corrals have been recorded to size large part of wadis. Dung areas are another characteristic of the Kel Tadrart settlements, and representing the clearest mark of animal husbandry. Large surface of ovicaprid excrements occur at every Kel Tadrart campsites and are extremely recognizable (Fig. 3-3).



Fig. 3-3. Dung area close to a cave used to pen sheep and goats in a Kel Tadrart settlement with detail of dung pellets morphology. Notice the large dispersion of the ovicaprid pellets (dark brown area). Courtesy and © The Archaeological Mission in the Sahara, Sapienza University of Rome.

The diet of the herds is based upon a few perennial plants: *Aristida pungens* and *Panicum turgidum* grasses (both used as fodder but only when green), and to, *Acacia seyal* and *Acacia raddiana* trees, considered by the Kel Tadrart as the most nourishing plants for their herds, *Tamarix* sp., and, to a lesser extent, *Zilla spinosa* herb (also used as fodder when other resources are lacking) (Biagetti 2014e). In

case of newborns, or after particularly dry years, the Kel Tadrart can purchase fresh fodder from the nearby oasis of Al-Awaynat. This strategy allows them to overcome the temporary scarcity of green pastures, and to sustain young ovicaprids.

3.5 Materials and Methods

3.5.1 Sampling

The settlement TAK_06/1 is set at the border between Libya and Algeria. It is located south of the Tadrart Acacus, the massif that hosts the Kel Tadrart. Our settlement TAK_06/1 lies thus at the very southern periphery of the Kel Tadrart customary territory. This campsite was intermittently inhabited before 2002 (Biagetti, 2014c), being one of the 'secondary settlements' (Biagetti, 2014b) recorded in the Tadrart Acacus. A virtual grid of 5x5m was built on the whole area of the settlement, and standard samples of sediment, which contained ovicaprine dung pellets were collected for the study of macro-remains. Twenty-one samples of loose sediment, block sediment and dung pellets have been later selected for the present work, along with two other samples coming from a tiny rock-shelter featuring hardened layers of dung (Fig. 3-4) (Table 3-2).



Fig. 3-4. The location of the samples.

Sample	Type	Analysis performed	Sample	Type	Analysis performed
1	Block sediment	Micromorphology	321	Loose sediment and dung pellet	LOI SIA
2	Block sediment	Micromorphology	322	Dung pellet	LOI
226-1/2	Dung pellet	LOI	331	Loose sediment and dung pellet	LOI SIA
227	Dung pellet	LOI	A29	Loose sediment and dung pellet	SIA
228	Dung pellet	LOI	B4	Loose sediment	SIA
229	Dung pellet	LOI	C28	Loose sediment and dung pellet	SIA
315	Loose sediment and dung pellet	LOI SIA CSIA	D3	Loose sediment	SIA
316	Dung pellet	LOI	F26	Loose sediment and dung pellet	SIA
317	Dung pellet	LOI	PA (318)	Dung pellets >1cm diameter	Micromorphology
318	Loose sediment and block sediment	LOI Micromorphology CSIA SIA	PB (318)	Dung pellets <1cm diameter	Micromorphology
319	Dung pellet	LOI			

Table 3-2. Sample summary and analyses performed: Loss On Ignition - LOI, Micromorphology, Stable Isotope analysis - SIA (C, N), Lipid analysis (*n*-alkanes) and Compound Specific Stable Isotope analysis – CSIA (C).

3.5.2 Bulk Soil Analysis

Thirteen samples of loose sediment (soil and ground dung pellets) were processed for loss-on-ignition (LOI). Soil sample refers to sample 318, a sediment block collected from the dark brown dung area in settlement TAK_06/1. The ground dung pellets were collected from the surface of different areas in settlement TAK_06/1 (Fig. 3). LOI was used as a proxy for soil moisture, soil organic matter (SOM), charcoal, calcium carbonate (CaCO_3), and mineral residue (MR), following 4 h cycles of drying at 105 °C, and combustion at 400 °C, 550 °C, and 925 °C (Heiri et al., 2001), and was done at the Institute for Ecosystem Research Laboratory (Christian-Albrechts Universität, Kiel).

3.5.3 Micromorphology of thin sections

Micromorphology analysis was conducted on five thin sections (4.8 x 2.8 cm, 30 μm thick) prepared from one undisturbed sediment block (sample 318); and two selected groups of ovicaprine pellets recovered from soil surface and collected from the same sedimentary context where the undisturbed block was taken: PA (four pellets >1cm diameter) and PB (five pellets <1cm diameter). Samples were embedded in epoxy resin, cut with an IsoMet low speed saw, mounted on glass slides, cut to a thickness of 1 mm, and finally polished to the desired thickness of 30 μm . After production, the slides were scanned on a flatbed scanner at high-resolution (1.200 dpi) in reflected light to allow detailed observations at mesoscopic level (Arpin et al., 2002).

Microscopic analysis was performed on a polarizing microscope (Nikon AZ100) at $\times 20$, $\times 100$, $\times 200$ and $\times 400$ magnifications under plane-polarized light (PPL) and crossed polarized light (XPL). Microphotographs were taken using a Nikon DS Fi2 camera. Micromorphological descriptions were carried out following guidelines from Bullock et al. (1985) and Stoops (2003), whereas interpretative concepts are from Courty et al. (1989) and Stoops et al. (2010). The microfacies described were defined by groundmass, microstructure, organic and inorganic composition, and pedofeatures regarding lithological changes within an individual thin section (Goldberg et al., 2009).

3.5.4 Bulk stable isotope analysis ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$)

Soil sediment samples and dung pellets were grinded and then lyophilised and analysed at Boston University Stable Isotope Laboratory (US). The analysis was performed in continuous flow mode for %C, %N, C/N, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ on a GVI IsoPrime isotope ratio mass spectrometer and a Eurovector elemental analyser, combined with a diluter and reference gas box. Samples for automated isotope analysis were first weighed out into tin boats to the nearest 0.01 mg on a Mettler AE240 or a Sartorius microelectronic balance. During a sequence run by the mass spectrometer, each sample was flash combusted at 1800°C in the Eurovector CN analyzer; the combustion products (CO_2 , N_2 and H_2O) were separated chromatographically and introduced into the mass spectrometer, with water removed in a chemical trap. The gases of interest were then introduced into the mass spectrometer for isotope analysis and the rest pumped away. The sample isotope ratio was compared to a secondary gas standard, whose isotope ratio has been calibrated to international standards. Samples ran in continuous flow mode and are currently within 0.2 ‰ precision for both nitrogen and carbon. Stable isotope abundance was expressed in standard δ notation relative to V-PDB and AIR (atmospheric N_2) for C and

N, respectively. In addition to carbon and nitrogen isotopes from the same sample, continuous flow also reported %C and %N data.

3.5.5 Lipid extraction and analysis (*n*-alkanes) and compound specific isotope analysis ($\delta^{13}\text{C}$)

2g of sediment and pellet samples were dried at 60°C during 48h, then ground and homogenized. The *n*-alkane identification and quantification were carried out at the Archaeological Micromorphology and Biomarkers - AMBI Lab of the Instituto Universitario de Bio-Orgánica Antonio González at Universidad de La Laguna, Spain. Lipids were extracted in 20mL 9:1 dichloromethane/methanol. 3 cycles of 30 minutes sonicator and 10 minutes centrifuge (4700rpm) were applied to the mixture. The total extraction was then evaporated and separated using solid phase extraction (SPE) on a silica gel column chromatography into five fractions of different polarity. In this paper, we only present results for the *n*-alkanes fraction, this one eluted with $\frac{3}{8}$ of dead volume in *n*-hexane. After addition of internal standard (IS) 5 α -androstane 8mg/L the volume was completed with 150 μL of DCM. The *n*-alkane fraction was identified and quantified by gas chromatography with a coupled detection and mass-selective detector (GC-Agilent 7890B, MSD Agilent 5977A) equipped with an HP-5MS capillary column (30m, ID: 250 μm , film thickness 0.25 μm). Compounds were identified by comparison of their retention times and mass spectra with those of reference compounds (mix C8-C40 and 5 α -androstane, Supelco) and comparison with the NIST mass spectra library. Quantification was based on calibration curves obtained by plotting the ratio $\text{Area}/\text{Area}_{\text{IS}}$ versus the concentration of each reference compound. Correlation coefficients were higher than 0.995. The *n*-alkane concentration is expressed as μg of individual compound per gram of dry sample ($\mu\text{g gds}^{-1}$).

Carbon isotope analysis by GC-IRMS was performed using a Thermo Scientific Isotope Ratio Mass Spectrometer Delta V Advantaged coupled to a GC Trace1310 through a Conflo IV interfaced with a temperature converter GC Isolink II. Samples were injected by means of a MultiMode injector (MMI) in splitless mode, with the temperature increasing from 79 °C (held 0.5 min) to 325 °C (held 3min) at a rate 10 °C·s⁻¹ and finally to 350 °C (held 3min) at 14 °C·s⁻¹. The GC was fitted with a Trace Gold 5-MS (Thermo Scientific) fused silica capillary column (30 m length x 0.25 mm i.d., 0.25 μm film thickness). Helium was used as the carrier gas at a flow rate set at 1.5 mL·min⁻¹. The combustion reactor temperature was maintained at 1000 °C. The temperature programme comprised a 2 min isothermal period at 70 °C increasing to 140 °C (held 2 min) at a rate of 12 °C·min⁻¹, followed by an increase period to 320 °C (held 15 min) at 3 °C·min⁻¹. Data acquisition and processing were carried out using the Isodat 3.0 software (Thermo Scientific). All the $\delta^{13}\text{C}$ values were normalized to VPDB (Vienna Pee Dee Belemnite) using an *n*-alkane Schiesselmann type A6 mixture (n-C₁₆ to n-C₃₀). The standard deviation of carbon isotope measurements was better than 0.5‰.

3.6 Results

3.6.1 Bulk soil and pellet analysis

Loss On Ignition (LOI) analysis was done by burning thirteen samples of loose sediment and ground pellets as described above. The main objective was to observe different percentages between the soil sample and the ovicaprine dung pellet samples. Results are shown in detail in Table 3-3. Water and organic matter percentages are higher in all pellet samples with mean values of 3.91% and 54.75% respectively, in comparison to the soil sample (1.73% and 23.93%). On the contrary, mineral residue percentage is higher in soil (73.05%) than in pellet samples (mean 42.08%). Calcium carbonate residue percentage have similar values in all samples with a mean of 2.92% for pellets and 2.60% for the soil sample. Results show as expected that the pellets themselves contain higher amounts of organic matter as it is related to the undigested plant remains content. The water percentage is also in accordance with that result. The loss of organic matter content in the soil is related to bacterial and mesofauna activity, also causing calcium carbonate residue to increase (e.g. organic matter passed through an earthworm's digestive system is broken up and neutralized by secretions of calcium carbonate from calciferous glands near the worm's gizzard) (Versteegh et al. 2014). The same mechanism of calcium carbonate addition is observed with fungal and mites larvae activity in the dung pellets (Norton and Behan-Pelletier, 1991).

Sample ID	Water %	Organic Matter %	Charcoal %	Carbonate %	Mineral Residue %
226-1 pellet	5.21	70.54	0.80	3.19	25.47
226-2 pellet	4.34	65.22	0.38	2.84	31.57
227 pellet	3.72	54.33	0.37	2.49	42.82
228 pellet	5.60	71.70	0.19	1.91	26.20
229 pellet	4.14	57.10	0.00	2.31	40.59
315 pellet	2.34	32.59	0.00	4.06	63.34
316 pellet	3.57	53.25	0.00	3.89	42.86
317 pellet	3.56	44.57	0.00	3.46	51.96
318 soil	1.73	23.93	0.42	2.60	73.05
319 pellet	3.46	62.09	0.00	1.49	36.42
321 pellet	3.83	51.99	0.00	2.84	45.17
322 pellet	3.98	50.00	0.73	3.17	46.10
331 pellet	3.18	43.71	0.29	3.43	52.57

Table 3-3. Loss On Ignition results showing percentages for water, organic matter, charcoal, carbonate and mineral residue.

3.6.2 Micromorphology of thin sections

Thin section of soil samples 1, 2 and 318 features three common microfacies (see Table 3-4 for a detailed description).

Sample 318 shows diffuse and soft contact between them: MF1, MF2, and MF3 (Fig. 3-5 and 3-6). Distinctive percentages of mineral coarse fraction are present in each of them resulting in 60% on the surface of the soil that decreases until 30% on the bottom. In general, the preservation of vegetal

remains is fairly good: seeds, phytoliths and stems are visible along some vegetal tissue with cellular structures with common presence of abundant fragments of sheep/goat excrements, oxalate druses, and massive presence of spherulites mixed in the sediment (Fig. 3-7).

MF1	60% coarse fraction, porphyric related distribution with a spongy loose microstructure composed of angular quartz grains (mm-sized). Very common channels and plane voids, no infillings, no gypsum. Preserved vegetal remains (seeds, phytoliths and stems are visible along some vegetal tissue with cellular structures). Common ovicaprine convoluted coprolite rich-spherulite fragments. Distribution of all materials included in the matrix is looser and less compressed than MF1 and MF2.
MF2	50% coarse fraction, porphyric related distribution with spongy microstructure composed of subangular quartz grains (mm-sized). Channels and plane voids are common. No gypsum presence. Some loose discontinuous gypsum infillings (tabular mosaic intergrowths). Very well preserved vegetal remains (seeds, phytoliths and stems are visible along some vegetal tissue and charcoal chunks). Presence of massive spherulites and common ovicaprine coprolite fragments. The distribution of all materials included in the matrix is horizontal and compressed, with some phytolith beddings.
MF3	30% coarse fraction, porphyric related distribution with a crumbly microstructure composed of subrounded quartz grains (mm-sized). Channels and plane voids (biological activity) are present with some loose discontinuous gypsum infillings (tabular mosaic intergrowths). Some calcitic druses spherulite-like (50-100µm range) Very well preserved vegetal remains (seeds, phytoliths and stems are visible along some vegetal tissue and charcoal with cellular structure also visible). Presence of black punctuations (charred organic matter), massive spherulites and common ovicaprine coprolite fragments. The distribution of all materials included in the matrix is horizontal and compressed, with some phytolith beddings.

Table 3-4. Microfacies identified in the soil sample thin sections (1, 2 and 318).

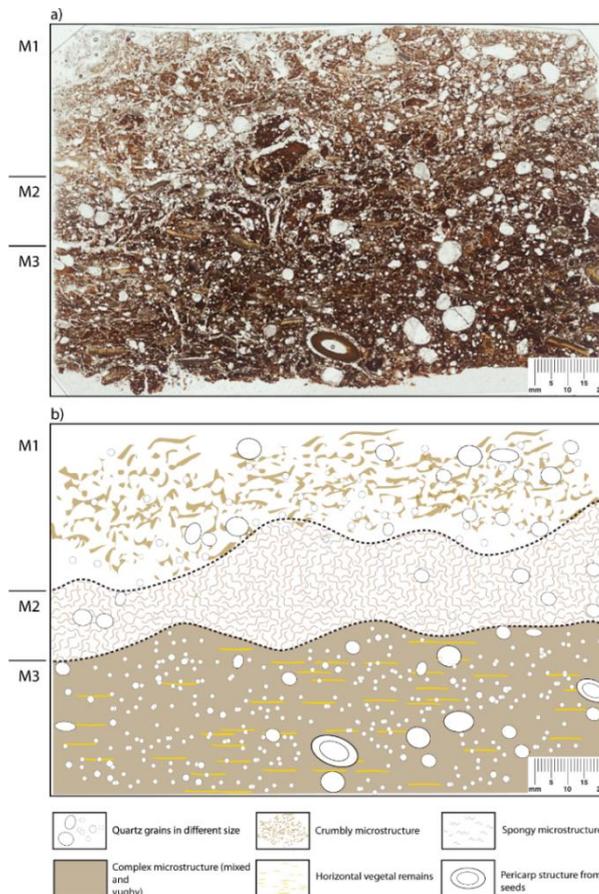


Fig. 3-5. Synthetic diagram of the micro-stratigraphic sequence in sample 318 (soil sample from stabling deposit) showing the microstructures and mineral compounds of each microfacies. The scale is 2cm.

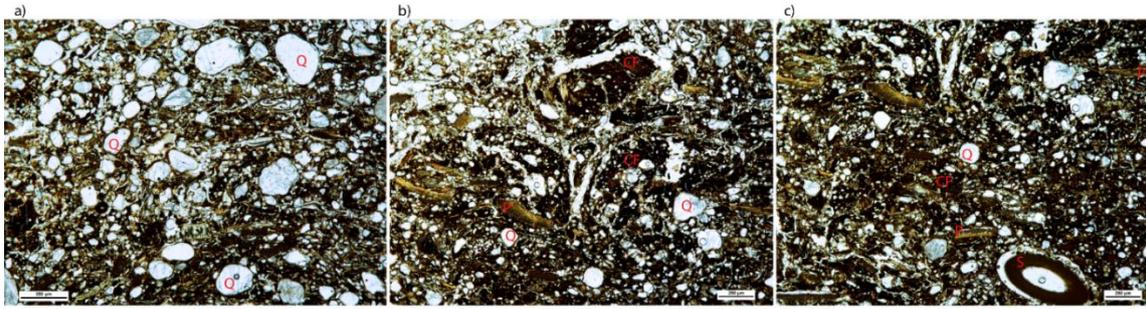


Fig. 3-6. Microphotographs showing microfacies a) 1, b) 2 and c) 3. Note the quartz (Q) abundances and size, the subangular coprolite micro fragments (CF), pericarp fragments (P) and a complete seed structure (S). All in PPL. The scale is 250µm.

The distribution of all materials included in the matrix is horizontal and compressed, most visible at the bottom (M3), where we observe some well-preserved phytolith beddings. Also, only at M3 we found some loose discontinuous gypsum infillings (with tabular mosaic crystals) and single crystal intergrowths, and spherulitic oxalate druses with mm-size. Thin sections from the pellets show the convoluted pattern characteristic of ovicaprine dung remains. PA (pellets >1cm diameter) and PB (pellets <1cm diameter) content (vegetal tissue as cellulose, phytoliths, oxalate druses, spherulites) does not differ from each other except for the amount of spherulites present (less massive in PB). Also, no gypsum infillings are present in any of the pellets samples and the spherulitic oxalate druses with mm-size are visible almost in all pellet samples (Fig. 3-7).

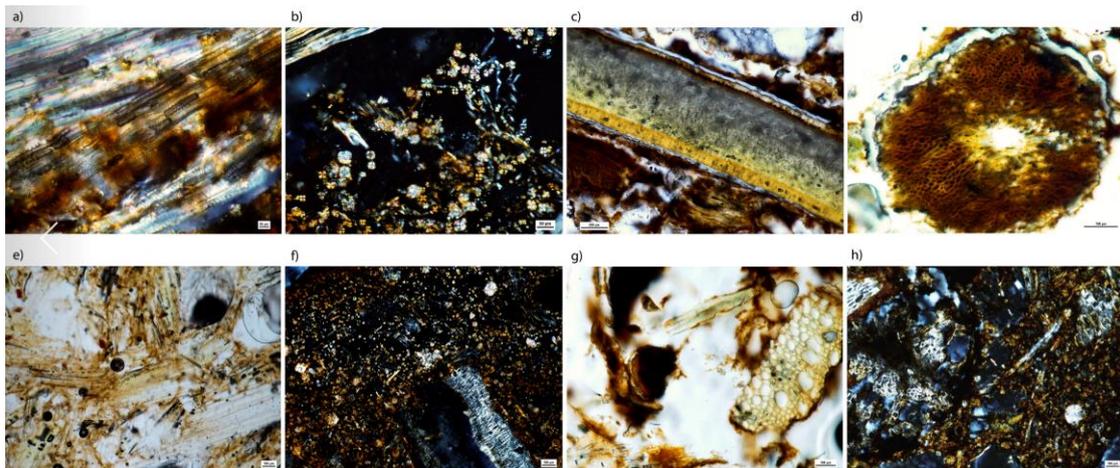


Fig. 3-7. Microphotographs showing a) phytolith bedding from echinate long cells (grass inflorescences and leaves; b) spherulites (20-50 µm sized) within the matrix sediment, image in XPL; c) detail of seed pericarp, image in XPL; d) detail of fresh grass stem, image in PPL; e) general view of pellet matrix from samples sized <1cm, note the rich phytolith beddings, image in PPL; f) detail of spherulite-rich matrix from pellet samples sized <1cm, note the calcium oxalates (80 µm), image in XPL; g) general view of pellet matrix from samples sized >1cm, note the micro-fragment of cellular vegetal tissue, image in PPL; h) detail of spherulite-rich matrix from pellet samples sized >1cm, note the calcium oxalates (100 µm), image in XPL. Scales between 100 and 50µm.

3.6.3 Bulk stable isotope analysis ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$)

Bulk stable isotope analysis from soil and pellet samples (undigested plant remains) show a general distribution for $\delta^{13}\text{C}$ in the range of C3 (carbon fixation pathway) plants, clustering around a mean carbon isotopic distribution of -23‰ (Fig. 3-8). Results for $\delta^{15}\text{N}$ show ranges between 5.24‰ and 10‰, with a mean of 7.9‰ (except for sample B4, with a N content too small for measurement), falling into the range of known values for shrubs and grasses in arid ecosystems (Sánchez-Zapata et al., 2007). Also, the grain or fruit tends to be depleted in $\delta^{15}\text{N}$ relative to the leaves and stems/stalks (Szapak, 2014), so

the analysed samples are more subject to be stem-rich, with abundant lignin and cellulose tissue that is difficult to digest. The general trend is a depleted nitrogen values for the pellet samples when compared to the soil samples, being this variation around a 2‰.

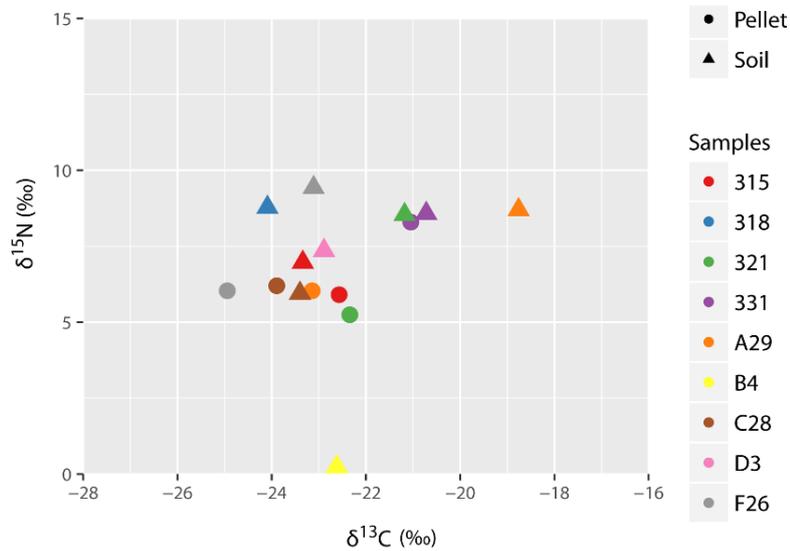


Fig. 3-8. Fig. 8. Bulk stable isotope analysis diagram showing values for soil and pellet samples. Note the general distribution of $\delta^{13}\text{C}$ (C3 plants), clustering around a mean carbon isotopic distribution of -23‰ . $\delta^{15}\text{N}$ show a mean value of 7.9‰ , falling into the range of known values for shrubs and grasses in arid ecosystems.

3.6.4 Lipid extraction and analysis (*n*-alkanes) and compound specific isotope analysis ($\delta^{13}\text{C}$)

The lipid profiles from both soil 318 and pellet 315 include medium- and long-chain *n*-alkanes (Fig. 3-9). The carbon number distribution from the plant wax-derived *n*-alkanes ranges from C_{19} to C_{35} , with a high odd over even predominance, shows predominant input of terrestrial higher plants (grasses, shrubs, and trees). The carbon-number maxima at C_{31} , both for the pellet and the soil sample, indicates a predominant presence of undigested vegetal remains originated from herbs, rather than woody plants, as shrubs or trees, being more evident in the pellet sample (Fig. 3-9). The $\delta^{13}\text{C}$ values of C_{25} , C_{27} , C_{29} , C_{31} , C_{33} and C_{35} *n*-alkanes in soil and pellet samples range from -28.3 to -33.6‰ , having the soil sample a lighter mean carbon isotopic composition (-31.6‰) than the pellet (-29.4‰) (Fig. 3-10). Moreover, all samples fall into the range of C3 (carbon fixation pathway) plants. The $\delta^{13}\text{C}$ values of C_{27} and C_{29} *n*-alkanes in the samples are consistent with the general isotopic distribution of long-chain *n*-alkanes in C3 plants previously reported (Collister et al., 1994; Lockheart et al., 1997; Chikaraishi and Naraoka, 2006; Pedentchouk et al., 2008).

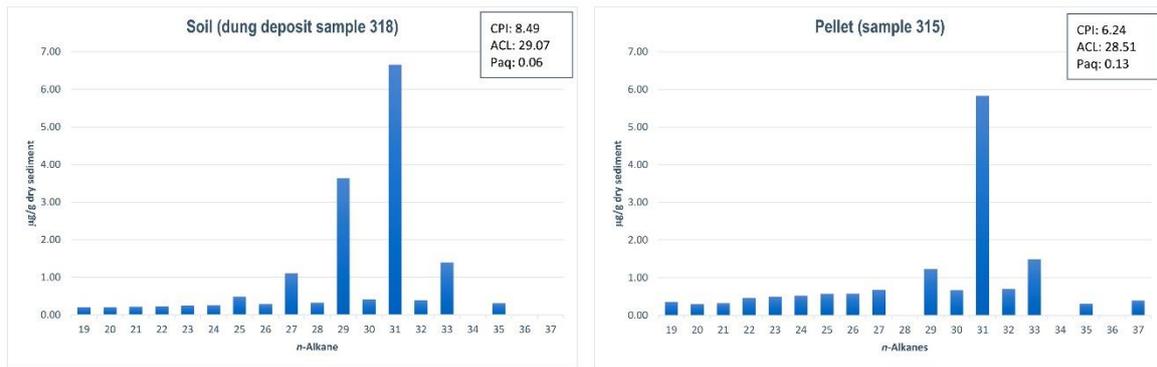


Fig. 3-9. Plant wax-derived *n*-alkanes lipid profiles from soil 318 and pellet 315 samples. Results include medium- and long-chain *n*-alkanes with a carbon number distribution with ranges from C₁₉ to C₃₅ (predominant input of terrestrial higher plants: grasses, shrubs, and trees). The mean carbon preference index (CPI) and average chain length (ACL) values show almost no difference between soil and pellet sample. The proportion of aquatic plants (P_{aq}) samples is equal or less than 0.1, meaning non emergent aquatic macrophyte input.

The mean carbon preference index (CPI) and average chain length (ACL) values show almost no difference between soil and pellet sample, with a mean of 7.36 and 28.79 respectively, which indicates good lipid preservation of higher terrestrial plants input in both samples. The proportion of aquatic plants (P_{aq}) ratio shows emergent and non-emergent aquatic macrophyte input, in the case of our samples is equal or less than 0.1, thus corroborates the terrestrial plant input with no algae or fungi input (Fig. 3-9).

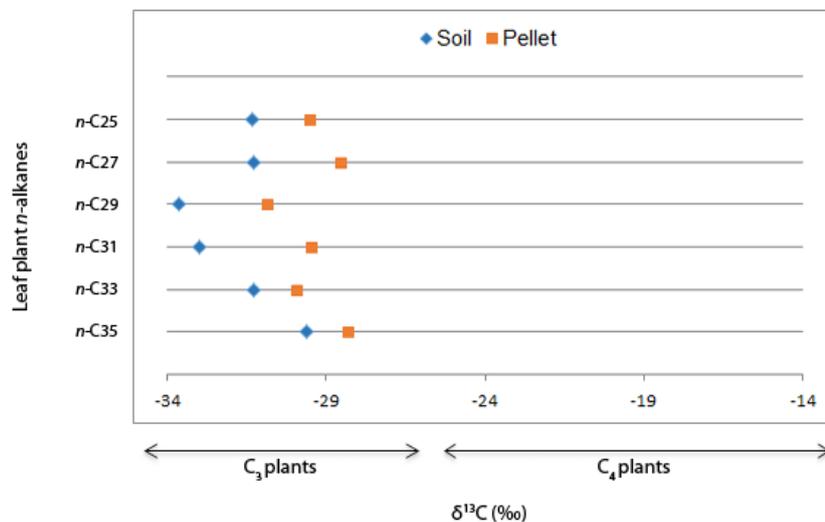


Fig. 3-10. Compound specific isotope analysis for $\delta^{13}\text{C}$ in *n*-alkanes. Values of C₂₅, C₂₇, C₂₉, C₃₁, C₃₃ and C₃₅ *n*-alkanes in soil and pellet samples show a lighter mean carbon isotopic composition of the soil (-31.6‰) than for the pellet (-29.4‰). All samples fall into the range of C₃ plants.

3.7 Discussion

Dung-derived organic matter evidence provides a tool for the investigation of patterns in seasonal movements or herd stabling among pastoral societies in arid environments. The Kel Tadrart graze their flocks along dried river valleys, where most common vegetation is made of *Aristida pungens* and *Panicum turgidum* (C₄ plants). Our results point to a clear presence of *n*-alkanes from C₃ non woody

plants, hinting at the fact that animals were present at the deposit in the time of excretion of pellets might have been fed on most likely *Zilla spinosa*. In addition, the presence of C3 woody species are observed in the soil sample (C₂₇ and C₂₉ *n*-alkanes), possibly due to the mixing and the accumulation of excrements from different animals that could have been fed also with the *Acacia* sp. trees at some point. Notably, the presence of C3 plants can also derive from the ingestion of fresh fodder bought in the neighboring villages, a common practice in case of newborns or seasonal reduced availability of natural pastures. Both possibilities, i.e the ingestion of purchased fodder or pastures of *Zilla* sp., are not mutually exclusive and point to a situation of environmental stress in the study area that could also have happened in the past. This confirms the role of TAK_06/1 as a secondary settlement (see Biagetti 2014b), to be seasonally occupied to graze on selected resources, possibly integrated by the intake of purchased fodder, likely in a very dry period, when the natural availability of other plants would have been reduced. Being not one of the Kel Tadrart main settlements, the occupation at TAK_06/1 fits into the Kel Tadrart opportunistic strategy of mobility, exploiting different locales according to the availability of natural resources. In this sense, the C3 plant signature detected in dung in a region, where C4 (or at least CAM) plants dominates the vegetal landscape (McClaran et al., 2000), can be interpreted as a proxy data for settling in the dry season or, at least, may suggest the occurrence of exceptionally arid years. Additionally, the phytolith images observed in thin section corroborate the ingestion of wild grasses or leaves. The presence of echinate long cells in the thin section is clear, being these reported for grasses and *Cyperaceae*. Also, there is a suspicious indication for the presence of dendritics (typical for the *Pooideae* in C3 grasses) but we cannot provide robust evidence, in this case, of a clear morphological identification (personal communication Luc Vrydaghs and Marta Dal Corso) because more 3D observation is needed.

$\delta^{13}\text{C}$ values for plant wax-derived *n*-alkanes show depleted numbers in the soil sample when compared to the pellet sample, being especially evident in the *n*-alkane C₂₉ (undigested woody plants, rich in lignin) and C₃₁ (undigested stems from grasses, also rich in lignin). This is indicative of decay and renewal of soil litter rich in the lignin and cellulose originated from the former living plant material, a process well observed in ecology research (Melillo et al., 1989; Wedin et al., 1995; Schweizer et al., 1999) and a potential indicator of anthropic activity at a site.

It is important to note that the organic matter is in a very good state of preservation due to the local extremely arid ecological setting. The high CPI index in both samples (pellet and soil) is similar of those regarding fresh plant material and modern soils that have not gone under strong processes of diagenesis or microbial reworking (Diefendorf et al., 2011). In addition, the CPI values, both higher than 5, show very slow microbial degradation that usually occurs in dry conditions (cold or warm) (Ortiz et al., 2010; Maheshwari, 2012). Dry conditions are also reflected in the bulk isotopic analysis of our samples. Studies in ecology show negative relationships between mean annual precipitation and foliar $\delta^{15}\text{N}$, with plants growing at arid sites with higher $\delta^{15}\text{N}$ values than those growing at wetter sites (Austin and Vitousek, 1998; Handley et al., 1999; Amundson et al., 2003). Furthermore, warmer ecosystems are characterized by higher $\delta^{15}\text{N}$ values in plants than colder ecosystems (Martinelli et al., 1999; Amundson et al., 2003; Pardo et al., 2006). Interestingly, our results show a variation of 2‰ between the mean values of the pellets and the soil samples, with an increase in the latter. This increase is probably due to

the addition of another trophic level in the soil from bacterial and/or insect decomposers source. Bulk $\delta^{13}\text{C}$ results indicate the presence of C3 plants, both in the soil and the pellets samples, although sample 315 soil shows probable input of CAM plants (plants with both pathways C3/C4).

Micromorphological analysis showed good preservation of convoluted micro-fragments of the ovicaprine pellets within the soil below the first centimeter, which is strongly affected by bioturbation and erosional processes. All diagnostic micro-features for stabling deposits (see Courty et al., 1989) are present in the soil sample: trampling, bedding, and presence of spherulites, druses, phytoliths, and micro-fragments of coprolites. The horizontal bedding of materials contained in the mid-lower layer is interpreted as evidence of trampling because of the compaction of organic particles with in situ breakages on the vegetal remains and the observation of a tendency towards a subangular mineral shape, suggesting some degree of crushing derived from human and/or animal trampling (Akeret and Rentzel 2001; Miller et al. 2009; Driscoll et al., 2014). The presence of large spherulitic calcium oxalates indicates the ingestion of a woody plant species producer (probably *Acacia* sp.), as the higher amounts of these are present in the pellets themselves and not mixed in the matrix sediment. Therefore, a post-depositional origin could be discarded although more research needs to be done in order to study oxalate amount and size production for Saharo-Arabian plants. On the contrary, the presence of isolated gypsum scatters that were identified at the very bottom of soil sample 318, relates to post-depositional and taphonomical processes. Gypsum has been previously associated with stabling deposits (Karkanis and Goldberg 2010; Égüez et al., 2016; Zerboni et al., 2017), where organic matter rich also in nitrites is accumulated. This organic matter reacts with the calcium carbonate available in the water that percolates to the bottom of the deposit and starts to precipitate the gypsum scatters in form of infillings, coatings, or crystals in a process that is very slow.

3.8 Conclusions

The recent development of geo-ethnoarchaeological studies dwells in a renewed enthusiasm for the relation between the present (ethno) and the past (archaeo), support by new analyses and approaches on hard data (geo). For many years archaeologists have been informed and misinformed by the observation of the ethnographic present, and in the very last decades geoarchaeologists as well have forayed into living communities looking for a methodological framework and references data, aiming at clarifying the relationships between human behavior and material by-products. Especially in the study of pastoral societies, geo-ethnoarchaeology has broken disciplinary barriers and opened new horizons for the interpretation of the allegedly 'invisible' and 'ephemeral' campsites. Precisely, this ongoing research is generating a more meaningful and accurate dataset to inform on and link back to archaeological material. The potential of the study for stabling deposits through a multi-proxy approach that combines the micro-scale and the molecular one is clear. The results of our research are relevant at the local scale, representing a comparison sample to interpret archaeological dung layers, whose investigation presented good results in terms of depositional and post-depositional formation processes (Cremaschi, 1998; Cremaschi and Zerboni, 2011). Nonetheless, there remain many unresolved questions in terms of landscape exploitation, patterns of mobility, and natural resources management across the Holocene. Ultimately, our data enables the design of approaches to dung deposits in drylands

worldwide, combining ethnoarchaeological and experimental data to explore the uniquely by-product bio-signatures of past and present animal husbandry on the ground.

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3.9 References

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Chapter 4

4 Carbon isotope ratios of plant *n*-alkanes and microstratigraphy analyses of dung accumulations in a pastoral nomadic winter campsite (Eastern Mongolia).

Abstract

Livestock faecal remains provide an important source of information on animal husbandry systems and use of dung in past societies. A combined micromorphological and biomolecular investigation of dung deposits brings new perspectives into past landscape land use and animal husbandry strategies by providing seasonal-scale information on livestock dietary intake as well as intensity of dung deposition in penning spaces. Here, we conducted microstratigraphic analyses and compound-specific carbon stable isotope analysis of plant *n*-alkanes of dung deposits associated with pastoral nomadic winter campsites in Mongolia in order to explore the floral origin of graze ingested by livestock and evaluate potential biomolecular signatures associated with the intensity and duration of dung deposition. Preliminary results suggest that intensity of stalling and the dietary composition of grazing herbivores are identifiable in dung samples recovered from corrals with this multi-proxy method. The $\delta^{13}\text{C}$ values of plant *n*-alkanes in dung deposits were unusually depleted compared to carbon isotope values of plant *n*-alkanes in soil control samples recovered from landscapes with minimal, if any, anthropic activity in the same ecoregion. These preliminary results highlight the importance of ethnoarchaeological studies in identifying biomarkers that convey information on pastoralist animal exploitation practices.

4.1 Introduction

Dung is a renewable secondary product rivaling in importance with other ante-mortem products of milk and fibre and is used by people to fertilize their crops, build their homes, and fuel their fires. Livestock dung is particularly important for pastoral nomads inhabiting treeless steppes or desert landscapes, providing both building material and a readily accessible fuel source that requires little, if any, processing before use.

Studies of dung recovered from the archaeological record rely heavily on ethnoarchaeological studies in order to link the precise activities involved with dung use and deposition with material correlates present in the archaeological record. In her seminal work exploring the use of dung as fuel in Elamite Bronze Age Iran, Miller (1984) observed plant use and disposal practices in an ethnographic context that helped identify the use of dung cakes for fuel on the basis of abundant and diverse weedy taxa present in archaeological hearths. Her work not only brought attention to the complex origins of palaeobotanical assemblages and the role of livestock in creating those assemblages – but, critically, also highlighted the importance of ethnoarchaeological studies as key to identifying dung use in the past. Subsequent work drew further from the ethnoarchaeological record in order to expand the repertoire of analytical tools available for identifying the presence of dung in archaeological deposits

(Jones 1984; Canti 1997, 1998; Alexandre et al. 1997; Matthews 2005; Shahack-Gross and Finkelstein 2008; Shahack-Gross 2011; Elliott et al. 2015). Carbonized seed remains and phytoliths recovered from purported dung deposits have also supplied crucial information on the taxonomic composition of browse and graze ingested by herbivorous livestock and seasonality of livestock pasture use and foddering practices (Portillo and Albert 2011).

Dung deposits are a characteristic feature of animal enclosures, which consist of defined areas where livestock are corralled on a regular basis in order to keep animals together (or apart) for breeding, milking, or to protect herds from the elements and wild animals. However, analytical approaches to identify specific activities associated with animal husbandry in archaeological contexts (such as intensity of livestock penning, re-use, cleaning, breaking of dung deposits, corraling of different livestock species, and foddering) remain relatively few. Here, we explore the utility of compound-specific carbon stable isotope analyses of *n*-alkanes from organic matter in dung deposits and micromorphology of dung remains recovered from livestock enclosures located in winter camps of contemporary Mongolian pastoralists in order to identify repetitious use of penning area and also to define taphonomical processes and stratigraphic deposition events in order to understand nomadic seasonal occupations and animal diet.

4.1.1 Micromorphological and organic chemistry approaches to identify corraling

Micromorphology has provided a means to better understand the regularity of dung deposition in corral environments in archaeological settings through identification of rich organic matter sediment microlayers. The number of microlayers present are indicative of successive occupation events (e.g. Courty et al. 1991; Brochier et al. 1992; Polo-Díaz 2009; Égüez et al. 2016; Alonso-Eguiluz et al. 2017). Dung deposits in archaeological sediments are isolated using a suite of characteristic features contained in microlayers, including amorphous organic matter, organic fibers, phytoliths, and spherulites (Macphail and Goldberg, 1995; Shahack-Gross et al. 2003). Corraling deposits are sharply distinct from natural sediment deposits in both their mineral composition and micro-structure. Natural sediments typically consist of a matrix comprised primarily of clay and silt mineral particles, low in organic matter, and homogenous in structure (i.e., lacking microlayers). Corraling deposits are characterized by laminated deposits with compacted and/or trampled layers, close packing and horizontal alignment of undigested vegetal remains, amorphous organic matter, massive presence of calcitic spherulites, opaline phytoliths and, calcium oxalate phytoliths (Brönnimann et al. 2017). Micromorphological analyses of dung deposits provide information on the frequency (number of microlayers) and intensity of animals corralled (the degree of trampling) (Miller et al., 2009; Miller and Sievers 2012).

Carbon and nitrogen isotopic analyses of undigested plant material recovered from sediments have also been used to identify dung deposition associated with livestock corraling and the dietary intake of penned animals (Shahack-Gross et al., 2008). One major challenge, however, in the application of carbon and nitrogen analyses to archaeological sediments in which dung is thought to have been deposited, is decoupling natural process isotopic change in soil nitrogen and inorganic carbon pools from human-mediated inputs in the form of animal penning and dung deposition (Linseele et al. 2013). Bulk stable isotope analysis ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of archaeological dung contexts have been seldom explored in part due

to the pre-existing inorganic carbon contained in the sediment and the nitrogen input (e.g. bacteria, insects or mesofauna) that could affect the isotopic composition of bulk dung (Staddon, 2004; Hobbie and Ouimette, 2009).

Finally, plant cuticular *n*-alkanes contained in the dung itself offer the potential to characterize the biosignatures associated with the intensity of animal corralling and the duration of dung deposition and plant diet origin. Alkanes are hydrocarbon molecules that are largely derived from epicuticular waxes of vascular higher plants (Eglinton and Hamilton, 1967; Rieley et al., 1991). These molecules form linear chains (*n*-alkanes) each with a different number of carbon atom (C-number). Most plant species have a characteristic pattern of alkane concentrations in their cuticular wax, with different species producing varying quantities of alkanes depending on season of growth. Therefore, the concentration pattern and distribution of *n*-alkanes in feces and plants permits the estimation of diet composition (Dove and Mayes 1996, 2005). For instance, *n*-alkanes have been successfully used to estimate diet composition in domestic animals (Duncan et al. 1999; Dungait et al., 2010; Égüez et al. 2018) and, less frequently, of wild herbivores (Bugalho et al. 2001, Hulbert et al. 2001). *N*-alkanes analysis have been also widely applied to study grassland ecosystems in Africa, South America and Western Eurasia but systematic regional studies on modern plant and topsoil material in Central Asia are still largely lacking (Strömberg, 2011), although recent attention has focused on grassland degradation and over-exploitation of grassland ecosystems in Inner Mongolia (Srivastava and Wiesenberg, 2018).

4.1.2 Livestock corralling by pastoral nomads in Mongolia

Livestock corrals are key pieces of pastoralist infrastructure regularly used by mobile herders in Mongolia today. In Sükhbaatar *aimag* (province) in the steppe-desert of southern Mongolia, herders graze their flocks during the summer months in distant pastures located about 40-50km from their winter campsites, accumulating fodder for winter from both summer and winter areas. Sheep, goats, cattle and horses are penned through the winter season (early November to late April) in multi-structured corrals built of wood or dung. Livestock is usually segregated by species, sex, and age. In Mongolia, livestock dung is the primary fuel source used by pastoral nomads even in regions where wood is abundant (Lkhagvadorj et al. 2013). While herders collect cattle and horse dung spread over the landscape for fuel for immediate use and store it in heaps outside of their *gers*, much of their winter fuel is provided by the dung harvested from thick accumulations deposited in the corrals during the winter months. Using shovels, herders remove compact dung in large blocks of up to 20 cm. height, which are used either as fuel, which burns steadier than loose dung, as well as for bricks used in the construction of the corrals (Figure 4-1). This cleaning, which takes place annually during the last months of summer or over every few years, also permits reuse of penning spaces.

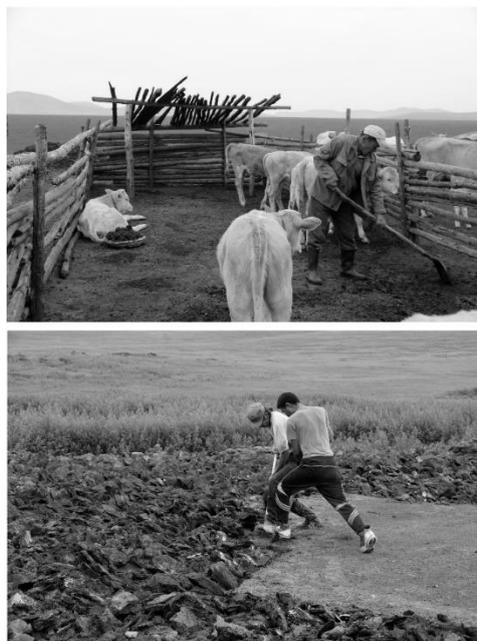


Fig. 4-1. Herders use shovels to collect cattle dung from the corrals and to remove the compact dung in large blocks to use it when dry as fuel.

4.2 Materials and Methods

4.2.1 Sampling and research area

Corral dung samples were obtained from one winter campsite (Campsite 46) located in the volcanic plateau of Dariganga *sum* in Sūkhbaatar *aimag* (Figure 4-2). Dariganga *sum* is situated within the Mongolian-Manchurian grassland steppe in a temperate grassland biome. The climate is strongly seasonal with winter temperatures averaging -23°C during the winter and $+28^{\circ}\text{C}$ during the summer months. The maximum annual precipitation is 100–150 mm the majority of which falls during the summer season (mid-May till late August) (Pyankov et al. 2000). Vegetation communities in eastern Mongolia consist largely of C_3 taxa, mostly annual grasses, although C_4 chenopods and grasses are also abundant. Pea shrubs (*Caragana microphylla*, *C. stenophylla*, *C. pygmaea*) and sagebrushes (*Artemisia* spp.) are also present. *Artemisia scoparia*, *A. macrocephala*, *Chenopodium album*, *Hyoscyamus niger*, and *Urtica cannabina* (Gunin et al. 1999) also flourish in the disturbed and organic rich soils surrounding winter camps.

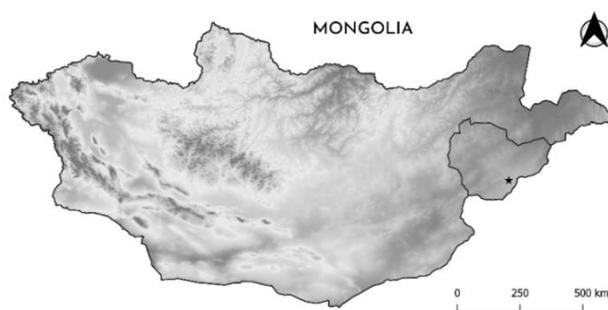


Fig. 4-2. Situation map of the campsite in the province of Sūkhbaatar, Eastern Mongolia.

To our knowledge, no data on the compound-specific carbon isotope composition of plant wax in Mongolia have been reported.

Campsite 46, approximately 250 m² in size, has been used on a yearly basis for at least three generations of the same family. The campsite is composed of 8 different features, including 1) the main corral, a rounded enclosure with a dung wall, that was used to gather goat and sheep; 2) a ovicaprine dung area that surrounds the main corral; 3) an adjacent small corral, also built with dung and used to gather goat and sheep; 4) another adjacent small corral but with visible horse dung on the surface; 5) a dung pile, or accumulation of cow and horse dung pellets in the western area of the campsite; 6) a dung pile of cow and horse dung pellets in the north area of the campsite; 7) a fodder corral, protected with wire; 8) a burnt area of different debris (bone, dung) (Figure 4-3).



Fig. 4-3. Detail of the sampled different features: 1) the main corral, a rounded enclosure with a dung wall; 2) an ovicaprine dung area that surrounds the main corral where vegetation has grown; 5) a dung pile in the western area of the campsite; 6) a dung pile of cow and horse dung in the north area of the campsite; 7) a fodder corral, protected with wire.

In order to explore possible differences in intensity of corral use and the composition of the diets of the livestock that used these corrals, we selected samples from two campsite features. In the main corral (feature 1) samples were taken from both the soil surface sediment (between 2 and 8 cm deep) which had a high content of dung remains and the dung wall of the corral. In the adjacent small corral (feature 3) samples were taken from surface sediments (between 2 and 8 cm deep) which had a high content of dung remains. Loose soil samples were extracted from the soil surface of the two features and a caprine dung pellets sample was recovered from the dung wall of pen 1. A control sample was extracted from the surface of a non-anthropogenic area that sits in the alluvial plane of Dariganga ca. 10km away from the campsite (Figure 4-4).

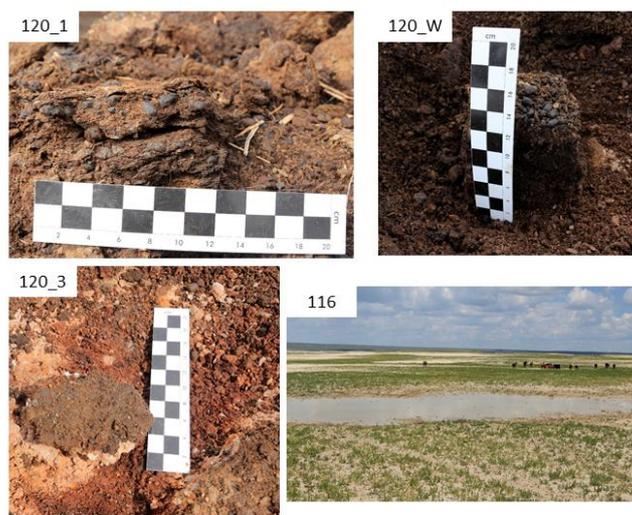


Fig. 4-4. Detail of the four samples analysed: 120_1 a block selected from feature 1, a rounded enclosure; 120_W the dung wall associated with the main corral; 120_3 an adjacent small corral also built with dung and used to gather goat and sheep; 116 control sample extracted from the surface of a non-anthropogenic area that sits in the alluvial plane of Dariganga ca. 10km away from the campsite.

4.2.2 Micromorphology of thin sections

We undertook micromorphological analyses of two thin sections. One, prepared from a sample block selected from feature 1, a rounded enclosure (sample 120_1), with a dung wall (sample 120_W) associated with the main corral (feature 1). A control sample was extracted from the surface of a non-anthropogenic area that sits in the alluvial plane of Dariganga (sample 116) ca. 10km away from the campsite (Fig. 4-4). The dung and control sediment blocks were embedded in epoxy resin, cut with an IsoMet low speed saw, mounted on glass slides, cut to a thickness of 1 mm, and the polished to 30 μm . After production, the slides were scanned on a flatbed scanner at high-resolution (1.200 dpi) in reflected light to allow detailed observations at the mesoscopic level (Arpin et al., 2002).

Microscopic analysis was performed on a polarizing microscope (Nikon AZ100) at $\times 20$, $\times 100$, $\times 200$ and $\times 400$ magnifications under plane-polarized light (PPL) and crossed polarized light (XPL) for the observation of isotropic and anisotropic materials. Microphotographs were taken using a Nikon DS Fi2 camera. Micromorphological descriptions were carried out following guidelines from Bullock et al. (1985) and Stoops (2003), whereas interpretative concepts are from Courty et al. (1989) and Stoops et al. (2010). The microfacies described were defined by groundmass, microstructure, organic and inorganic composition, and pedofeatures regarding lithological changes within an individual thin section (Goldberg et al., 2009).

4.2.3 Lipid extraction and analysis (*n*-alkanes) and compound specific isotope analysis ($\delta^{13}\text{C}$)

We analysed two loose soil samples from campsite 46, extracted from the soil surface of two different penning features (1 and 3) and a caprine dung pellets (named 120_W) sample recovered from the dung wall of pen 1. Another loose soil sample was extracted from the block of the control sample number 116 (Fig. 4-4). 2g of each of these sediment samples were dried at 60°C during 48h, then ground

and homogenized. Non-volumetric material (including glassware and glass wool) was calcined at 450 °C during 10 h before extraction to eliminate any possible organic compounds. Lipids were extracted in 20mL 9:1 dichloromethane/methanol following a modified version of a previously published methodology (Bhattacharya et al., 2017). 3 cycles of 30 minutes sonicator and 10 minutes centrifuge (4700rpm) were applied to the mixture. The total extraction was then evaporated and separated using solid phase extraction (SPE) via silica gel column chromatography into six fractions of different polarity. In this paper, we present results for the *n*-alkanes fraction, eluted with 3/8 of dead volume in *n*-hexane. After addition of internal standard (IS) 5 α -androstane 8mg/L the volume was completed with 150 μ L of DCM.

The compounds in the *n*-alkane fraction were identified and quantified by gas chromatography with a coupled detection and mass-selective detector (GC-Agilent 7890B, MSD Agilent 5977A) equipped with an HP-5MS capillary column (30m, ID: 250 μ m, film thickness 0.25 μ m). The GC was programmed to an initial temperature of 70 °C for 2 min, heated with a heating rate of 12 °C/min to 140 °C and to final temperature of 320 °C with a heating rate of 3 °C/min and held for 15 min, with helium as the carrier gas (2 mL/min). The total run time was 82.83 min to avoid overlap or interferences with other compounds. The multimode injector was held at a split ratio of 5:1 at an initial temperature of 70 °C during 0.85 min and heated to 300 °C at 720 °C/min. All measurements were repeated two times. The MS was operated in full scan mode (*m/z* 40–580) with an electron ionization energy of 70 eV and with the temperatures of the ion source and quadrupole set at 230 °C and 150 °C, respectively. Quantification was carried out taking the four most intense fragment ions (*m/z* 43, 57, 71 and 85, and *m/z* 67, 95, 81 and 245 for IS) for *n*-alkanes and the total ion chromatogram for the rest of the analytes. Compounds were identified by comparison of their retention times and mass spectra with those of reference compounds (mix C8-C40 alkanes and 5 α -androstane, Supelco) and comparison with the NIST mass spectra library. Quantification was based on calibration curves obtained by plotting the ratio Area/Area_{IS} versus the concentration of each reference compound. Correlation coefficients were higher than 0.995. The *n*-alkane concentration is expressed as μ g of individual compound per gram of dry sample (μ g gds⁻¹).

Carbon isotope analysis by GC-IRMS was performed using a Thermo Scientific Isotope Ratio Mass Spectrometer Delta V Advantage coupled to a GC Trace1310 through a Conflo IV interfaced with a temperature converter GC Isolink II. Samples were injected by means of a MultiMode injector (MMI) in splitless mode, with the temperature increasing from 79 °C (held 0.5 min) to 325 °C (held 3min) at a rate 10 °C·s⁻¹ and finally to 350 °C (held 3min) at 14 °C·s⁻¹. The GC was fitted with a Trace Gold 5-MS (Thermo Scientific) fused silica capillary column (30 m length x 0.25 mm i.d., 0.25 μ m film thickness). Helium was used as the carrier gas at a flow rate set at 1.5 mL·min⁻¹. The combustion reactor temperature was maintained at 1000 °C. The temperature programme comprised a 2 min isothermal period at 70 °C increasing to 140 °C (held 2 min) at a rate of 12 °C·min⁻¹, followed by an increase period to 320 °C (held 15 min) at 3 °C·min⁻¹. Data acquisition and processing were carried out using the Isodat 3.0 software (Thermo Scientific). All the δ ¹³C values were normalized to VPDB (Vienna Pee Dee Belemnite) using an *n*-alkane Schiimmelmann type A6 mixture (*n*-C16 to *n*-C30). The standard deviation of carbon isotope measurements was better than 0.5‰.

The *n*-alkane extraction, identification and quantification were carried out at the Archaeological Micromorphology and Biomarkers - AMBI Lab of the Instituto Universitario de Bio-Organica Antonio González at Universidad de La Laguna, Spain.

To evaluate the *n*-alkane distribution we calculated the Carbon Preference Index (CPI) using the Bray and Evans equation (1961) and the average chain length (ACL) was calculated following the recommendation of Freeman and Pancost (2014).

4.3 Results

The thin section of the block selected from feature 1 (soil sediment surface) exhibited two microfacies (M1 and M2) with a very diffuse contact between these. Mineral size distribution was quite heterogeneous, ranging from 1mm to 1 cm in size, being coarser and subangular in the lowest microfacies. Mineral and organic materials were horizontal and trampled, most visible at the bottom (M2), where elongated pores and channels along some well-preserved phytolith beddings were present. The preservation of vegetal remains was good: seed fragments, phytoliths and stems were visible along with some vegetal tissue with preserved cellular structures. In addition, a high density of caprine dung, both whole and fragmented pellets, and spherulites were visible, especially in M2. The coprolite pellets were characterized by a convoluted pattern characteristic of ovicaprine dung remains with similar content in all of them including vegetal tissue (e.g. cellulose, lignin, phytoliths, and oxalate druses) and spherulites (Figure 4-5). No gypsum infillings were identified indicating that no active leaching of CaCO_3 occurred despite the high organic matter accumulation.

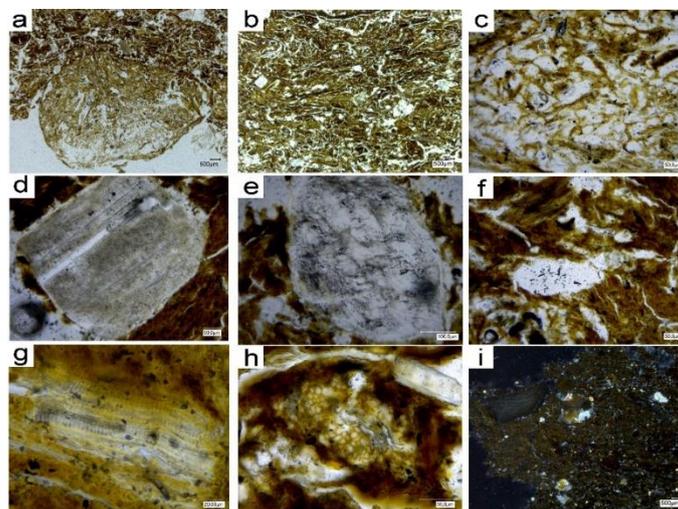


Fig. 4-5. Microphotos of thin section from the block recovered in campsite 46 (main corral). a) preserved ovicaprine coprolite, note the convoluted pattern of the partially digested plant tissue. In PPL; b) general view of the horizontal disposition of the elements contained within the matrix, also indicating trampling action. In PPL; c) detail of humified amorphous vegetal material. In PPL; d) detail of a subrounded feldspar grain. In PPL; e) detail of a subrounded quartz grain. In PPL; f) detail of mesofaunal activity (earthworm). In PPL; g) detail of long articulated phytoliths. In PPL; h) detail of grass stem in a cortical view. In PPL; i) detail of massive fecal spherulites, some obscured by organic matter. In XPL. Scale is in μm .

The thin section of control sample (116) revealed a very low organic content with the sample characterized by a ca. 80% mineral fraction. The mineral size distribution is heterogeneous, consisting of mostly rounded and subrounded 1mm-sized quartz grains consistent with a strong aeolian and

alluvial deposition. The matrix is composed of fine silty clay and displays desiccation marks from freezing and defrosting cycles. The sample shows characteristics of the natural sedimentation and low permeability of the saline deposits of periodic alluvial fans, characteristics of this area.

The lipid profile from the central penning feature includes long-chain *n*-alkanes. The carbon number distribution from the plant wax-derived *n*-alkanes show a range of C₂₇-C₃₃ with a predominance of odd carbons. C₃₁ is most abundant with a concentration of 80 µg/g of dry sediment. The isotopic composition of the δ¹³C *n*-alkanes fall into the range of C₃ plants with a total mean of -34.88‰ over the four *n*-alkanes measured (Figures 4-6 and 4-7).

The lipid profile from the ovicaprine pellets recovered from the dung wall of penning feature 1 show the presence of medium- and long-chain *n*-alkanes. The carbon number distribution from the plant wax-derived *n*-alkanes show a range of C₁₉-C₃₅ again a predominance of odd-numbered carbons. The C_{max} is at C₃₁ with a concentration of 79 µg/g of dry sediment. The isotopic composition of the δ¹³C *n*-alkanes fall into the range of C₃ plants with a total mean of -34.27‰ (Figures 4-6 and 4-7).

The lipid profile from the small penning feature number 3 includes long-chain *n*-alkanes. The carbon number distribution from the plant wax-derived *n*-alkanes show a range of C₂₇-C₃₃, with odd over even predominance. The maximum is in C₃₁ with a concentration of 60 µg/g of dry sediment. The isotopic composition of the δ¹³C *n*-alkanes fall into the range of C₃ plants with a total mean of -34.88‰. See Fig. 4-6 and 4-7 for detail.

The lipid profile from the sediment control sample include medium- and long-chain *n*-alkanes. The carbon number distribution from the plant wax-derived *n*-alkanes show a range of C₁₇-C₃₅ with even over odd predominance. There is no C_{max} as the profile shows a flat pattern diagnostic of high natural degradation of the organic matter. Notably, the concentration of organic matter is very low, 0.5 µg/g of dry sediment. The isotopic composition of the δ¹³C *n*-alkanes fall into the range of C₃ (carbon fixation pathway) plants with a total mean of -32.62‰ (Figures 4-6 and 4-7).

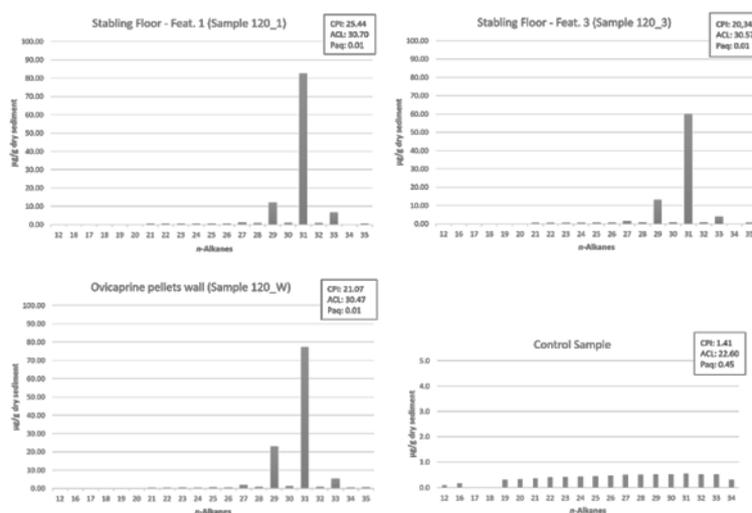


Fig. 4-6. Detail of *n*-alkanes histograms of the four samples analyzed with CPI, ACL and Paq values. The carbon number distribution from the plant wax-derived *n*-alkanes for all samples, except the control sample, show a range of C₂₇-C₃₃ with a predominance of odd carbons. C₃₁ is also the most abundant for the anthropic features. Control sample include medium- and long-chain *n*-alkanes. Notably, concentration of organic matter is very low, 0.5 µg/g of dry sediment, when compared with the penning features.

We used the carbon preference index (CPI), which measures the relative abundance of odd over even carbon chain lengths, and the average chain length (ACL), which is the weighted average of the various carbon chain lengths, to better characterize the *n*-alkanes distribution. The CPI and ACL ratios are far higher than 1 (mean 22.28) in all samples from the penning features of the winter campsite. In contrast, the control sample shows a CPI ratio of 1.41.

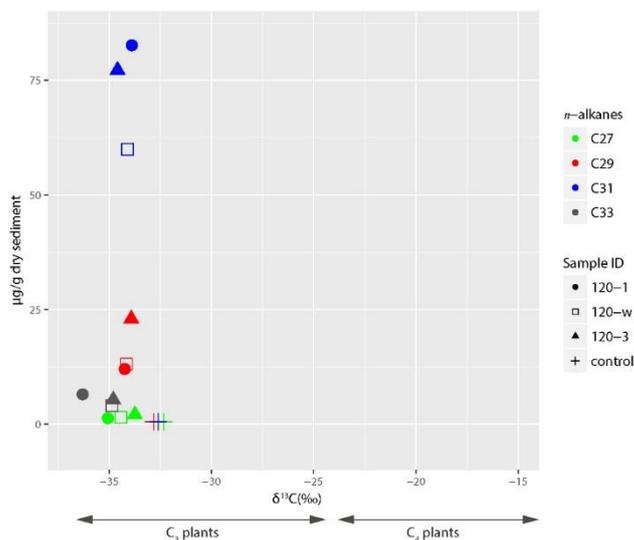


Fig. 4-7. Biplot showing the carbon isotope values from C_{27} , C_{29} , C_{31} and C_{33} alkanes (colors) from the four samples analysed (shapes) in relation to their concentrations ($\mu\text{g/g}$ of dry sediment). The isotopic composition of the $\delta^{13}\text{C}$ *n*-alkanes in all samples fall into the range of C_3 plants. Note the difference in $\delta^{13}\text{C}$ values exhibited by the control sample and the depleted dung deposits. C_{max} of alkane C_{31} is observed for all the dung related samples in contrast to the control sample. The indicative whole plant $\delta^{13}\text{C}$ ranges for C_3 and C_4 plants in arrows are values for Central Asian grasslands (Wittmer et al. 2008).

4.4 Discussion

Alkanes are present in soils and sediments and are relatively resistant to biodegradation due to their hydrophobic and indigestibility condition (Otto and Simpson, 2005). The distribution of C-numbers in *n*-alkanes within soil and sediments deposits identifies its source material with terrestrial higher plants producing long-chain *n*-alkanes ratios (carbon number C_{27} to C_{35}), while aquatic plants exhibit short- and medium-chain *n*-alkanes ratios (C_{17} to C_{25}); both aquatic and terrestrial plants exhibit a predominance of odd C-numbers. The presence of even C-number alkanes in terrestrial and aquatic plants is uncommon, and when present in sediments, is usually related to bacterial, microbial and algal detritus natural degradation and its microbial or geochemical alteration (Bush and McInerney, 2013). The carbon number distribution from the plant wax-derived *n*-alkanes from our penning features ranges from C_{27} to C_{33} with a high odd C over even predominance, and show predominant input of terrestrial higher plants (as grasses, shrubs, or trees) (Carr et al., 2014). Remarkably, the presence of C_{max} at C_{31} in all penning features may indicate that animals fed largely on C_3 grasses during the winter months. The predominance of total alkane concentrations of C_{31} in winter season dung deposits may reflect the seasonality of harvest of winter fodder. Grass species, such as *Stipa* sp and *Artemisia* sp. have their maximum total concentration of *n*-alkanes during summer (July and August) due to the increase of solar radiation in these months (Lin and She 2006; Lin et al., 2012). Indeed, the carbon-number maxima at C_{31} suggests a predominant presence of undigested vegetal remains originated from herbs and grasses,

rather than woody plants as shrubs or trees (Cranwell, 1973; Cranwell et al., 1987). This suggestion is in accordance with our ethnographic interviews reported in the field, in which herders stated that animals in winter, especially cattle, are only fed on grasses provided as fodder. In the same line, Makarewicz (2014) reported that Mongolian pastoralists in the Gobi steppe-desert collect fodder, typically grasses and some chenopods, during the late summer and store it in their winter camps to feed select animals during the winter months. Moreover, these results are congruent with those from the few studies on *n*-alkane patterns for grasses available for the Inner Mongolia (China) region, where C₃₁ was detected being always the carbon number maxima for herbaceous plants like *Stipa* sp. and C₂₇ and C₂₉ for woody plants like *Phragmites* sp. (Duan and He, 2011). Previous research comparing long-chain *n*-alkane ratios with pollen records reports C_{max} at C₂₇ and C₂₉ for a woody plant proxy and C₃₁ and C₃₃ for a grass proxy (Cranwell, 1973; Schwark et al. 2002; Meyers, 2003; Bai et al. 2009). Nonetheless, Duan and He (2011) point out some inconsistencies between their findings and previous reports of herbaceous plants' C_{max} of C₃₁ and woody plants' C_{max} of C₂₇ or C₂₉, and more recently, Bush and McInerny (2013) specifically caution about the use of the relative abundance of long-chain alkanes to distinguish between input from graminoids and woody plants. In the light of these potential constraints, our current ongoing work is aimed to strengthen our hypothesis of a foddered grass diet by analysing *n*-alkanes from grasses and shrubs commonly used for fodder in this region (Égüez et al., in preparation).

As stated in the results section, to assess the source biomass and degree of degradation we used the carbon preference index (CPI) and the average chain length (ACL). Our results, higher than 1 (mean 22.28), indicates good lipid preservation of higher terrestrial plants input in all samples from the penning features of the winter campsite (Eglinton and Hamilton 1967). In contrast, the control sample shows a CPI ratio of 1.41, a value which is close to algae and fungi input values and indicative of degraded organic matter (Cranwell 1981). However, we acknowledge that CPI values could also be influenced by climatic factors (Sachse et al., 2004). To further investigate this possible aquatic or bacterial source of organic matter in the sediment we used the P_{aq} index, that indicates submerged fungal and floating aquatic macrophyte input if higher than 0.1 (Ficken et al., 2000). The control sample has a P_{aq} index of 0.45, thus corroborating this fungal input of organic matter that is also mixed with the terrestrial one when the alluvial plain receives the annual little rainfall. P_{aq} ratios from the winter campsite samples are 0.01, confirming a high terrestrial plant input with no algae or fungi input.

4.4.1 $\delta^{13}\text{C}$ signature of stabling deposits

The extremely low $\delta^{13}\text{C}$ values visible in plant *n*-alkanes recovered from winter camp dung deposits (-32.35 to -36.30‰) slightly differ from the expected lower edge range values for the arid environments of the Gobi steppe-desert (Fig. 4-7). Comparative *n*-alkane $\delta^{13}\text{C}$ values in Inner Mongolia for C3 plants average -27‰ with a range of -22 to -33‰ reflecting in large part physiological responses to aridity and, for C4 plants an average of -13‰ and smaller range of -9 to -16‰, reflecting small-scale response to environmental factors (Gao et al., 2004; Lin and She 2006; Wittmer et al. 2008, 2010; Lin et al. 2012). Nonetheless, Diefendorf and Freimuth (2017) established that the mean *n*-alkane fractionation factor (i.e. the difference between the $\delta^{13}\text{C}$ values of alkanes and whole leaf) is between -4.8 and -6.3‰ for graminoids and between -6.9 and -8.2‰ for forbs. Thus, our results are likely within the normal range

for C3 plants. Likewise, the depleted $\delta^{13}\text{C}$ values in our samples may also reflect diagenetic alteration of the in vivo carbon isotope signature in the burial environment. Precipitation and aridity levels strongly influence plant carbon isotope values with C3 plants enriched ca 1-3‰ in ^{13}C when under water stress (Korol et al. 1999; Wang et al. 2010).

Major biochemical components of plant tissues, such as polysaccharides or cellulose, are isotopically distinct due to differences in carbon isotopic fractionation rates for different tissues (see Benner et al. 1987). In our samples, recovered from a low rainfall area, plant wax-derived *n*-alkanes from penning features are depleted ca. 2-3‰ in $\delta^{13}\text{C}$ compared to the wall pellets sample and ca. 2-4‰ when compared to the control sample. This pattern is particularly pronounced in the *n*-alkane C_{29} (undigested woody plants, rich in lignin) and C_{31} (undigested stems from grasses rich in lignin). A predominance in dung of ^{13}C depleted plant compounds such as lignin typical for woody C3 plants may explain the low $\delta^{13}\text{C}$ values observed in the Mongolian winter camp dung deposits (Hobbie and Werner 2004). Moreover, the 2–3‰ enrichment in ^{13}C visible in the control sample when compared to the dung (both in the wall and corral surface) samples is also explained by the low content of organic matter and, especially low lignin levels, within the natural sediments of this region. However, this does not necessarily mean that livestock dietary intake is mostly composed of woody plants as C3 shrubs, as the lipid results show a major input of C3 ingested grasses. Instead, low $\delta^{13}\text{C}$ values are likely due to high concentrations of undigested plant remains of the grasses that were deposited in the sediment, which are rich in lignin and cellulose as those are the most abundant structural materials of plants found in herbivore dung. Plant lignin-C is strongly depleted in ^{13}C when compared with the bulk plant material by up to 2.5% in legumes and up to 4.7% in grasses (Schweizer et al. 1999).

In addition, in our samples, continuous litter deposition in sediment that is rich in lignin seems to be affecting the carbon fractionation of decaying undigested plant material, as the extreme low values of -36 to -34‰ from the penning deposits are abnormal for natural soils in this arid grassland eco-region. Control sample from the alluvial plain has values of -32‰, which is in accordance with the reported range values for the Chinese Inner Mongolian and northern China grasslands.

The mechanism that governs the observed depletion in ^{13}C of lignin likely derives from isotopic discrimination associated with two amino acids: phenylalanine and tyrosine. As tyrosine is heavily depleted in ^{13}C and is a major precursor of lignin in grass species, it explains the larger depletion in lignin-C of the grasses analyzed in this study. Continuous and massive accumulation of lignin from ingested grasses in the penning structures influence the isotopic fractionation during decomposition. This massive accumulation would produce a recycling unit in which the resulting litter is more depleted in ^{13}C over time because of the fast loss of more isotopically enriched soluble constituents (Asada et al. 2005). Also, a recycling effect has been found most often in vertical variations of stable isotope composition in tropical forests where both plant tissues and litter in the forest floor are more depleted in ^{13}C (Benner et al. 1987; Cotrufo et al. 2013).

Isotopic values are indicative of a continuous decay and renewal of soil litter rich in the lignin and cellulose that originated from the former living plant material, being the previous explained process well observed in ecology research (Wedin et al. 1995; Schweizer et al. 1999). The variation in $\delta^{13}\text{C}$ values between dung samples obtained from different places within the winter camp may be due to differences

in the intensity of corral use. Lower $\delta^{13}\text{C}$ values, as observed for sample 120_1 (-34 to -36) may indicate more animals were gathered and hence deposited more dung and possibly in thicker layers in corral number 1. This would be in accordance with the micromorphology analysis, which shows for this sample very strong trampling features including layering and in situ breaking of vegetal micro remains. The $\delta^{13}\text{C}$ values 1-2‰ lower observed in corral 2 from sample 120_3, meaning less decomposing organic matter in the soil and therefore, fewer gathered animals in the corral at the time of deposition.

4.4.2 Challenges for future research

While holding great potential, the application of soil lipids as molecular proxies need to take into account intra- and inter-plant variability in the molecular composition of plant-derived organic matter, and the transformation or degradation of (some of) the molecules once present in the soil. Effectiveness of *n*-alkanes biomarkers depends in part on characterization of *n*-alkane profiles at regional and local levels as environmental conditions may influence the pattern of the cuticular wax profile of plant species growing in different places (Samuels et al., 2008).

As Chang (1992) noted, archaeology cannot begin to formulate the development of pastoralism without a more basic understanding of how pastoralists use landscapes today. We would also add the importance of understanding the primary biosignatures that pastoralists leave in the landscape. Certainly, the ethnoarchaeology of mobile pastoralism lacks a large body of collected knowledge that could make us possible to accurately distinguish its unique characteristics and problems (e.g. ephemerality of habitation remains, re-use of space, or mobility patterns, among others). Still, the use of multi-scale ethnoarchaeological data and scientific analysis of the remains of contemporary pastoral societies stands as a powerful tool for understanding past nomadic pastoral sites.

4.5 Conclusion

We examined the sediment dung deposits from an Eastern Mongolian winter campsite to identify its molecular and isotopic fingerprints in order to potentially recognise husbandry management and animal diet. The results showed that:

- *n*-alkane patterns of herbivore excrements contained in the sediment are sufficiently different from natural signatures from the same area. Therefore, this result is clearly reflecting differences in the particular plant species that contribute to the *n*-alkanes in the sediment (natural versus an anthropic deposit).
- Changes in $\delta^{13}\text{C}_{\text{alkane}}$ values could provide information about diet and possible herd management. Dung deposits and dung-derived organic matter *n*-alkanes in our samples provided useful data suitable for the investigation of dietary intake by livestock and husbandry practices among pastoral societies.

Without being exhaustive, this work is a contribution to setting the bases for developing a more robust framework to understand pastoral husbandry management strategies in a long-term perspective. Our exploratory study introduces innovative molecular analytical proxies that complements more traditional approaches (e.g. the study of archaeological artefacts). The potential identification of the spatial distribution of penning spaces at a local scale highlights the relevance of sediment distinctive

biomarkers (e.g. high content and well-preserved OM along alkane $\delta^{13}\text{C}$ depleted values) and offers new scenarios to detect past pastoral habitation sites and foddering practices.

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Chapter 5

5 *n*-Alkanes and their carbon isotopes ($\delta^{13}\text{C}$) reveal seasonal foddering and long-term corralling of pastoralist livestock in eastern Mongolia

Abstract

n-Alkanes compound-specific $\delta^{13}\text{C}$ signatures from anthropic pastoralist winter campsites in Mongolia have great potential to reconstruct to dietary seasonality of domesticated livestock and local climatic conditions. We have analysed leaf wax *n*-alkanes and their $\delta^{13}\text{C}$ signature in five common fodder plant species (*Artemisia mongolica*, *Chenopodium album*, *Elymus dahuricus*, *Elymus sibiricus* and *Stipa sibirica*), and topsoils (0–8 cm) in four livestock corrals in northern and southern Mongolia. *n*-Alkanes identified in fresh plant parts reflect signatures for vascular plant waxes with mean $\delta^{13}\text{C}$ stable isotope values of individual *n*-alkanes typical for C3 plants (-34.1‰). Plants from forest and desert steppe exhibit similar mean *n*-alkane $\delta^{13}\text{C}$ values but significantly wider isotopic variation in desert steppe flora reflecting the interannual variation in water availability and evapotranspiration that characterise arid grassland ecosystems. The non-anthropogenic control samples exhibit $\delta^{13}\text{C}$ mean values significantly higher from that of dung deposits and fresh plants from the same region (-28.5‰). Dung deposits have *n*-alkane profiles dominated by vascular plant waxes with mean *n*-alkane $\delta^{13}\text{C}$ values of -34.5‰ for forest and -31.2‰ for desert steppe corrals. Geographical differences in the fodder harvest areas were detected by the ACL and CPI indexes, reflecting small-scale local mobilities and associated dietary intake as well as better preservation of organic matter in forest than in desert steppe deposits. Our data support previous research that *n*-alkanes profiles and their carbon isotopic values vary depending on the environment, while simultaneously demonstrating how anthropogenic activity, in this case corralling of livestock, is recorded in the *n*-alkane record validating the use of biomarker studies in pastoralist archaeological contexts. Our study shows that $\delta^{13}\text{C}$ *n*-alkane values from herbivore excrements contained in sediment are sufficiently different from both fresh plants and from natural sedimentary signatures from the same area. In addition, this paper contributes to the notion that *n*-alkanes profiles and their isotopic values vary geographically depending on the environment. Finally, we show the viability in applying biomarker studies in pastoralist archaeological contexts.

Keywords: *n*-alkanes, carbon isotope analysis, plant biomarkers, dung, Mongolia.

5.1 Introduction

Pastoralism is a fundamental subsistence strategy that has supplied food to people since the early Holocene (Hammer and Arbuckle, 2017; Miller et al. 2019). The term “pastoralism” usually refers to an outsized importance of bovid livestock in the overall subsistence economy, but a broad range of animal

husbandry practices may or may not include mobility and some amount of agriculture, as well as a significant variation in community size and social organisation (Cribb 1991; Khazanov 1994; Honeychurch and Makarewicz 2016). In general, archaeologists use the term to refer to mobile communities that rely on herd animals for the majority of their subsistence and practice little or no agriculture, often being treated as a separate entity from settled communities (Chang 2015). This approach is problematic as pastoralism is a complex human lifeway that encompasses many forms and can be affected by many variables (Salzman 2004).

In order to maximize available pasturage for the benefit of their animals, pastoralists typically move their herds on a seasonal basis, with the frequency and distance of moves adapted to local climate, landscapes and resources environment and resources, from which they are dependent: climate, topography, flora and water sources (Abdi, 2003). Seasonal mobility in pastoral societies is a critical factor that enables herders to provide quality food for their domesticates (e.g. cattle, yaks, goats, sheep, horses, camels) and, consequently, to successfully obtain human food resources – meat, fat, marrow, blood, and milk, and secondary products -milk by-products, dung for fuel and fertiliser and textile fibres (Sherratt 1981). In addition, pastoralist mobility facilitates social and political opportunities for networking and trade (Ventresca-Miller and Makarewicz 2018). To date, most work on pastoralist herd management strategies in ancient pastoralist societies has focused on establishing mobility and seasonal foddering through stable isotopic analyses of livestock bones and teeth which offer insights on pastoral livestock mobility and animal dietary intake at lifetime and seasonal scales (Makarewicz 2014, Hermes et al. 2018), but offer less information on the intensity of key practices like corralling animals in winter camps which helps promote animal survivorship in harsh environments.

Establishing the seasonality of pastoralist campsite occupation and the local climatic conditions in which mobile communities wintered their animals are still unresolved major questions in the archaeology of pastoralism. Here, we explore animal management strategies involving seasonal foddering through the investigation of *n*-alkane carbon isotopic composition of dung deposits and fresh forage plants from two distinct ecosystems within the Mongolian steppe. In doing so, we characterise the origin of the ingested plants by the animals that were corralled during winter and to corroborate fodder and occupation seasonalities. This approach sets the stage for the analyses and interpretation of *n*-alkanes concentrations in archaeological dung-rich sedimentary deposits.

5.1.1 *n*-Alkanes as a record of animal diet and local environmental conditions

Detecting pastoralist activities in the archaeological record is often challenged by the low visibility of often ephemeral habitation sites associated with mobile communities (David and Kramer 2001; Salzman 2004; Biagetti 2014). However, pastoralists frequently invest heavily in infrastructures such as wells, enclosures, and winter campsites designed to enhance the management of their mobile livestock, and, these modifications leave visible traces on the landscape (Hammer 2014; Rosen 2016). Several of these features, in particular corrals, provide biomolecular archives of pastoralist activity that potentially offer a means document ancient livestock herding practices. Dung deposits – a ubiquitous feature of pastoralist campsites around the world, in particular offer a rich record of past livestock management practices associated with seasonality of occupation, stocking rate, separation of animals by species, age,

or sex within enclosures, and dietary intake (Chang and Koster 1986; Macphail et al., 2004; Shahack-Gross et al., 2008; Marshall et al., 2018). To date, these aspects have been investigated through analysis of sediment micromorphology, macrobotanical remains and phytoliths, and isotope analyses of bulk organic fraction recovered from deposits associated with dung deposition (Macphail and Goldberg 1995; Shahack-Gross et al., 2003; Shahack-Gross, 2011; Linseele et al., 2013; Gur-Arieh et al., 2018).

These high organic-matter dung deposits contain carbon-based compounds including lipids, cellulose, proteins, chitin and lignin undigestible in animal rumen. Wax lipid *n*-alkanes are among the carbon-based compounds that are well-preserved in dung deposits (Carr et al., 2010; Égüez and Makarewicz 2018). *n*-Alkanes are chemically inert, non-polar saturated hydrocarbon molecules that primarily originate from epicuticular waxes of vascular higher plants (Eglinton and Hamilton, 1967), these form a barrier against uncontrolled water evaporation, mechanical damage, and fungi, bacteria, insects and parasite action. Intracuticular waxes prevent uncontrolled transpiration while epicuticular waxes regulate albedo and wettability (Eglinton and Hamilton, 1967; Koch and Ensikat 2008). *n*-Alkanes are indigestible, relatively resistant to microbial degradation and easily extractable by chemical methods from plant tissues, excrements and sediments (Mayes et al., 1986; Malossini et al., 1994; Duncan et al., 1999; Dove and Mayes 2006; Ardenghi et al., 2017), and are well preserved in soils and lacustrine sediments (Huang et al., 1995; Otto and Simpson 2005; Smith et al., 2007; Garcin et al., 2012). In addition, due to their non-polar single covalent bonds and the lack of functional groups, *n*-alkanes often preserve authentic stable isotope ratios that can be stable over a geological time scales (Cranwell, 1981; Schimmelmann et al., 2006; Vajda et al., 2017; Seki et al., 2012; Ardenghi et al., 2017). These properties make *n*-alkanes one of the most reliable biomarkers for animal diet estimation (Duncan et al., 1999; Dove and Mayes 2005; Dungait et al., 2010) and paleoenvironmental reconstructions (Sachse et al., 2006; Niedermeyer et al., 2010, 2016; Schemmel et al., 2016).

The composition and abundance on *n*-alkanes in plants vary among species, tissues and growth stage (Wiesenberg et al., 2004, Bush and McInerney, 2013, Diefendorf and Freimuth, 2017). Plants typically produce a range of *n*-alkanes of different chain lengths and typically exhibit a strong predominance of odd-over-even numbered chains due to decarboxylation from even-numbered fatty acid molecules (Kolattukudy 1980). Odd carbon numbered long-chained (>C₂₅) alkanes are characteristic components of the epicuticular waxes of higher plants, while odd carbon numbered short-chained (<C₂₁) alkanes derive from lower plants (aquatic algae, mosses and ferns) and microbial organisms (Eglinton and Hamilton, 1967; Eckmeier and Wiesenberg, 2009). The predominance of even-over-odd chains in the wax alkanes is typically presented in hydrocarbons formed by diagenetically altered material such as detrital organic matter (Eglinton and Hamilton, 1963; Simoneit 1986). Leaf wax carbon chains also responds to different environmental regimes so that *n*-alkane profile derived from sediments provides a specific biota biomarker (Samuels et al., 2008; Bush and McInerney, 2015).

5.2 Materials and Methods

5.2.1 Geographical and ethnographical setting

Mongolia is characterized by strongly seasonal environments characterized by relatively short summers, and long, extremely cold winters. Most precipitation falls in the later summer months. There is a north-south gradient in precipitation and temperature levels, with annual precipitation ranging from less than 50 mm per year in the warmer Gobi steppe desert to 400 mm, the cooler northern forest steppe (Vandandorj et al., 2015). Pastureland covers more than 80% of Mongolia, which is heavily grazed by sheep, goat, cattle, horses and, in the north, yaks, herded by pastoral nomads.

Livestock corrals are key pieces of pastoralist infrastructure regularly used by mobile herders in Mongolia today. Herders graze their flocks during the summer months in distant pastures located about 40–50 km from their winter campsites while accumulating fodder during the late summer months and storing it at their winter camps where pastures are also reserved (Makarewicz 2014). Sheep, goats, cattle, and horses, segregated by species, sex, and age, are penned through the winter season (early November to late April) in multi-structured corrals built of wood or dung, although the herders allow the animals free roaming periods during daytime. Over the winter season, dung is rapidly laid down in corral structures but left in place, resulting in thick deposits that accumulate over years (Fig. 1). This compacted dung is then harvested, typically in large blocks, from corral deposits for fuel and construction (Égüez and Makarewicz, 2018).



Fig. 5-1. Mongolian herders breaking and collecting dung blocks for drying. Photo: N. Égüez.

5.2.2 Dung-rich penning deposits samples

Penning deposits from enclosed corrals were sampled from winter campsites located in two contrasting phytogeographical zones during July 2015: Khentii (forest steppe) and Dornogovi (desert steppe) aimags (Fig. 2a). A total of four well-preserved penning deposits, defined here as spatially confined accumulations of droppings from domesticated livestock, were sampled from two contemporary winter campsites where sheep and goats were penned. Campsite 7, is located in the Khentii province, while campsite 54, is situated in the Dornogovi province (Fig. 2b and 2c). For each campsite two corrals were sampled (A and B).

Campsite 7 is closely located to a riparian forest within the rich grasslands of Bayan-Ovoo in the forest steppe of Khentii province. In this area, vegetal communities are dominated by *Stipa* and *Leymus* spp. Sheep and goat corrals are not roofed with visible good preservation of organic matter with dung accumulations reaching ca. 30cm in depth. Campsite 7 is sheltered between the rocky outcrops that configure the semi-arid landscape of Dornogovi province. Low-lying areas appear as marshes in years when there is a great deal of precipitation, but are most often dry with stands of feathergrasses (mostly *Stipa* spp.). Here, corrals were also not roofed and supported visible, albeit poorly preserved, dung deposits ca. 8cm in depth.

In addition, two non-anthropogenic areas about 1 km away from each campsite were sampled for natural organic matter content control (1 and 2) (Table 1).

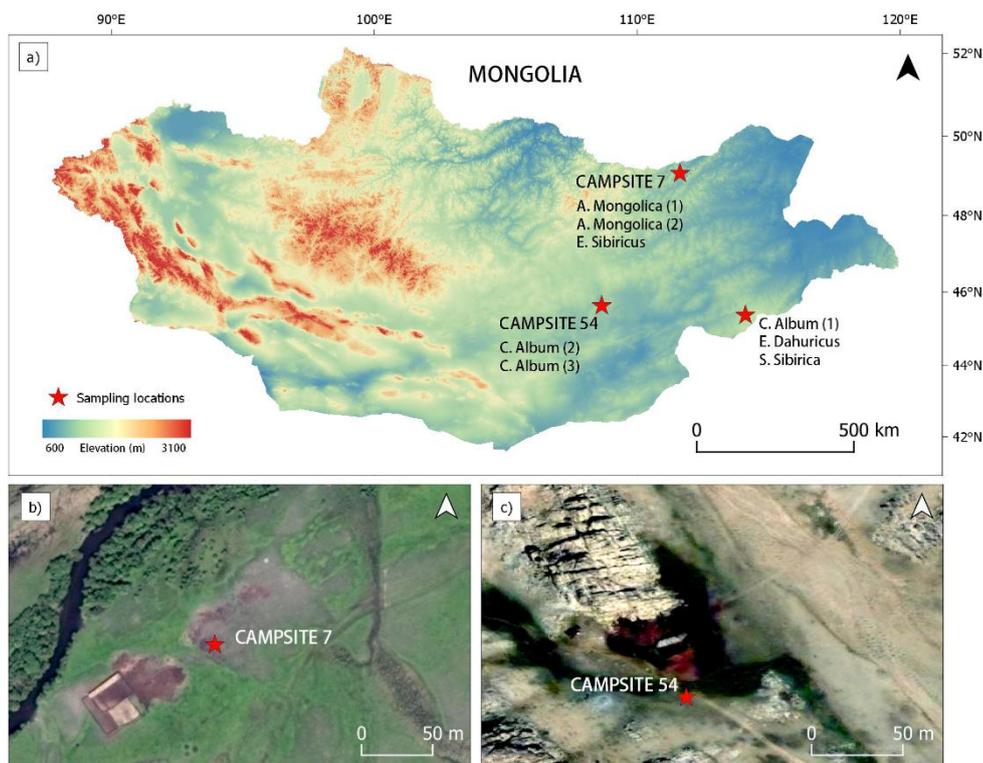


Fig. 5-2. General map of Mongolia (a) showing sampling locations (base map: elevation SRTM 3.0 1 arc second). Subsets (a) and (b) show the location and surroundings of Campsite 7 and Campsite 54, respectively. Note the organic-rich dark brown dung deposits in Campsite 7 in contrast to the poor-preserved dung deposits in Campsite 54.

Sample	Natural zone	Province	Lat	Long	Plant family	Photosynthetic pathway
Campsite 7	Forest steppe	Khentii	49.070275	111.627992		
Campsite 54	Desert steppe	Dornogovi	45.631038	108.651634		
Control sediment 1	Forest steppe	Khentii	49.074467	111.640175		
Control sediment 2	Desert steppe	Dornogovi	45.624320	108.658120		
<i>Artemisia mongolica</i> 1	Forest steppe	Khentii	49.070366	111.627833	Asteraceae	C3
<i>Artemisia mongolica</i> 2	Forest steppe	Khentii	48.966686	111.491767	Asteraceae	C3
<i>Chenopodium album</i> 1	Desert steppe	Sukhbaatar	45.372728	114.119124	Chenopodiaceae	C3
<i>Chenopodium album</i> 2	Desert steppe	Dornogovi	45.582287	108.624598	Chenopodiaceae	C3
<i>Chenopodium album</i> 3	Desert steppe	Dornogovi	45.582287	108.624598	Chenopodiaceae	C3
<i>Elymus dahuricus</i>	Desert steppe	Sukhbaatar	45.327778	114.121405	Poaceae	C3
<i>Elymus sibiricus</i>	Forest steppe	Khentii	49.070366	111.627833	Poaceae	C3
<i>Stipa sibirica</i>	Desert steppe	Sukhbaatar	45.372728	114.119124	Poaceae	C3

Table 5-1. Summary of the studied samples with basic parameters. Coordinates were recorded in the field by the authors with a handheld GPS.

5.2.3 Plant samples

Here, we take advantage of the continental climatic seasonality that influences floral growth and plant tissue isotopic composition in order to detect the potential range of $\delta^{13}\text{C}$ variation in plant *n*-alkanes in different environments. We also take advantage of different *n*-alkane compositions and concentrations that characterized different plant parts such as leaves, stems, roots, flowers and fruits in order to further establish the dietary origins of bovid dung (Dove et al., 1996; Smith et al., 2001; Wiesenberg et al., 2004; Jansen et al., 2007; Huang et al., 2011; Jambrina-Enríguez et al., 2018). Here, a total of eight plant samples were collected in order to establish the range of carbon isotopic variation in the *n*-alkanes in floral growth from the steppe-desert and forest steppe. These included five different species that are commonly used during winter for animal fodder: *Artemisia mongolica* (n=2), *Chenopodium album* (n=3), *Elymus dahuricus* (n=1), *Elymus sibiricus* (n=1) and *Stipa sibirica* (n=1). The samples were gathered in Dadal sum (Khentii province), Dariganga sum (Sukhbaatar province) and the similar latitudinal desert steppe of Dornogovi province. Plants were selected on the basis of stage of growth, and only large mature plants were gathered as *n*-alkane production is stable at this plant life-stage (Tipple et al., 2013).

5.2.4 Lipid extraction, analysis and quantification.

1g of each of the plant sample parts (16 in total) was dried at 60°C over 48h, ground with liquid nitrogen and then homogenised with an agate mortar. 1g of each dung-rich sediment samples was dried at 60°C over 48h, and then homogenised using an agate mortar. Before extractions, all glassware and glass wool was placed in a muffle oven at 450 °C for 10 h to eliminate any possible organic compounds. Lipids were extracted in 20mL 9:1 dichloromethane/methanol following a modified version of a previously published methodology (Bhattacharya et al., 2017). 3 cycles of 30 minutes in a sonicator and 10 minutes in a centrifuge (4700rpm) were applied to the mixture. The total extraction was then evaporated and separated into six fractions of different polarity using solid phase extraction (SPE) via silica gel column chromatography. The *n*-alkane fraction was eluted with 3/8 of dead volume in *n*-hexane. After addition of internal standard (IS) 5 α -androstane 8mg/L to the resulting fraction the volume was completed with 150 μ L of DCM.

The compounds in the *n*-alkane fraction were identified and quantified by gas chromatography with a coupled detection and mass-selective detector (GC-Agilent 7890B, MSD Agilent 5977A) equipped with an HP-5MS capillary column (30m, ID: 250 μ m, film thickness 0.25 μ m). The GC was programmed to an initial temperature of 70 °C for 2 min, heated with a heating rate of 12 °C/min to 140 °C and to final temperature of 320 °C with a heating rate of 3 °C/min and held for 15 min, with helium as the carrier gas (2 mL/min). The total run time was 82.83 min to avoid overlap or interferences with other compounds. The multimode injector was held at a split ratio of 5:1 at an initial temperature of 70 °C during 0.85 min and heated to 300 °C at 720 °C/min. All measurements were repeated two times. The MS was operated in full scan mode (*m/z* 40–580) with an electron ionization energy of 70 eV and with the temperatures of the ion source and quadrupole set at 230 °C and 150 °C, respectively. Quantification was carried out taking the four most intense fragment ions (*m/z* 43, 57, 71 and 85, and *m/z* 67, 95, 81 and 245 for IS) for *n*-alkanes and the total ion chromatogram for the rest of the analytes. Compounds were identified by comparison of their retention times and mass spectra with those of reference compounds (mix C8-C40 alkanes and 5 α -androstane, Supelco) and comparison with the NIST mass spectra library. Quantification was based on calibration curves obtained by plotting the ratio Area/Area_{IS} versus the concentration of each reference compound. Correlation coefficients were higher than 0.995. The *n*-alkane concentration is expressed as μ g of individual compound per gram of dry sample (μ g gds⁻¹).

Several indexes have been developed for helping the environmental interpretation of occurring wax *n*-alkanes abundances. Among these methods, the most common are the average chain length (ACL) and the carbon preference index (CPI). ACL is the weighted average of the various carbon chain lengths, whereas CPI measures the relative abundance of odd over even carbon (Duan and He, 2011; Bush and McInerney, 2015). The *n*-alkane distribution was calculated according to the Carbon Preference Index (CPI) using the Bray and Evans equation (1961):

$$\text{CPI}_{25-33} = [(\sum \text{C}_{25-33\text{odd}} / \sum \text{C}_{24-32\text{even}}) + (\sum \text{C}_{25-33\text{odd}} / \sum \text{C}_{26-34\text{even}})] \times 0.5$$

The average chain length (ACL) was calculated following the recommendation of Freeman and Pancost (2014). For total *n*-alkanes we used the C15–C33 interval:

$$ACL_{15-33}total = \sum(C_i \times [C_i]) / \sum[C_i]; 15 \leq i \leq 33$$

The value $[C_i]$ is the concentration of the *n*-alkane with *i* carbon atoms.

5.2.5 Compound-specific carbon isotope analysis

Carbon isotope analysis by GC-IRMS was performed using a Thermo Scientific Isotope Ratio Mass Spectrometer Delta V Advantaged coupled to a GC Trace1310 through a Conflo IV interfaced with a temperature converter GC Isolink II. Samples were injected by means of a MultiMode injector (MMI) in splitless mode, with the temperature increasing from 79 °C (held 0.5 min) to 325 °C (held 3min) at a rate 10 °C·s⁻¹ and finally to 350 °C (held 3min) at 14 °C·s⁻¹. The GC was fitted with a Trace Gold 5-MS (Thermo Scientific) fused silica capillary column (30 m length x 0.25 mm i.d., 0.25 μm film thickness). Helium was used as the carrier gas at a flow rate set at 1.5 mL·min⁻¹. The combustion reactor temperature was maintained at 1000 °C. The temperature programme comprised a 2 min isothermal period at 70 °C increasing to 140 °C (held 2 min) at a rate of 12 °C·min⁻¹, followed by an increase period to 320 °C (held 15 min) at 3 °C·min⁻¹. Data acquisition and processing were carried out using the Isodat 3.0 software (Thermo Scientific). All the δ¹³C values were normalized to VPDB (Vienna Pee Dee Belemnite) using an *n*-alkane Schiimmelmann type A6 mixture (n-C₁₆ to n-C₃₀). The standard deviation of carbon isotope measurements was better than 0.5‰.

5.2.6 Statistical analyses

A set of statistical tests was performed to compare means of carbon isotopic values and *n*-alkane concentrations between four categorical groups: three groups from surveyed fresh plant parts (i.e. flower, grain and stem samples) and a fourth group from recovered dung samples, respectively. A preliminary exploration of the data included sensitivity analyses and a Shapiro-Wilk test was applied to check for normality. Normally-distributed carbon isotopic values were analysed using One-way analysis of variance (ANOVA), followed by a post-hoc Tukey's test to identify stochastic dominance among specific groups. In contrast, the analysis of *n*-alkane concentrations (non-parametric) of the same four categorical groups used a Kruskal-Wallis Test and a post-hoc Dunn's Test (Dinno 2017).

A similar approach was selected to identify statistical significance between distinct environmental effects in carbon isotopic values and *n*-alkane concentrations from fresh plant parts and dung samples. Two categorical means were compared accordingly to the geographic origin of our samples: the desert steppe region and the forest steppe region. A two-sample Student's t-Test was performed in carbon values, whereas a non-parametric Mann-Whitney U Test was used for the two groups' *n*-alkane concentrations. All the analyses were performed in R 3.5.1 (R Core Team, 2018). Data description and graphics used the packages psych (Revelle, 2018), ggplot2 (Wickham, 2016), gridExtra (Aguje 2017).

5.3 Results

Distribution and abundance of *n*-alkanes for each sample recovered from forest and steppe desert zones are reported in Fig. 3 and Fig. 4. $\delta^{13}\text{C}$ values are reported in Fig. 5 and Fig. 6. Supplementary data about distribution, abundance and $\delta^{13}\text{C}$ values of *n*-alkanes for all samples is given in the Annex.

5.3.1 *n*-Alkane distributions and $\delta^{13}\text{C}$ in fresh plants

All plant lipid profiles include –mid and long-chain *n*-alkanes (>*n*C21) and predominance of odd carbon numbers. The range of carbon numbers is slightly wider in stems compared to flowers and grains (C21-C35 vs C21-C33). Almost all plant species demonstrate a *C*_{max} at C29 in stem parts, except for *Artemisia mongolica* where maximum values are visible at C31. Flowers from all species exhibit *C*_{max} at C29 while *C*_{max} values in seeds are variable among species: with max values at C23 for *Elymus dahuricus*, C27 for *Stipa sibirica* and C29 for *Elymus sibiricus* (Fig. 3).

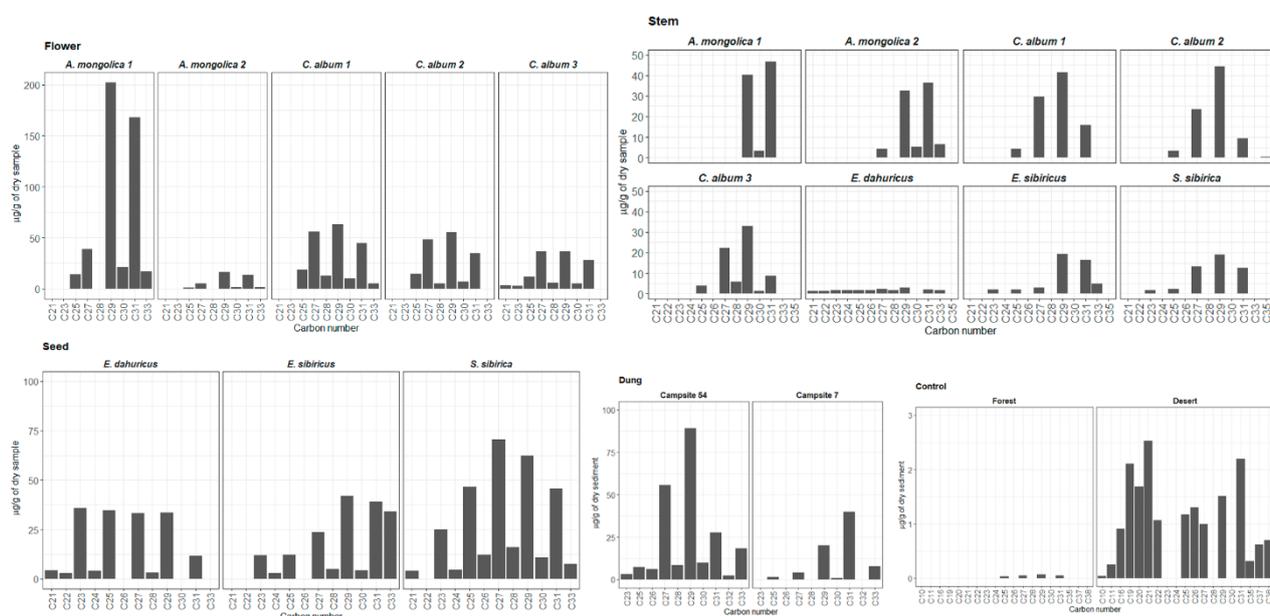


Fig. 5-3. Histograms indicating the distribution and abundance of *n*-alkanes in flowers, grains, stems, coralls from campsites 7 and 54 and control samples. The concentrations are given in µg per g of dry sample (µg/g sample).

n-Alkanes are most abundant in flower parts, followed by seeds and stems ($\bar{x} = 30.4\mu\text{g/g}$, $\bar{x} = 22.1\mu\text{g/g}$ and $\bar{x} = 11.9\mu\text{g/g}$ respectively). When all plant parts are grouped together, *n*-alkanes abundances between forest and desert steppe show similar ratios ($\bar{x} = 21.1\mu\text{g/g}$ and $\bar{x} = 15.7\mu\text{g/g}$ respectively) (Fig. 4). Statistical observation for pair-wise comparisons, resulted in significance ($p < .05$) for all the possible pair groups, except for the stem-flower grouping. No statistical significance is observed for geographical variation when the Mann-Whitney U Test was performed for *n*-alkane concentrations (*w*-statistic = 62, *p*-value = 0.07022).

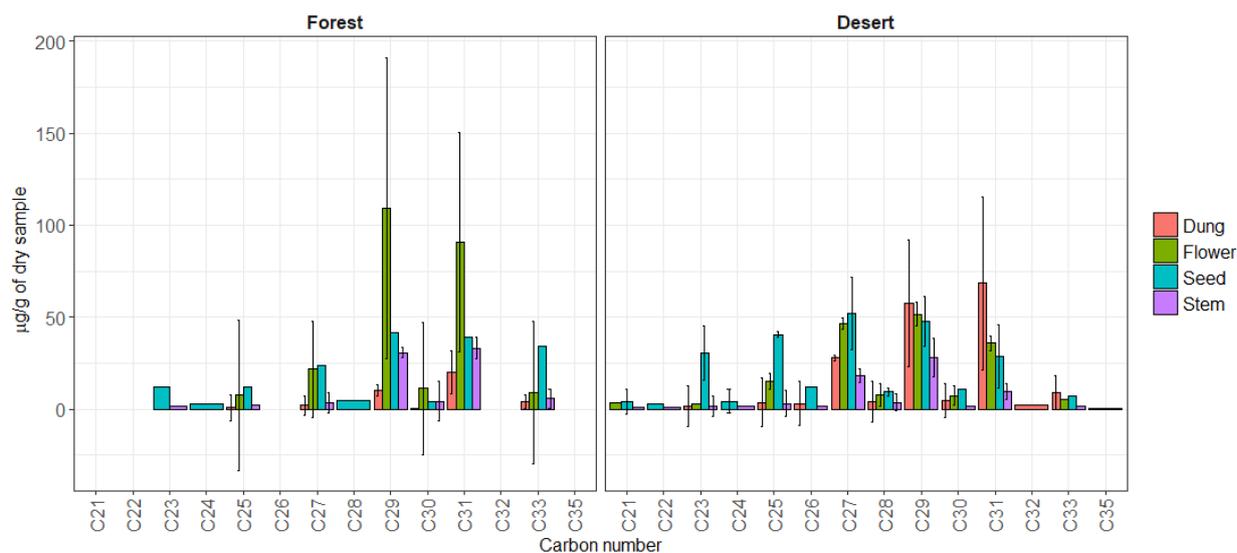


Fig. 5-4. Bloxplots showing the distributions and abundance of *n*-alkanes in fresh plants parts and dung deposits of the two campsites with the associated error value ($\alpha = 0.05$).

Overall, $\delta^{13}\text{C}$ values in plant tissues show isotopic significance between plant parts as well as between geographical regions. *n*-alkane carbon isotope values measured plants from dung from the forest steppe and desert steppe exhibit similar means averaging -34.4‰ and -31.2‰ respectively, but plants from the desert steppe region, which include *C. album*, *E. dahuricus* and *S. sibirica*, demonstrate a wider variation in carbon isotope values from -26.4 to -40.4‰ compared to those from the forest region which include *A. mongolica* and *E. sibiricus* and range from -30.7 to -37.3‰ .

Different plant parts exhibit different carbon isotopes ratios in *n*-alkanes. The combined forest steppe and desert steppe mean $\delta^{13}\text{C}$ values for flowers are -36‰ , grains -32.6‰ and stems -34.9‰ (Fig. 5). When separated by geographical areas the total mean carbon isotopic values for plant parts are: flowers -35.7‰ , -33.6‰ for grains and -35.7‰ for stems in the forest area. For the desert steppe area $\delta^{13}\text{C}$ values are: flowers -36‰ , grains -32.1‰ and stems -34.6‰ (Fig. 5).

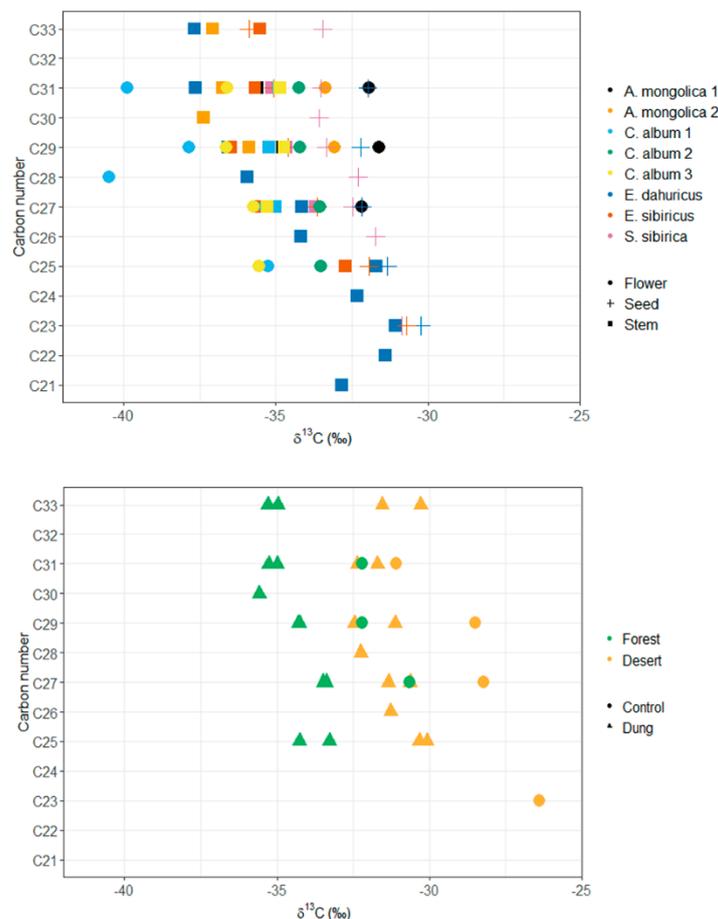


Fig. 5-5. Biplot showing the *n*-alkane carbon isotope values for all samples. Shapes are for type of sample and colors for plant species. The isotopic composition for all samples fall into the range of C3 plants. The mean carbon isotopic values for fresh plants is -34.5‰ and -32.8‰ for the dung deposits. Note the difference in $\delta^{13}\text{C}$ values exhibited by the control sample and the rest of samples, difference is 2-4‰ higher.

5.3.2 *n*-Alkane distributions and $\delta^{13}\text{C}$ in dung deposits

Penning deposits averaged $14.5\mu\text{g/g}$ of *n*-alkanes and yielded the following *n*-alkane distribution: C29, C31 and C33 for forest steppe region samples and C27, C29, C31 for desert steppe region samples (Fig. 3). The lipid profile obtained from penning deposits from both campsites includes medium and long-chain *n*-alkanes ($>n\text{C}23$) with a predominance of odd-over-even carbon number, thus compatible with profiles of terrestrial vascular plants. Specifically, the forest steppe campsite 7, C31 yields the highest concentrations in dry dung-rich sediment of $33.1\mu\text{g/g}$, while the desert steppe campsite 54, yielded the highest concentrations in *n*-alkane C29 at $89.3\mu\text{g/g}$.

The lipid profiles of the sediment control samples averaged $1.1\mu\text{g/g}$ of *n*-alkanes and includes short, medium and long-chain *n*-alkanes ($>n\text{C}10$). The carbon number distribution from the plant wax-derived *n*-alkanes show a range of C10-C38 with a predominance of higher concentration in the odd carbons.

C21 is the most abundant carbon number in sample "control desert", while C29 is the most abundant for "control forest" (Fig. 3).

Forest steppe dung n-alkanes exhibited on average -34.5 ‰ $\delta^{13}\text{C}$, while n-alkanes from dung samples from the desert steppe were significantly enriched in ^{13}C on average 3.3 ‰. $\delta^{13}\text{C}$ values show similar intra-site range variation between corrals A and B (ca. 2‰) on each of both campsites (Fig. 6). $\delta^{13}\text{C}$ from the "control forest" non-anthropogenic sediment sample range between -30.6 to -32.6 ‰ and ^{13}C enriched ca. 2-4‰ related to campsite 7. $\delta^{13}\text{C}$ values from the "control desert" non-anthropogenic sediment sample range between -26.4 ‰ to -31.07 ‰ and are significantly enriched in ^{13}C (ca. 4-6‰) when compared to campsite 54 (Fig. 5).

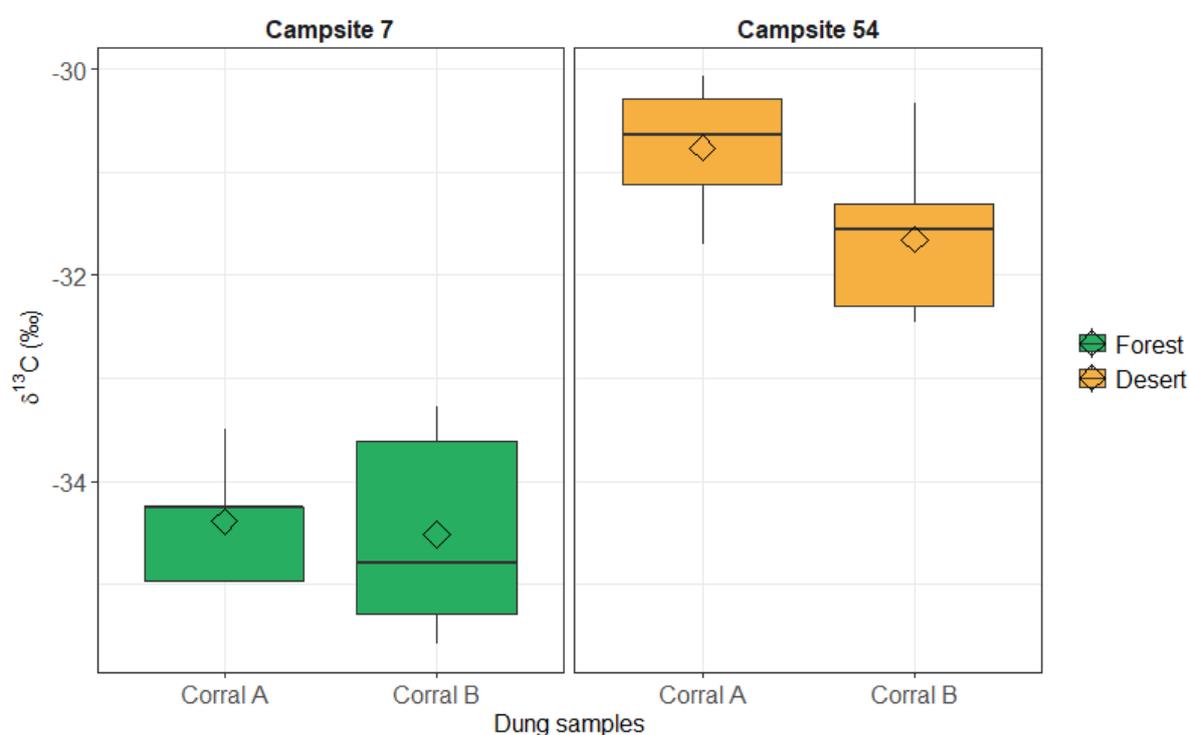


Fig. 5-6. Boxplot showing the n-alkane carbon isotope values for dung deposits from campsite 7 and 54. The isotopic composition for all samples fall into the range of C3 plants.

5.3.3 CPI and ACL values

Results for CPI and ACL values are presented in Table 2, Fig. 7, Fig. 8 and Fig. 9. CPI values of long-chained n-alkanes extracted from all samples ranged from 2.18 to 61.28 with values increasing according to depositional environment and plant part following: control < stem < grain < flower < dung. CPI values are higher in forest samples when compared to those of desert samples (mean of 23 vs 8 respectively). The average chain length (ACL) mean values range are higher in forest plant samples than in desert plants (ca. 29 vs 27). When $\delta^{13}\text{C}$ values were plotted regarding CPI and ACL values for forest and desert this pattern was still observable except for seed sample *E. dahuricus* (desert), which is higher than most forest samples (see Fig. 7).

Significant differences between dung deposits from campsites in forest and desert are observable, both for CPI and ACL (Fig. 8). CPI values obtained from campsite 7 displays a wide range of values (19 to 61), while in contrast, campsite 54 values show a narrow range (6 to 18) thus reflecting lower intra-campsite animal diet variation. In contrast, the reverse pattern occurs for ACL values, showing a narrow range in campsite 7 and a large range in campsite 54 with more intra-site variation between corrals 1 and 2 (29.61 and 27.97 respectively).

Grouping	CPI				ACL			
	\bar{x}	\tilde{x}	Min.	Max.	\bar{x}	\tilde{x}	Min.	Max.
Type								
Flower	14.76	12.59	8.23	24.10	28.82	28.57	28.23	29.63
Seed	11.21	13.77	6.05	14.97	26.63	26.11	25.10	28.81
Stem	8.89	9.63	2.18	25.75	27.14	28.51	21.23	30.16
Dung	21.20	16.95	7.02	61.28	29.31	29.61	27.97	30.35
Control	3.81	3.81	3.81	3.81	24.40	24.40	20.54	28.27
Natural Zone								
Forest	23.78	19.65	12.66	61.28	29.26	29.42	28.27	30.35
Desert	8.38	7.02	2.18	16.95	25.93	27.97	20.54	29.61

Table 5-2. CPI and ACL mean and median values for samples and natural zones (forest and desert).

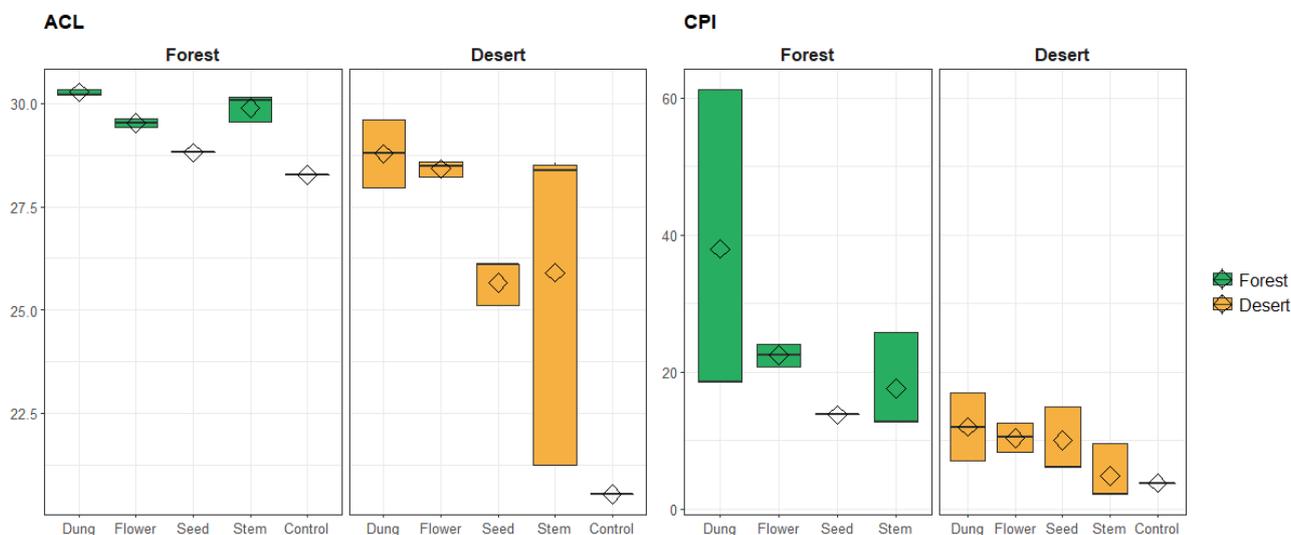


Fig. 5-7. Set of boxplots showing ACL and CPI values for all samples, grouped by natural zones (forest and grassland).

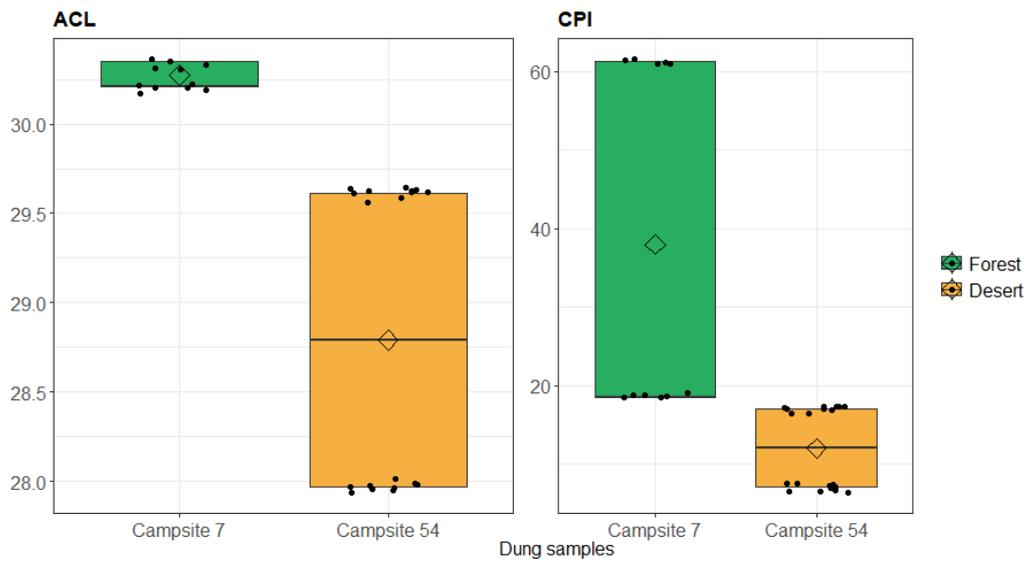


Fig. 5-8. Boxplot showing ACL and CPI indexes for dung deposits from campsite 7 and campsite 54.

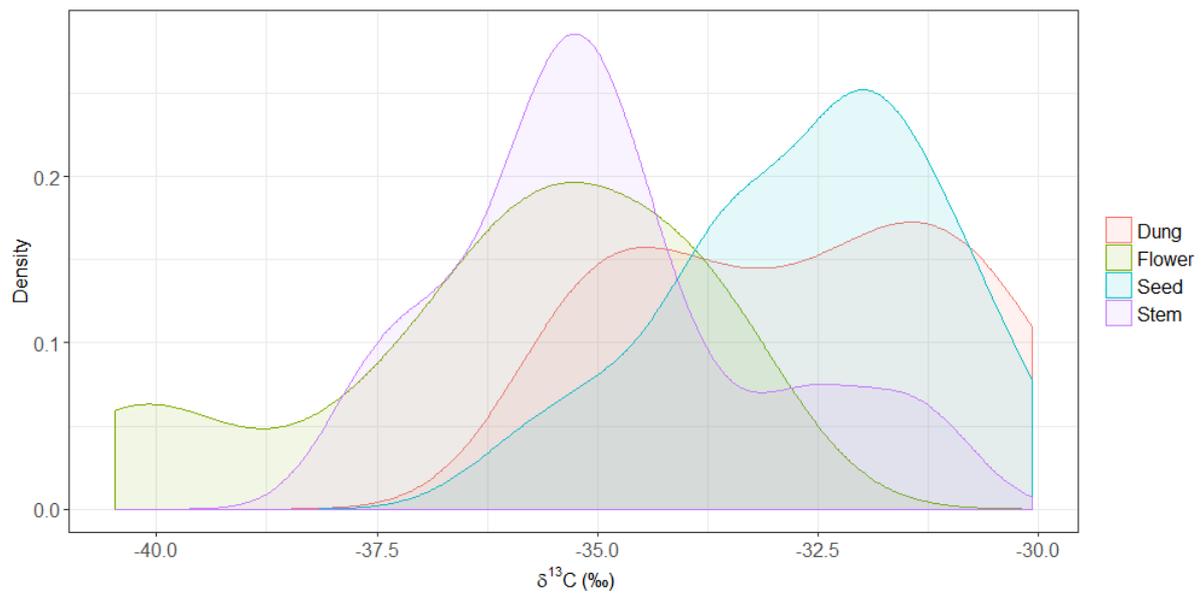


Fig. 5-9. Kernel density estimates for carbon values grouped by fresh plant parts and dung samples.

5.4 Discussion

5.4.1 ACL and CPI indexes indicate geographical variation in foddering and organic matter maturation

The considerable differences between dung deposits in forest steppe and desert steppe ACL values may be due to differences in the plant species and parts incorporated into dung deposits. Forest plants yield on average shorter chain lengths than plants from grasslands or deserts, because woody plants produce leaf waxes with shorter ACL values due to the plant development under long periods of lower temperatures. On the contrary, non-woody plants that would have grown in environments with periods of higher temperatures, would show higher ACL values (Cranwell, 1973). Moreover, it has been suggested that the modal carbon number of a higher plant *n*-alkane distribution is broadly related to latitude (Poynter et al., 1989; Poynter and Eglinton, 1990), with higher modal carbon numbers occurring at lower latitudes. However, opposite to previous studies, our samples show higher modal carbon chain numbers in higher latitudes (forest steppe) and lower in low latitudes (desert steppe) (Fig. 7) suggesting that extreme continental climate that involve large diurnal and seasonal ranges of temperature, small annual precipitation totals, and low relative humidities influences modal carbon chain representation. In addition, our results show that the dung variation in modal carbon numbers, for both campsites, emulate those numbers of the fresh, summer season plants. Thus, herders are providing fodder diets that originate from the same ecosystem where the winter campsite is located. The small variation in ACL ranges for the forest steppe suggests lower evapotranspiration, while the ACL great variation in the desert steppe samples is reflecting large rates of evapotranspiration due to more climatic variations (temperature and rainfall) within this specific ecosystem (Rao et al., 2009; Sakari et al., 2008), a pattern that is also observed in the dung deposits (Fig. 8).

CPI values in all our samples, except the non-anthropogenic sediment control sample, exhibit high odd-to-even predominance, distinguishing between alkanes from vascular land plants and the alkanes belonging to bacteria. In this study, CPI index is used as a reference indicator for organic matter maturity. Our results show that the *n*-alkane CPI data extracted from fresh plants in Mongolia exhibits gradual increase with the increasing latitude (Fig. 7), and again, samples from the corrals of winter campsites also follow this criterion (Fig. 8). Therefore, our results indicate better preservation of organic matter in forest deposits than in desert steppe contexts, since the latter are strongly affected by erosional processes. Forest soils have more soil organic matter than grasslands or croplands (Tsui et al., 2004; Wang et al., 2008) due to the quality of litterfall and the lower rate of decomposition in forest ecosystems. High long-chain *n*-alkane carbon preference index values (>1) have been widely accepted as an indicator for terrestrial sources of sedimentary organic matter and as a palaeoenvironmental indicator (Peters et al., 2005), as higher CPI values usually correlate with cold and dry environments. Moreover, CPI values generally decreases (≈ 1 / < 1) during the maturation of organic matter, which is attributed to the dilution of *n*-alkanes without the odd-over-even predominance from thermal cracking of hydrocarbons (Luo et al., 2011). The values of CPI close to one are due to more input of recycled organic matter and bacterial and marine microorganisms (Kennicutt et al., 1987).

5.4.2 *n*-Alkane distributions and $\delta^{13}\text{C}$ values

The significant differences in $\delta^{13}\text{C}$ values among plant parts (except for the stem-flower group that showed no significant differences) as well as between plants from forest steppe and desert steppe is likely caused by plant growth stage and diverse environmental variables that are known to impact floral $\delta^{13}\text{C}$ values such as temperature and precipitation. The composition and abundance on *n*-alkanes vary between plant species, tissues and growth stage (Wiesenberg et al., 2004, Bush and McInerney, 2013, Diefendorf and Freimuth, 2017). This heterogeneity in *n*-alkane composition and concentration is largely controlled by stage of tissue development, level of humidity and wax production and regeneration stage (Richardson et al., 2005; Sachse et al., 2012; Kahmen et al., 2011; Gao et al., 2015; Ardenghi et al., 2017). Altogether, variation in $\delta^{13}\text{C}$ values in leaf wax may depend on plant type or water availability which influences the $\delta^{13}\text{C}$ values of plant tissues caused by shifts in CO_2 uptake and fixation (Δleaf). The diffusion of CO_2 from the atmosphere into the air-filled spaces within the leaf together with the plant enzymatic activity, produces a ^{13}C variation that can range between -6‰ and -37‰ (Chikaraishi et al., 2004; Marshall et al. 2007; Sachse et al., 2009). Therefore, leaf water availability derived isotopic signals can be useful for assigning geographic origins to plant materials and plant-derived products (Dawson et al., 2002; West et al., 2006).

The carbon number distribution from the plant wax derived *n*-alkanes from Mongolian penning deposits ranges from C23 to C33, maximising in campsite 7 at C31 and in campsite 54 at C29, indicating animals ingested herbaceous plants in the forest steppe area and shrubby plants or stem-rich plants in the desert steppe area (Fig. 4). As *n*-alkanes originate from leaf waxes of higher terrestrial plants and their homologue pattern allow to discriminate between different vegetation forms, *n*-alkanes derived from sites with grassland/herbs are mainly dominated by C31, while *n*-alkanes derived from sites with deciduous trees/shrubs show high abundances of C29 (Cranwell 1973; Cranwell et al., 1987; Bliedtner et al., 2018). Furthermore, previous research in Mongolian pastoral winter campsites reported penning deposits rich in C29 and C31 *n*-alkanes reflecting ingestion of herbaceous plants and excretion and later deposition of the of stem-rich tissue undigested vegetal remains (Égüez and Makarewicz 2018).

Our results for sample *n*-alkane composition (i.e. vascular plants) and concentrations (i.e. higher in flowers and seeds than in stems) also suggest that these are reflecting the moment in which the plant was collected, in this case, the summer season. Our findings indicate that animals fed on plants that had reached maturity during the summer months and were fed to caprines during the winter months because of the lack of differences in the *n*-alkane concentrations observed in stems of fresh plants and dung deposited during the winter months, in both the forest- and desert- steppe regions. In Mongolia, the combination of very short spring and summer seasons, lasting between May and August and most total precipitation falling between June and July, forces floral growth and rapid fluorescence to produce seeds before the onset of cold temperatures in early September (Shinoda et al. 2007). Previous research suggests that *n*-alkanes accumulate rapidly during plant maturation during spring and early summer, and then remain relatively constant through the rest of the growing season (Eglinton and Hamilton, 1967; Avato et al., 1984; Tipple et al., 2013). Moreover, ethnographical work has shown that tall grasses, chenopods, and some herbaceous growth from summer season pastures are commonly collected by

Mongolian herders, stored in winter camps, and then provided to animals as winter fodder (Makarewicz and Tuross 2006; Makarewicz 2014).

The similarity in the $\delta^{13}\text{C}$ values between seed and dung *n*-alkanes is unexpected but as herders provide their animals with mature plants collected in late summer, it is possible that seeds and grains attached to the stems of grasses and herbs were ingested by animals. Although not dominant, high proportions of the C27 *n*-alkane (C_{max} in our seed samples and present in the stems) are present in the dung deposits, especially on campsite 54 (desert steppe). Thus, this signal may also be reflecting seasonal seed input into the diet together with the stems, for example from *Stipa sibirica* and *Chenopodium album*, abundant species in the desert steppe. It has been long established that carbon number maxima at C27 in sediments is indicative of forests related to cold climates and woody species presence (Cranwell 1973; Zech et al., 2009). But, the higher presence of C27 in our desert-steppe deposits supports instead the provision and ingestion of grass fodder rich in seeds and stems collected in summer rather than representing a litter depositional effect.

The low values for dung deposits are not in accordance with reported $\delta^{13}\text{C}$ range values for the Chinese Inner Mongolian and northern China grassland natural soils, between ca. -20 and -28‰ (Chen et al. 2007; Yao et al. 2016), where high $\delta^{13}\text{C}$ values reflect high local aridity levels. In contrast, low $\delta^{13}\text{C}$ values in Mongolian dung deposits data resemble those of tropical forests where both plant tissues and litter on the forest floor are depleted in ^{13}C due to recycling effects associated with continuous litter deposition over active soils (Benner et al. 1987; Cotrufo et al. 2013).

Two mechanisms explain the low values for dung deposits when compared to natural soils. First, low $\delta^{13}\text{C}$ values in dung-rich sediments are due to the high concentration of undigested plant remains rich in lignin (i.e. stems). Plant lignin-C is strongly depleted in ^{13}C when compared with the bulk plant material by up to 2.5% in legumes and up to 4.7% in grasses (Schweizer et al. 1999). Due to isotopic discrimination associated with the amino acids phenylalanine and tyrosine, the carbon isotope composition of lignin detritus gradually changes during biogeochemical processing in the decomposition phase, leaving a material that is relatively depleted in ^{13}C (Benner et al. 1987).

Continuous and massive accumulation of lignin from ingested vegetal material in the penning structures appear to be undergoing similar isotopic fractionation during decomposition. The massive accumulation of dung would produce a recycling unit in which the resulting litter is more depleted in ^{13}C over time because of the fast loss of more isotopically enriched soluble constituents (Schweizer et al. 1999; Asada et al. 2005). We also suggest continuous litter deposition in corral dung deposits, which generally accumulate over the course of several years, is also responsible for ^{13}C depletion in corral *n*-alkanes.

The post-depositional depletion in ^{13}C of plant waxes within dung is likely a result of diagenesis and organic matter maturation. The products of plant decomposition, the evolved CO_2 and the newly formed soil organic matter, may vary in their ^{13}C signature from that of the original plant material (Schweizer et al. 1999). Thus, carbon isotopic fractionations in plant materials and those occurring during decomposition have direct implications in studies of short- and longer-term organic matter dynamics in dung deposits.

5.5 Conclusion

Jansen and Wiesenberg (2017) recently pointed out three main limitations when applying molecular biomarkers in sediments to estimate environmental variation: 1) the variability in the molecular composition of plant-derived organic matter as a result of genetic or life stage variations or external environmental factors; 2) the variability in input pathways into the soil; and 3) the transformation and/or selective degradation of the molecules once present in the soil. Our results showed that preservation of *n*-alkanes in modern dung deposits is very good and that, *n*-alkane homologue distributions, coupled with the *n*-alkane $\delta^{13}\text{C}$ are a good proxy to identify penning deposits in ecologically distinct sites in Mongolia and that those are isotopically distinct from the natural soils. Our ACL and CPI values directly reflect an open-range diet in desert environments, and a more restricted diet in forest environments with environmental differences between forest and desert steppes detected. However, the variability in the alkane composition could be a result of both genetic or life stage variations or external environmental factors and caution should be taken in using ACL as proxy for past changes in vegetation, based only in temperature and rainfall, as other factors could be influencing the presence of longer chain lengths.

This study is an example of how current advances in lipid and stable isotope analytical techniques can set the stage to advance the archaeology of pastoralist societies. We have shown that sedimentary dung deposits preserve digested plant lipids and that these can be characterised using molecular markers for distinguishing seasonal foddering, local environmental data and to an extent grazing habitats. Further biomarker research on this topic should: 1) involve larger data sets with different Mongolian plant species to corroborate the environmental differences observed here and 2) incorporate other proxies such as *n*-alkane specific δD analysis to provide further insights into palaeoenvironmental conditions, as δD variation is related to evapotranspiration and soil evaporation-induced fractionation (Kahmen et al., 2011). Research in this direction will furnish compound-specific stable isotope datasets in Mongolia and allow us to explore important aspects of pastoralist societies such as husbandry, mobility and pasture land use in the past.

5.6 References

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Chapter 6

6 Conclusions

The papers presented in this dissertation focus on applying methods of the Geo and Bio-sciences. Especially in the study of pastoral societies, geo-ethnoarchaeology has broken disciplinary barriers and opened new horizons for the interpretation of the allegedly 'invisible' and 'ephemeral' campsites. Hence, the potential of the study for stabling deposits through a multi-proxy approach that combines the micro-scale and the molecular one is clear.

The recent development of geo-ethnoarchaeological studies dwells in a renewed enthusiasm for the relation between the present (ethno) and the past (archaeo), supported by new analyses and approaches on hard data (organic chemistry). For many years archaeologists have been informed and misinformed by the observation of the ethnographic present, and in the very last decades geoarchaeologists as well have forayed into living communities looking for a methodological framework and references data, aiming at clarifying the relationships between human behavior and material by-products.

Results yielded in the present articles show that proof of concept studies are necessary to understand how the isotopic record displays pastoralist husbandry practices and how can we untangle these from environmental inputs, animal and plant physiology and metabolism.

The micro-contextual evidence for synchronous domestic activity and sheep penning at Cueva de El Toro, is just an example of how much molecular analyses are needed. The Early Neolithic of the Iberian Peninsula is a complex period that deals with the emergence of new technologies but also the interplay between pastoral and sedenterised communities. Our microstratigraphic approach coupled with short-life radiocarbon dates provides a temporal framework and its context at a scale suitable for approaching behavioural change, which is necessary to understand the regional evolution of Neolithic societies. We provided empirical evidence to set forth a model of Neolithic settlement dynamics involving human groups that lived in caves and shared their living space with goat/sheep. Further geoarchaeological investigation comprising a large sample size for the identification of site formation processes and n-alkane analysis in the characteristic dung deposits of the period (i.e. *fumiers*) is necessary to test our hypotheses.

The case of pastoralism in desert landscapes is a hot topic today in archaeology and the fact that most of dung deposits are shallow and poor-preserved make these case-scenarios in a especial need of molecular approaches. The results of our research are relevant at the local scale, representing a comparison sample to interpret archaeological dung layers, whose investigation presented good results in terms of depositional and post-depositional formation processes (Cremaschi, 1998; Cremaschi and Zerboni, 2011). Nonetheless, there remain many unresolved questions in terms of landscape exploitation, patterns of mobility, and natural resources management across the Holocene. Ultimately, our data enables the design of approaches to dung deposits in drylands worldwide, combining ethnoarchaeological and experimental data to explore the uniquely by-product bio-signatures of past and present animal husbandry on the ground.

In this thesis, we also examined the sediment dung deposits from a continental environment. Eastern Mongolian winter campsites were analysed to identify their molecular and isotopic fingerprints in order to potentially recognise husbandry management and animal diet. The results showed that *n*-alkane patterns of herbivore excrements contained in the sediment are sufficiently different from natural signatures from the same area. Therefore, this result is clearly reflecting differences in the particular plant species that contribute to the *n*-alkanes in the sediment (natural versus an anthropic deposit). Moreover, the changes in $\delta^{13}\text{C}_{\text{alkane}}$ values could provide information about diet and possible herd management. This exploratory study introduced innovative molecular analytical proxies that complemented more traditional approaches (e.g. the study of archaeological artefacts). The potential identification of the spatial distribution of penning spaces at a local scale highlights the relevance of sediment distinctive biomarkers (e.g. high content and well-preserved OM along alkane $\delta^{13}\text{C}$ depleted values) and offered new scenarios to detect past pastoral habitation sites and foddering practices.

Finally, this research examined fresh plants and pastoral dung deposits from two distinctive natural zones in Eastern Mongolia to identify molecular environmental variation in plants and rich organic deposits. The aim was to assess the potential to locate former pastoral habitation sites, foddering practices and latitudinal differences. The results showed that $\delta^{13}\text{C}$ alkane values from herbivore excrements contained in the sediment are sufficiently different from natural signatures from the same area. In addition, ACL values directly reflected an open-range diet in grassland environments and a more restricted diet in forest environments.

While holding great potential, the application of soil lipids as molecular proxies need to take into account intra- and inter-plant variability in the molecular composition of plant-derived organic matter, and the transformation or degradation of (some of) the molecules once present in the soil. Effectiveness of *n*-alkanes biomarkers depends in part on characterization of *n*-alkane profiles at regional and local levels as environmental conditions may influence the pattern of the cuticular wax profile of plant species growing in different places. Dung deposits and dung-derived organic matter *n*-alkanes in our samples provided useful data suitable for the investigation of dietary intake by livestock and husbandry practices among pastoral societies, contributing to advance the research on archaeological pastoralist contexts by showing the feasibility of applying biomarker analysis in sedimentary dung deposits.

Certainly, the ethnoarchaeology of mobile pastoralism lacks a large body of collected knowledge that could make us possible to accurately distinguish its unique characteristics and problems (e.g. ephemerality of habitation remains, re-use of space, or mobility patterns, among others). Still, the use of multi-scale ethnoarchaeological data and scientific analysis of the remains of contemporary pastoral societies stands as a powerful tool for understanding subsistence strategies and human-domesticates economic relationships.

Accordingly, the results of this thesis provide a fundamental much-needed model for the investigation of mobile pastoralist households.

Annex

Chapter 5 raw data with *n*-alkanes values.

Grouping	<i>n</i> -alkanes ($\mu\text{g/g}$ of dry sample)						
	\bar{x}	s	\bar{x}	Min.	Max.	C _{range}	C _{max}
Sample							
Campsite 7							
Dung corral A	12.06	13.31	6.27	0.86	33.11	C25-C33	C31
Dung corral B	2.24	2.35	1.29	0.61	6.71	C25-C33	C31
Campsite 54							
Dung corral A	26.17	40.11	4.81	1.40	108.98	C23-C33	C31
Dung corral B	11.24	10.21	5.43	1.81	27.71	C23-C33	C31
Campsite control Forest							
	0.04	0.01	0.04	0.03	0.06	C25-31C	C29
Campsite control Desert							
	1.16	0.74	1.06	0.04	2.53	C10-C38	C21
<i>A. mongolica</i> 1							
Flower	76.83	85.00	29.86	13.97	202.13	C25-C33	C29
Stem	30.14	23.40	40.28	3.38	46.75	C29-C31	C31
<i>A. mongolica</i> 2							
Flower	6.54	6.70	3.30	1.33	16.21	C25-C33	C29
Stem	16.95	16.08	6.47	4.06	36.41	C29-C31	C31
<i>C. album</i> 1							
Flower	30.01	23.85	18.66	5.36	63.04	C25-C33	C29
Stem	22.74	16.34	22.65	4.07	41.59	C25-C31	C29
<i>C. album</i> 2							
Flower	27.57	21.65	24.75	5.07	55.59	C25-C31	C29
Stem	16.21	18.09	9.49	0.31	44.32	C25-C35	C29
<i>C. album</i> 3							
Flower	16.24	14.89	8.61	2.82	36.49	C21-C31	C29
Stem	12.49	12.34	7.34	1.42	32.80	C25-C31	C29
<i>E. dahuricus</i>							
Seed	18.14	15.55	11.64	3.00	35.92	C21-C31	C23
Stem	1.78	0.45	1.68	1.24	2.79	C21-C33	C29
<i>E. sibiricus</i>							
Seed	19.35	15.60	12.17	2.73	41.84	C23-C31	C29
Stem	7.94	7.84	4.00	1.89	19.36	C23-C33	C29
<i>S. sibirica</i>							
Seed	27.74	24.29	15.87	4.14	70.61	C21-C33	C27
Stem	9.79	7.48	12.55	1.76	18.93	C23-C31	C29
Type							
Flower	30.48	43.90	14.89	1.33	202.13	C21-C33	C29
Seed	22.16	19.21	12.18	2.73	70.61	C21-C33	C29
Stem	11.96	13.87	4.07	0.31	46.75	C21-C35	C31
Dung	14.55	25.21	4.35	0.61	108.98	C23-C33	C31
Natural Zone							
Forest	21.11	38.10	6.59	0.61	202.13	C23-C33	C31
Desert	15.75	20.59	5.44	0.04	108.98	C10-C38	C31