

Unlocking the Past: Exploring Moroccan Lake Sediments Through Ancient DNA and Palynology for Insights into Paleoenvironment, Land Use, and Practices

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1 Unlocking the Past: Exploring Moroccan Lake Sediments through Ancient DNA and 2 Palynology for Insights into Paleoenvironment, Land Use, and Practices 3 Authors: Morgane Ollivier^{1*\$}, Frédérique Barloy-Hubler^{1*}, Sebastián Pérez-Díaz², Régis 4 Debruyne³, José Utge Buil⁴, Françoise Dessarps⁴, Dominique Marguerie¹, Philippe Lanos^{5,6}, 5 6 José Antonio López-Sáez⁷, Larbi Boudad⁸, Jean-François Berger^{9\$} 7 8 * These authors contributed equally to this work. 9 ^sCorresponding authors: morgane.ollivier@univ-rennes.fr, jean-francois.berger@univ-10 lyon2.fr 11 12 **Affiliations :** ¹: University of Rennes, CNRS, ECOBIO (Ecosystèmes, biodiversité, évolution)–UMR 6553, 13 Rennes, France. 14 15 ²: Universidad de Cantabria, Santander, España. 16 ³: MNHN - CNRS, UMR 7209 AASPE (Archéozoologie, Archéobotanique, Sociétés, Pratiques 17 et Environnements), Paris, France. 18 ⁴: Unité Eco-anthropologie, Muséum national d'Histoire naturelle, CNRS UMR 7206, 19 Université de Paris, Musée de l'Homme, 17 place du Trocadéro 75016 Paris, France. 20 ⁵: CNRS, UMR 6034 Archéosciences Bordeaux, Université Bordeaux-Montaigne 21 ⁶: CNRS, UMR 6118 Géosciences-Rennes, University of Rennes 22 ⁷: José Antonio López-Sáez. Instituto de Historia, CSIC, Madrid, España 23 ⁸: Université Mohammed V, Faculté des Sciences, Rabat, Morroco 24 ⁹: CNRS, UMR 5600 EVS/IRG, University of Lyon 25 26 **Authors contributions:** 27 Morgane Ollivier: Conceptualization, methodology, formal analysis, investigation, Writing-28 Original draft preparation, funding acquisition; Frédérique Barloy-Hubler: formal analysis, 29 data curation, Writing- Original draft preparation; Sebastián Pérez-Díaz: formal analysis, 30 investigation, Writing- Original draft preparation; José Antonio López-Sáez: investigation; 31 Régis Debruyne, Jose Utge-Buil, Françoise Dessarp: investigation (experimental lab work); 32 Dominique Marguerie, Philippe Lanos: formal analysis; Jean-François Berger: 33 Conceptualization, resources, investigation, Writing- Original draft preparation. All authors:

34 Writing- Review and editing.

35 Abstract:

36 Over the last 12,000 years, the adoption of an agro-pastoral lifestyle has become prevalent, 37 leading to gradual change in landscapes. In northwestern Morocco, typical elements of an agro-38 pastoral economy emerged around 7,400 cal BP in the Tingitana Peninsula. This was associated 39 with Cardial ceramic style and the presence of key cultivated plant species and domestic 40 mammals. However, in this region, the archaeological record of the last five millennia remains 41 fragmented, with significant regional variations that challenge our understanding of the 42 neolithization processes. This study introduces a new five-meter-long core from Lake Iffer in 43 the North African Middle Atlas. Our objective is to assess the site's potential to reveal insights 44 into the impacts of climate and human activities on the ecosystems and the landscape of the 45 Middle Atlas during the Holocene. Employing a pluridisciplinary approach, we combined paleolimnology and palynology with, for the first time in this context, ancient DNA analysis 46 47 focusing on sedimentary samples spanning from Late Neolithic to Bronze Age.

48 Our findings reveal an environmental opening between 4.1-3.9 ka BP likely triggered by a 49 drought episode, possibly associated with the so-called "4.2 ka cal. BP event". During the same 50 period, we present evidence of increased anthropic pressure, reflected by the presence of ruderal 51 taxa and Bovidae, despite the absence of archaeozoological material. It attests of pastoral 52 activities at least 4,371 years ago at Iffer lake site while no evidence of cultivated plant taxa 53 was found. Moreover, our study demonstrates that both climatic fluctuations and human 54 activities contributed to the local decline of Elephantidae, as indicated by ancient sedimentary 55 DNA Our findings offer new perspectives for exploring and studying Mediterranean sites with. 56 limited material evidence.

57

58 Key-words: pastoralism, Late Neolithic, Bronze Age, Morocco, Middle Atlas Lake, ancient
59 sedimentary DNA, Palynology

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61 Highligths:

- Drought episode between 4,133-3,992 cal. BP, possibly linked to the "4.2 ka cal. BP event."
- Evidence of pastoralism demonstrated by Bovidae aDNA detection
- Presence of Elephantidae in the anthropological system since the late Neolithic.
- Anthropization and climate variations influence land use and wildlife variations.
- Multidisciplinary approach unveils new perspectives for exploring limited Mediterranean material.
- 67

- 68 **1. Introduction**
- 69

70 Over the past 12,000 years, human has seen his way of life change drastically. From nomadic 71 he gradually became sedentary and the adoption of an agro-pastoral regime became widespread. 72 This transition was possible from the end of the Late glacial in Near East in parallel with climate 73 changes (Bar-Yosef and Belfer-Cohen, 1989), and spreads across the Mediterranean from the 74 9th millennium BP onwards (Berger and Guilaine, 2009; Weninger et al., 2006). Intensification 75 of the economy of production and increasing anthropization have gradually evolved over the 76 last millennia, resulting in a gradual change in landscapes. 77 The Mediterranean region, situated between 30 and 40°N, is one of the most sensitive zones to 78 climatic fluctuations which contributed to shape the ecosystems. Its geographical situation lies

in a zone of influence of the North Atlantic oceanic circulation (Ait Brahim et al., 2019). In the
Mediterranean basin, past environmental changes have resulted from critical climate
oscillations and growing ecosystem instability exacerbated by human activities over the past
9,000 years (Cheddadi et al., 2016; Fletcher and Zielhofer, 2013; Mercuri et al., 2012; Sadori
et al., 2013).

84 In northwestern Morocco, typical elements of an agro-pastoral economy appeared in the 85 Tingitana Peninsula around 7,400 cal BP (Gibaja et al., 2012; Linstädter et al., 2018; Marinval 86 and Ballouche, 2003; Morales et al., 2016, Simoes et al. 2023), associated with Cardial ceramic 87 style similar to that of the eastern coast of the Iberian Peninsula. This event is accompanied by 88 the appearance of the main cultivated species of cereals and legumes, as well as domestic 89 mammals common to the early Iberian Neolithic, (Manen et al., 2007; Martínez-Sánchez et al., 90 2018), suggesting that the Neolithic wave diffused from the southern coast of the Iberian 91 Peninsula via sea crossing following a north to south axis (Martínez-Sánchez et al., 2018). 92 However, the archaeological record of the last five millennia in northwestern Morocco remains 93 very fragmented and poorly studied, with great regional contrasts that complicate the 94 understanding of the chronology of the neolithization processes (Lucarini et al., 2021; Martínez-95 Sánchez et al., 2018).

96 Lake sediment changes and variations in sedimentation rate offer valuable information about 97 past climate influences, water depth, and paleoenvironments (Damnati et al., 2016; Magny et 98 al., 2007). These elements associated with the study of ancient remains and chrono-cultural 99 frameworks helps understand biodiversity fluctuations and past land use impacts (Cheddadi et 100 al., 2015; Simonneau et al., 2013). Over the last two decades, lake sedimentary DNA has

101 complemented pollen and macro-remains, proving valuable for studying past community-level

102 changes (Capo et al., 2021). It enables direct identification of organisms in sedimentary archives

103 and helps understand changes in their representation over time. To date, such studies have never

104 been performed in northwestern Maghreb contexts.

105 In Morocco, valuable information on ecosystem changes and species dynamics has primarily 106 come from studies focused on the northern coasts, the Tingitane peninsula, and the Middle and 107 High Atlas Mountains. Several multi-proxy studies of Middle Atlas lakes (such as 108 Tigalmamine, Sidi Ali, Iffrah, Afourgagh) have documented the main hydroclimatic variations 109 and the evolutionary trend of regional climate during the Holocene (Lamb and van der Kaars, 110 1995; Rhoujjati et al., 2010; Zielhofer et al., 2019). Despite pioneering paleolimnological and 111 pluridisciplinary works in the Middle Atlas lakes since the 1990s (Cheddadi et al., 1998; Lamb 112 and van der Kaars, 1995), continuous and long Holocene records with robust absolute 113 chronologies in Mediterranean North Africa remain incomplete, despite recent 114 paleoenvironmental studies and anthropogenic impacts identification has been performed 115 (Cheddadi et al., 2019, 2015; López-Sáez et al., 2023; Nourelbait et al., 2016). Recently, a 116 continuous study from Lake Sidi Ali archive reconstructs Western Mediterranean hydro-117 climatic variability, seasonality and forcing mechanisms. The study reveals low lake levels at 118 6.6-5.4 ka and from 3 ka, a multi-centennial-scale NAO-type pattern and Western 119 Mediterranean, and a winter rain maximum generally associated with solar minima (Zielhofer 120 et al., 2019, 2017).

121 Due to the scarcity of well-dated fossil records, the absence of reliable absolute chronologies, 122 and incomplete archaeological studies in Mediterranean North Africa, a comprehensive 123 analysis of spatial and temporal patterns of environmental variations and species dynamics, 124 which could provide insights into land use and practices, remains challenging. In consequence, 125 the process of progressive anthropization and the development of agro-pastoral practices which, 126 in parallel with the climate, have shaped the environment in northwestern Morocco over the 127 past 7,000 years, remain not fully understood. Therefore, these processes cannot be 128 contextualized and integrated into the known patterns in southern Europe and the Near East 129 edges of the Mediterranean.

In this study, we present a newly recovered five meters long core from Lake Iffer in the North African Middle Atlas. Our objective is to assess the site's capability in providing insights into the impacts of climate and human-induced changes on the Holocene ecosystems and landscape of the Middle Atlas. To achieve this, we employed a pluridisciplinary approach combining paleolimnology, palynology and, for the first time in this context, ancient sedimentary DNA

135 (*seda*DNA) analysis on a few samples to explore the potential of paleogenomic methods on136 such Mediterranean sites.

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139 **2.** Study area

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141 Lake Iffer (33°36'30"N, 4°54'30"W), located in northeastern Morocco (Figure 1A), is one of 142 several lake systems in the tabular Middle Atlas Mountains at the boundary of the Imouzzère 143 and Amekla causses (Rhoujjati et al., 2012). Of moderate size (3.5 ha), it is located in a tectono-144 karstic zone of middle altitude (Baali, 1998), and occupies the center of a watershed of endoreic type whose surface of 4.5 km² extends between 1500 to 1846 m of altitude (Figure 1B). The 145 146 bein of the lake is composed of a calcic bicarbonate facies (Benkaddour et al., 2008; Damnati 147 and Taieb, 2003), associated with the intense karstification of the liasic dolomites lying on an impermeable substratum formed by the Trias argillites (Baali, 1998). The lake is supplied by a 148 149 network of gullies that function periodically during the rainy season, particularly well-marked 150 on its eastern shore, connected to a vast lacustrine terrace. Its level fluctuates according to the 151 seasons and the annual rainfall. The climate is Mediterranean sub-humid with cold winters. 152 Remnants of ancient red Mediterranean soils (terra rossa) are observed on the slopes, more or 153 less heavily colluviated and brunified (rendziniform) by Holocene pedogenesis (Alaoui et al., 154 2020). The surrounding forest vegetation is composed of holm oaks (Quercus ilex) with a few 155 feet of Aleppo and maritime pines (Pinus halepensis and P. maritimus). The matorrals are 156 dominated by Thymeleae tartonraira and Adenocarpus (Lecompte, 1986). The lake has 157 significant water deficits because of recurring drought and human pastoral pressure since the 158 1980s, leading to the decrease in water level and increasing salinity, with relatively alkaline 159 water (Damnati et al., 2016). The aquatic flora of the lake is dominated by cattail (Typha 160 latifolia), white water lily (Nymphaea alba), pondweed (Potamogeton pectinatus) and Eurasian 161 watermilfoil (Myriophyllum spicatum), which are very dense near the shores (Chillasse et al., 162 2001).



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Figure 1: Geographic, topographic and lithostratigraphic data of the studied samples at Iffer lake (Morocco). A. Location of the study site in the Moroccan Middle Atlas mountains; B. Topographic restitution of the lake perimeter with position of the studied core; C. Panoramic view of the eastern shore of the lake with the position of the core (red star) and the steep western and northern wooded slopes; D. Photograph and lithostratigraphy of core-C1 with position of radiocarbon dates, ancient sedimentary DNA and pollen samples; E. Photograph of the lake's western shoreline during a low-water event in autumn 2022, with flocks of sheep and cattle.

171	Morpho-stratigraphic analysis of the catchment was performed by Alaoui et al., (2020), who
172	confirmed an upper Pleistocene age at the base of the upper terrace lacustrine chalk formations
173	to the east (29,360+/-490 BP). Traces of nesting of highly oxidized and pedogenized peaty
174	lacustrine formations, rich in Neolithic flints and shards, are identified in the middle part of the
175	beine.
176	First geochemical and magnetic analyses of detrital formations stored in the lake reflect the
177	nature of the dolomite bedrock and from forest developed soils (Rhoujati et al. 2012, Damnati
178	et al. 2015). These preliminary studies describe warm and dry climate (6500-4600 cal. BP),
179	wetter climatic conditions with high lake level (4600-2000 cal. BP), increasing pastoral and
180	agricultural activities (e.g. Figure 1E) caused the decline of forest linked to increased soil
181	erosion from 2,000 years.
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184	3. Material and methods
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186	3.1 Archeological context and Geomorphology of the site
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188	The new field study conducted at Lake Iffer combined surveys of natural sections (from
189	regressive erosion gullies) and a transect of six cores (from 3 to 6m), carried out with a "Cobra"
189 190	regressive erosion gullies) and a transect of six cores (from 3 to 6m), carried out with a "Cobra" thermic corer, which complete a series of three deep cores (10m) located in the middle of the
189 190 191	regressive erosion gullies) and a transect of six cores (from 3 to 6m), carried out with a "Cobra" thermic corer, which complete a series of three deep cores (10m) located in the middle of the lake, which have been the subject of sedimentological analyses and a palynological study
189 190 191 192	regressive erosion gullies) and a transect of six cores (from 3 to 6m), carried out with a "Cobra" thermic corer, which complete a series of three deep cores (10m) located in the middle of the lake, which have been the subject of sedimentological analyses and a palynological study (Damnati et al., 2016; Rhoujjati et al., 2012). The 500-cm-long sediment core IF-C1 was
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 189 190 191 192 193 194 195 196 197 198 	regressive erosion gullies) and a transect of six cores (from 3 to 6m), carried out with a "Cobra" thermic corer, which complete a series of three deep cores (10m) located in the middle of the lake, which have been the subject of sedimentological analyses and a palynological study (Damnati et al., 2016; Rhoujjati et al., 2012). The 500-cm-long sediment core IF-C1 was extracted from the eastern shore of the lake (Figure 1C). On IF-C1, six accelerator mass spectrometry radiocarbon dates were measured on charcoals at the Artemis-Lyon/Saclay and Poznan Radiocarbon Laboratory (Table S1). We used a Bayesian approach implemented in the new version of ChronoModel (Lanos & Dufresne, 2022) and the calibration data set IntCal20 (Reimer et al, 2020) to estimate an average age-depth curve (MCMC; 3,000,000 iterations, SI, Figure S1). From the age-depth curve obtained by
 189 190 191 192 193 194 195 196 197 198 199 	regressive erosion gullies) and a transect of six cores (from 3 to 6m), carried out with a "Cobra" thermic corer, which complete a series of three deep cores (10m) located in the middle of the lake, which have been the subject of sedimentological analyses and a palynological study (Damnati et al., 2016; Rhoujjati et al., 2012). The 500-cm-long sediment core IF-C1 was extracted from the eastern shore of the lake (Figure 1C). On IF-C1, six accelerator mass spectrometry radiocarbon dates were measured on charcoals at the Artemis-Lyon/Saclay and Poznan Radiocarbon Laboratory (Table S1). We used a Bayesian approach implemented in the new version of ChronoModel (Lanos & Dufresne, 2022) and the calibration data set IntCal20 (Reimer et al, 2020) to estimate an average age-depth curve (MCMC; 3,000,000 iterations, SI, Figure S1). From the age-depth curve obtained by ChronoModel 3.2.2, the sediment accumulation rate was estimated. The estimated curve with a
 189 190 191 192 193 194 195 196 197 198 199 200 	regressive erosion gullies) and a transect of six cores (from 3 to 6m), carried out with a "Cobra" thermic corer, which complete a series of three deep cores (10m) located in the middle of the lake, which have been the subject of sedimentological analyses and a palynological study (Damnati et al., 2016; Rhoujjati et al., 2012). The 500-cm-long sediment core IF-C1 was extracted from the eastern shore of the lake (Figure 1C). On IF-C1, six accelerator mass spectrometry radiocarbon dates were measured on charcoals at the Artemis-Lyon/Saclay and Poznan Radiocarbon Laboratory (Table S1). We used a Bayesian approach implemented in the new version of ChronoModel (Lanos & Dufresne, 2022) and the calibration data set IntCal20 (Reimer et al, 2020) to estimate an average age-depth curve (MCMC; 3,000,000 iterations, SI, Figure S1). From the age-depth curve obtained by ChronoModel 3.2.2, the sediment accumulation rate was estimated. The estimated curve with a confidence interval allows to calculate a date interval whatever the depth (posterior probability
 189 190 191 192 193 194 195 196 197 198 199 200 201 	regressive erosion gullies) and a transect of six cores (from 3 to 6m), carried out with a "Cobra" thermic corer, which complete a series of three deep cores (10m) located in the middle of the lake, which have been the subject of sedimentological analyses and a palynological study (Damnati et al., 2016; Rhoujjati et al., 2012). The 500-cm-long sediment core IF-C1 was extracted from the eastern shore of the lake (Figure 1C). On IF-C1, six accelerator mass spectrometry radiocarbon dates were measured on charcoals at the Artemis-Lyon/Saclay and Poznan Radiocarbon Laboratory (Table S1). We used a Bayesian approach implemented in the new version of ChronoModel (Lanos & Dufresne, 2022) and the calibration data set IntCal20 (Reimer et al, 2020) to estimate an average age-depth curve (MCMC; 3,000,000 iterations, SI, Figure S1). From the age-depth curve obtained by ChronoModel 3.2.2, the sediment accumulation rate was estimated. The estimated curve with a confidence interval allows to calculate a date interval whatever the depth (posterior probability density region HPD at a given confidence level (95%)) and an age (Mode A Posteriori) was
 189 190 191 192 193 194 195 196 197 198 199 200 201 202 	regressive erosion gullies) and a transect of six cores (from 3 to 6m), carried out with a "Cobra" thermic corer, which complete a series of three deep cores (10m) located in the middle of the lake, which have been the subject of sedimentological analyses and a palynological study (Damnati et al., 2016; Rhoujjati et al., 2012). The 500-cm-long sediment core IF-C1 was extracted from the eastern shore of the lake (Figure 1C). On IF-C1, six accelerator mass spectrometry radiocarbon dates were measured on charcoals at the Artemis-Lyon/Saclay and Poznan Radiocarbon Laboratory (Table S1). We used a Bayesian approach implemented in the new version of ChronoModel (Lanos & Dufresne, 2022) and the calibration data set IntCal20 (Reimer et al, 2020) to estimate an average age-depth curve (MCMC; 3,000,000 iterations, SI, Figure S1). From the age-depth curve obtained by ChronoModel 3.2.2, the sediment accumulation rate was estimated. The estimated curve with a confidence interval allows to calculate a date interval whatever the depth (posterior probability density region HPD at a given confidence level (95%)) and an age (Mode A Posteriori) was assigned for each sample (Table S2).

203 This core was sampled every 10 cm for pollen analyses, and was passed through the core 204 scanner for XRF analysis every centimeter. Hydroclimatic fluctuations have succeeded one 205 another leading to the oscillation of lake levels for more than 6 millennia, visible on both sides 206 of the lake, by the alternation between silty-organic and chalky facies. The yellowish sandy or 207 silty facies, more or less rubefied, testify to more detritic phases, connecting the slopes and the 208 lake. The samples were collected between 2.3 and 3.03m depth, i.e. according to the age-depth 209 model between 4,600 and 3,900 BP (late Neolithic-early Bronze Age). This part of the core 210 corresponds globally to a period of higher water levels (positive water balance), associated with 211 lacustrine deposits containing numerous carbonate biomarkers (tubes and plates) (Figure 1D). 212 To document human occupation near the lake, fifteen windows of archaeological survey were 213 carried out over 1.5 to 2 ha with the support of a GPS on the eastern terrace of the lake, rich in 214 chipped flints, with scattered ceramics on the surface. Three sectors indicate an occupation of 215 the Epipaleolithic period (flakes with cut edges and bladeletts also pointed by abrupt retouches). 216 Five other sectors indicate a debitage rather of Mesolithic age (with segments of circle, 217 unidirectional nuclei with negatives of narrow bladeletts). Eight places show evidence of 218 Neolithic occupation sensu lato. The presence of larger flint pieces with more imposing 219 modules indicates productions attributable to a Late Neolithic, possibly corroborated by the 220 presence of some beige ceramic shards with incised decorations of the campaniform culture

type (study in progress R. Guilbert-Berger). Concentrations of more recent ceramics (non-

turned and turned) testify to proto-historic to antique human occupations (studies in progress).

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225 3.2 Palynological analysis

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227 A total of 48 samples have been studied in Iffer-C1 core sequence. The chemical 228 treatment of the samples has been carried out following the so-called classical method (Burjachs 229 and Renault-Miskovsky, 1992; Faegry and Iversen, 1975; Girard and Renault-Miskovsky, 230 1969; Goeury and De Beaulieu, 1979; Moore et al., 1999), resulting in a final portion of the 231 sediment preserved in glycerin gelatin (detailed in SI). An optical microscope (Nikon Eclipse 50i model), with 40X, 60X and 100X objectives, the latter with immersion oil, has been used 232 233 for the identification of the pollen and non-pollinic microfossils. The reference collection of the 234 Environmental Archaeology Research Group of the CSIC (Madrid), in addition to various 235 bibliographic sources on the morphometric characterization of palynomorphs have been used

for the identification of pollen morphotypes (Bonnefille, 1980; Moore et al., 1999, 1991; Moore

and Webb, 1978; Reille, 1995, 1992).

238 Non-pollen microfossils (algal spores, cyanobacteria, fungal spores and talus remains, fungal

bodies of fungi, fragments of bryophytes or pteridophytes, animal micro-species, microfossils
of unknown biological nature, etc.) were also identified based on abundant bibliographic
references (Bakker and Van Smeerdijk, 1982; Lopez-Saez et al., 1998; Pals et al., 1980;
Pantaleon-Cano et al., 1996; Sáez-López et al., 1999; van Geel, 1978; van Geel et al., 2003;
van Geel et al., 1989, 1980). For the elaboration of the pollen diagram, hydro-hygrophilic taxa,
non-pollen microfossils, as well as *Aster* type, Cardueae and Cichorioideae have been excluded

245 from the base sum, due to their zoophilic character being usually overrepresented (Bottema,

246 1975; López-Sáez et al., 2003; Sáez-López et al., 1999). The relative percentage of these
247 excluded palynomorphs has been calculated with respect to the total sum.

In this study we describe results (Table S3) of the four samples (at 300, 279, 250 and 236 cm
depth) that coincide with ancient DNA (aDNA) samples.

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252 3.3 Ancient DNA analysis

The entire Iffer-C1 core was opened in a dedicated room in the Palgene platform (ENS Lyon). DNA samples were taken each nine centimeters. We first scraped the sediment surface using sterile scalpels (5-10mm), then sampled inner uncontaminated sediments with a new sterile scalpel and transferred them to sterile tubes. Subsampled sediment was stored at -20° C for subsequent extractions. For this study, four samples were chosen for analysis (Figure 1D, Table S2). All the extractions were then conducted on the P2GM platform (MNHN Paris) equipped with specialized facilities devoted to aDNA analysis.

260 The four extractions of *seda*DNA were performed from 100 mg of sediment (+/- 10%), in one

batch, including one extraction blank following Dabney et al (2013) protocol (detailed in SI).

262 We prepared libraries following a slightly modified protocol from Meyer and Kircher (2010)

using the double indexing strategy of Kircher et al. (2012) (detailed in SI).

All samples underwent enrichment using custom probes (MyBaits custom design) designed to target 19 mammal and 22 plant taxa (Table S4, SI). The enrichment utilized complete mitogenome for mammals and *matk*, *rbcl*, and *trnl-trnt* chloroplast loci for plants. Libraries were captured per batch of two following the MYbaits manual V3, with 48 hours hybridisation at 56°C. The captures were amplified using KAPA Hi-Fi polymerase following the MyBaits 269 manual instructions. Captured libraries were all sequenced in paired-end on a partial Illumina
270 NovaSeq 6000 S4 XP lane (300 cycles, PE 150).

- 271 Reads were processed through the Paleomix pipeline (Schubert et al., 2014), (Table S5) 272 allowing 1) adapters removal, read pairing and merging, and sequence filtering using 273 AdapterRemoval (Lindgreen, 2012) (min length reads>=35bp, trimns: yes, triml qualities: yes, 274 mm: 5); 2) duplicates filtering and 3) reads mapping (Li and Durbin, 2009), (BWA aln; -1 1024; 275 -o 2; -n 0.01) against a database comprising one sequence per genus and targeted loci (retaining 276 the longest sequence) from available mammal and viridiplantae in the GenBank Database 277 (march 2020). Reads were secondary analyzed with Prinseq (Schmieder and Edwards, 2011) to remove low complexity sequences (-lc method dust -lc threshold 7 -trim tail left 5 -278 279 trim tail right 5). Bam files were filtered with samtools (Danecek et al., 2021) using tag "X0:i:" 280 to generate two sequence files, one with unique best hit and one with multiple best hit. All reads 281 with matches were identified through DNA homology searches using BLAST (Altschul et al., 282 1990) against the nr database (08/2022) to validate or determine the best taxonomic assignment. 283 Significant homologies obtained by Blast (best query cover and e-value) that refer to a single 284 taxon are retained. If scores are equivalent for multiple taxa, the taxonomic rank is adjusted 285 until a clear result is obtained (from genus to family depending on the case). To achieve precise and unambiguous taxonomic classification, biocurators performed this analysis manually. Taxa 286 287 present in the control (extraction blank: sample without DNA) were excluded from the study 288 and regarded as experimental contamination.
- 289 Mosses, ferns, algae and hornworts were not included in this study. We have selected taxa that 290 represent more than 1% of the total number of reads attributed to plant or mammal taxa in at 291 least one of the four samples studied (Table S6). However, taxa that do not fill the 1% criterion 292 but described in the pollen analysis (Table S6) were added in figure 2. Given the capture design 293 and the results obtained for both *seda*DNA and palynology, we have chosen the following four 294 categories: mammals, trees, aquatic plants and terrestrial plants. The relative evolution of the 295 proportion of each taxon (>1% of total reads assigned) is then considered intra-categories 296 (normalized in %, Figure 2). The results were illustrated as charts using R version 4.2.2 (2022-297 10-31) via Rstudio version 2022.12.0+353 and ggplot2, hrbrthemes and dlpyr packages. 298 Finally, sedaDNA deamination pattern and fragment length distributions were inspected with 299 mapDamage2 (Jónsson et al., 2013) for taxa with sufficient read counts after mapping of the 300 reads against each reference genomes (Table S7, Figure S3). 301
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4. Results and Discussion

- 305 *4.1 Landscape dynamics*
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307 At the molecular level, less than 1% of total sequences could be assigned (Table S5). In 308 sample SL383394 at 302cm depth, showed no identified mammals, and very low number of 309 plant reads (n=161) (Table S2, S6), reaching a maximum of four reads for taxa of interest. For 310 this reason, the relative proportions of plant families for this sample are biased and difficult to 311 interpret and we decided to not present and discuss sedaDNA results for this sample. This 312 period corresponds to one of the four arid abrupt events identified around 4.5-4.4 ka BP, in 313 Lake Tigalmamine (about 100 km from Lake Iffer, El Hamouti, 2014). The dates also mark the 314 end of Lake Iffer's initial filling and the existence of a shallow water body due to low water 315 inputs, with very high carbonate content (Rhoujjati et al, 2012). This high alkalinity, combined 316 with aridity, may have led to greater DNA degradation, as already reported in other studies 317 (Capo et al, 2021). This deficiency of DNA may explain the low sequencing yields for this 318 sample (approximately 4 to 30 times less sequences than the other three samples, Table S5) and 319 the difficulties in taxonomic identification. (Parducci et al, 2017). Sequenced DNA quantities for the three other samples are adequate (Table S5). They indeed cover the 4.3-3.9 ka BP period, 320 321 that corresponds to declining carbonate contents and temperatures in Lake Iffer, due to rising 322 lake water levels linked to wetter conditions. Regional references also indicate episodes of low 323 lake levels with reduced precipitation/evaporation ratio during this time (Rhoujjati et al, 2012).

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325 Three major genera of trees (Pinus, Quercus and Arbutus) can be clearly identified within 326 sedaDNA during the considered period (Figure 2A, Table S6). *Pinus* and *Quercus* are currently 327 present on the site and their DNA as well as pollen remains are present throughout the period 328 studied. Arbutus is only detected by sedaDNA analysis at 4,371 cal. BP (30% of the tree and 329 shrub population) before drastically decrease. Its presence is in accordance with a previous 330 study at Benzú Cave, located in the northernmost tip of the Tingitan Peninsula, 360 kilometers 331 north of Iffer Lake, where it was used as fuel together with Quercus sp., Olea sp., Fraxinus sp. 332 among others (Muñoz et al., 2013; Vijande-Vila et al., 2019). Its absence in the pollen register 333 could be explained by its entomophilous pollination, the fact that *Arbutus* is a poor pollen 334 producer since its mode of pollination is autogamy (Hagerup, 1957; Faida et al., 2023) and







Taxons



Taxons : Ceratophyllum Cyperaceae Myriophyllum Ruppia



Portion in %

100

Portion in %

among four groups: A) trees and shrubs, B) herbs, C) aquatic plants and D) mammals; and E) : relative proportions of tree/herbs/aquatic plants identified. In A, B, C and D panels: Up: Relative proportion of each plant family/genus among all assigned plant/ mammal reads - for which the number of reads >1%of the total number of reads assigned to either plant or mammal taxa in at least one sample or present in the pollen records ; bottom: relative proportion of the main plant and mammal taxa assigned (>1%); red: Plant taxa for which the percentage is <1% and identified in pollen remains.

Taxons : Bovidae Elephantidae This preprint research paper has not been peer reviewed. Electronic copy available at: https://ssrn.com/abstract=4534181





335 because its pollen is large and heavy and does not travel far from the tree and, moreover, it is 336 similar to other pollens of the Ericaceae family and difficult to distinguish (Almeida et al, 2022). 337 Nowadays in Morocco, Arbutus occurs in scrublands, oak forests, and dense maquis. Its density 338 depends on a gradient with densest populations where the climate is subhumid to humid as this 339 species prefers fresh, well-drained soils (Wahid et al, 2019). However, Arbutus shows strong 340 resistance to hard environmental conditions and has the ability to regenerate after forest fires 341 (El Haouari et al, 2021). The significant decline in its population, in proportion to that of 342 Quercus and Pinus after 4.2 ka BP could be explained by the so-called "4.2 cal. ka BP event" 343 corresponding to an interval of cooling and drying conditions (Bini et al, 2019). During this 344 period, the percentages of tree pollen show, in Morocco, a moderate increase in tree cover 345 between 4.5 and 4.2 ka BP followed by a decline, creating an opening of the forests between 346 4.2 and 3.9 ka BP (di Rita et al, 2022). As Arbutus is part of the understory vegetation and its 347 growth has been shown to be inversely related to the presence of holm oak (Badal et al, 1994), 348 we can hypothesize that the growth of the forest cover between 4.5 and 4.2 ka BP, followed by 349 its opening, also observed with our *seda*DNA results, is one of the causes of the decline of this

350 species after 4.2 ka BP.

351 This forest development around the 4.2 ka BP event is clearly evidenced by our Iffer Lake 352 sedaDNA analysis. The proportion of tree DNA increases from 12% to 30% between 4.3 ka 353 and 4.1 ka BP and then stabilizes (Figure 2E). This dominant association of *Quercus* and *Pinus* 354 is also corroborated by palynological analyzes which confirm that this *Pinus-Quercus* mixed 355 forest ecosystem is well developed during this period (Table S3, Figure 3). They are associated 356 with Olea europea around 4.5 ka BP and then riparian taxa at 4.3 ka BP (Alnus and Fraxinus) 357 while Cedrus remains constant. We first observe a predominant presence of Quercus, that 358 decline together with Cedrus or Olea europea, indicating some deforestation around 4.1 ka BP. 359 This episode is followed by a re-expansion of oak forests at 3.9 ka BP and *Olea europaea* and 360 the progressive disappearance of riparian taxa, (Figure 2A, Figure 3).

361 This significant disturbance in the relative composition of mixed oak and pine forests between 362 4.1 and 3.9 ka BP could be attributed to drought but also probably to possible fires. In Ait Ichou, 363 70 km from Ifter lake, the number of micro-charcoals increased considerably after ~4.5 ka BP with maximum values between ~3 and 1.5 ka BP (Tabel, 2015). As human activities in the 364 365 Middle Atlas were rather minor until ~2-1.5 ka BP (Cheddadi et al, 2015; Tabel, 2015), these 366 fires are likely of natural origin, given the more arid environment. A recent study, carried out 367 in the northeast of Spain, shows that, while these two species regenerate effectively after fire, 368 *Pinus* recolonizes disturbed areas via seedlings, whereas *Quercus* regrows vigorously after

disturbances. Therefore, in mixed forests, even small-scale fires can lead to significant probabilities of transitioning from mixed forests to monospecific forests, with *Quercus* dominating at higher altitudes and *Pinus* at lower altitudes, particularly in drier and warmer conditions (Broncano et al, 2005). We can therefore hypothesize that the relative deforestation and the decrease in Pine DNA in favor of oak DNA between 4.1- 3.9 ka BP would correspond to the consequence of fires, themselves linked to an episode of drought.

375 At Dayat Hachlaf, a few kilometers from lake Iffer, Quercus and Pinus forest contingencies 376 have been recorded since 6 ka BP, with a noticeable change in forest composition around 3.5 377 ka BP (Nourelbait, 2016). These *Quercus-Pinus* variations were not really recorded via the 378 palynological study. Pinus is a well-known quantitatively and extensively dispersed pollen 379 producer (Poska and Pidek, 2010; López-Sáez et al., 2013), dominating Iffer lake record and 380 complicating the correlation between its presence on the site and its pollen abundance (Bell & 381 Fletcher, 2016), well in less proportion. Other trees are detected by the pollen (Cupressaceae, 382 Cedrus, Olea europea and riparian taxa (Fraxinus), only found as traces for some of them 383 among sedaDNA data, indiscernible among other representatives of the same families (co-best 384 hits and <2% of assigned reads).

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Figure 3: Palynological diagram reconstituted from four samples at Iffer lake, covering a 380 years
period (Late Neolithic to Bronze Age)

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393 2B). We noticed a slight variation in the proportion of these taxa over time where Fabaceae is 394 the most abundant around 4.3 ka BP whereas Asteraceae dominates at 4.1 ka BP in detriment 395 of other families, and in association with other ruderal taxa in the palynological record 396 (*Plantago lanceolata, Rumex, Medicago;* Figure 3). The palynological rests are dominated by 397 Poaceae, showing probably the presence of grasslands in the vicinity.

398 Finally, we could determine two major genera of aquatic plants among sedaDNA sequences 399 (Figure 2C, Table S6): Myriophyllum (Haloragaceae) and Ceratophyllum (Ceratophyllaceae), 400 along with low amount of Ruppia (Cymodoceae) and Carex (Cyperaceae). Myriophyllum and 401 *Carex* were also determined in palynological remains with the same relative evolution of their 402 proportion (Figure 3). As already demonstrated in previous studies, aquatic plants, which grow 403 in and around the lake, dominate the assemblage (Figure 2E) to the detriment of terrestrial plants 404 which are often under-represented (Birks, 2003, Alsos, et al 2018). Myriophyllum appears in 405 the pollen record of the four samples with constant values of 4-9% (Figure 3), indicating a stable 406 presence throughout this time, as well as shallow and somewhat eutrophic waters. sedaDNA 407 results (Figure 2C, Table S6) show a decrease of *Ruppia* and *Carex* over the time since these 408 two species, represent respectively 3.7% and 4.6% of aquatic plants at 4.3 ka BP then pass to 409 the state of traces.

410 Myriophyllum dominated in the lake during the whole studied period even if we observe, at 411 4,133 cal. BP, an increase of the proportion of Ceratophyllum sp. Myriophyllum and 412 Ceratophyllum are two species of submerged macrophytes with common characteristics (fast 413 growth, low preference for light). However, Myriophyllum is a rooted species, while 414 *Ceratophyllum* does not form roots and is found free-floating. Consequently, nutrient uptake by 415 Ceratophyllum is almost entirely foliar whereas Myriophyllum can absorb nutrients from 416 sediments and the water column, thereby limiting nutrients available to floating plants (Cao & 417 Wang, 2012). Although Nourebailt et al (2016) seems to denote that the proportion of aquatic 418 plants cannot be used as indicators of lake level variations but only presence of water in the 419 site, a paleoecological analysis of the macro-remains of Lake Iffer for 6000 years (Middle Atlas, 420 Morocco, Huillery, 2022) shows, between 4,350 and 3,320.5 cal. BP, a progressive installation 421 of relatively humid conditions. Same study pointed out abundance of microcharcoals between 422 4.1-3.9 ka BP that testifies local fires (which corroborates our hypothesis concerning the cause 423 of the Quercus-Pinus shift). We can hypothesize that Ceratophyllum benefited from favorable 424 conditions (light, dissolved nutrient) at 4.1 ka BP but that then, plant degradation following 425 fires and humidification of the environment favored Myriophyllum. This hypothesis 426 corresponds to the observations showing that, in Lake Iffer, between 4.6 and 3.6 ka BP, the

427 organic matter content marks a very significant return in terms of contribution compared to the428 previous period (Rhoujjati et al, 2012).

In total, all taxa identified by pollen were retrieved in sedaDNA (sometimes as traces) but *seda*DNA identified seven taxa absent in pollen (Ericaceae, Convolvulaceae, Fabaceae, Lamiaceae, Rosaceae Ceratophyllaceae and Cymodoceae), either because pollen diffusion mode (anemogamy and entomogamy) or low quantity of pollen produced and/or detected. The absence of total overlap between the two approaches as well as the relative differences between trees/shrubs and herbaceous is not surprising. Pollen reflects local and regional landscape while *seda*DNA explain local vegetation variation (Capo et al., 2021, Parducci, 2013, 2019).

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Altogether, results indicate an environment dominated by evergreen oak and Poaceae. Around 4.3 ka BP, increased environmental humidity is characterized by the rise of *Fraxinus* and Cyperaceae. This precedes a relative opening of the environment, between 4.1- 3.9 ka BP, with relative deforestation, the gradual disappearance of riparian taxa and the decrease in Pine in favor of oak. This may have resulted from a drought episode, possibly linked to the "4.2 ka cal. BP event." Additionally, we observe greater anthropic pressure during the same period, as evidenced by the presence of ruderal taxa.

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446 4.2 Evidence of increased anthropization and pastoralism

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Morocco, especially the Western Maghreb, is the most well-dated and documented region of Mediterranean Africa (excluding Egypt) between 6,000-2,900 years BP. However, information remains scarce, particularly about the ways of life that supported people in this area during the preceding fourth and early third millennia BP (Lucarini et al., 2021).

Among *seda*DNA sequences, the major part of mammal reads mapped on Elephantidae and Bovidae mitogenomes. Two main genera identified within Bovidae are *Ovis* and *Bos*, but differentiation between the two genera was not always possible due to the similarity of the genomic regions mapped to the reads. In the palynological record, in sample 300, fungal ascospores of coprophilic ecology (*Sordaria* sp., Figure 3), a fungi that develop in animal excrement, have been identified (van Geel, 1978, López Sáez and López Merino, 2007). Its scarce dispersion undoubtedly determines the existence of animals on a local scale.

459 Domesticated animals, mainly caprines and cattle (all already present during the eighth-seventh 460 millennia BP), remained widely attested throughout Mediterranean Africa. In Western 461 Maghreb, their presence is reported in coastal sites during the early to mid-sixth millennium BP 462 with evidence of cattle, caprines, and pigs (Linstädter et al., 2018; Morales et al., 2013; 463 Schmieder and Edwards, 2011). Later, the site of Oued Beh (late sixth millennium BP), 464 indicates a possible socially complex agricultural society (Rus et al., 2012). Evidence of 465 pastoral activity and crop cultivation remains diffuse for the first half of the fifth millennium 466 BP (Lucarini et al., 2021). Finally, during the fourth millennium BP until the start of the third 467 millennium BP only few remains attest of pastoral way of life with the presence of domestic 468 cattle and caprines (Wengler et al., 1994; Wengler and Vernet, 1992).

469 Here we evidence, for the first time, presence of Bovidae on the Iffer site, since 4,371 cal. BP 470 and continuously during at least 380 years. The site and the approach to the lake as an access 471 to the water could have constituted a favorable environment for grazing. We observe the 472 proportion of this taxa increasing since 4,133 cal. BP at the expense of the Elephantidae, 473 detected since 4,371 cal. BP before disappearing 140 years later (Figure 2D). This corresponds 474 to a slight landscape opening and increased human influence, marked by the presence of ruderal 475 taxa. However, there is no indication of cultivated plant taxa at this site. This is not unexpected 476 as it is well-established that agriculture was delayed in most of Mediterranean Africa, due to 477 pastoral groups that have traditionally exhibited robust and resilient lifestyles, influenced by 478 Saharan foraging and pastoral practices (Lucarini et al., 2021). Also, these populations probably 479 exploited wild plants such as acorns, pine nuts, and arbutus (Barker et al., 2010, 2009; Morales, 480 2018; Morales et al., 2015) that were crucial sources of food along the Saharan belt (Dunne et 481 al., 2016; Lucarini, 2014; Mercuri et al., 2018; Wasylikowa, 2001), and the Mediterranean coast 482 (Lucarini et al., 2016) even during Neolithic.

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485 **4.3** Presence of Elephantidae is impacted by anthropic pressure and climatic

486 *variations*

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The presence of elephants is attested by *seda*DNA identified in two of the four samples, spanning 140 years (from 4,371 to 4,133 cal BP, Figure 2D). This detection is noteworthy as elephants was not a targeted taxon in the capture. Unlike plants, detecting animals through *seda*DNA is often challenging and incomplete, given the limited traces they leave. Thus, their detection suggests a significant population on the site, sufficient for their DNA to be deposited and later detected thousands of years later (Giguet-Covex et al., 2019). 494 In the Plio-Pleistocene, proboscidean species in Morocco include the genera Anancus, 495 Mammuthus (the species M. africanavus) and Elephas. In Morocco, Elephas taxa include the 496 species Elephas recki Dietrich 1915, a widespread Pleistocene African species that ranged from 497 Eritrea (Martínez-Navarro, 2010) to the Atlantic coast of Morocco (Arambourg, 1979) and the 498 species Elephas iolensis excavated from the mid-pleistocene Anchrif fossil site (Marinheiro et 499 al., 2014; Ouchaou et al., 2017). These specialized grazing species became extinct in the 500 Middle-Upper Pleistocene (Manthi et al., 2020), likely due to the intensity and increasing rate 501 of climatic fluctuations, which favored the generalist and mixed feeder Loxodonta africana. 502 The subspecies Loxodonta africana pharaohensis, also called Atlas elephant, was present in 503 North Africa and therefore in Morocco, until its extinction in Roman times. In particular, the 504 'African Humid phase' in North West Africa (c.10.5-5.5 ka cal BP) offered favorable 505 conditions permitting its far wide distribution (Schuhmacher, 2017).

506 The exact determination of the genus and the species of Elephantidae at Iffer site was impossible 507 due to the similarity of the genomic regions mapped to the reads in both *Elephas* and *Loxodonta* 508 genus. Although paleontological evidence suggests the absence of *Elephas* genus in this region 509 during that period, we could hypothesize they could belong to Loxodonta taxa. Moreover, none 510 of the reads attributed to Elephantidae matches 100% with the available sequences of current 511 representatives (mitogenomes available for seven different species). It suggests that these 512 sequences could be attributed to a species not represented in the current databases and therefore 513 possibly ancient, as it is the case for Loxodonta africana pharaohensis.

514 During the Neolithic and Bronze Age period, the presence of elephants in Morocco has been 515 documented through various archaeological artefacts and rock art (eg: petroglyph of an 516 elephant, cave site of Aït Ouazik, late Neolithic, engravings of Hassi Ghilan; (Boudouhou, 517 2020; Daugas, 2010; Schuhmacher, 2017). While these representations and archeological 518 elements could testify of their environment, of the richness of the fauna in the Atlas and of the 519 use of elephants (hunting, transportation, trade, ivory) (Daugas, 2010; Fregel et al., 2018; 520 Schuhmacher, 2017), the scarce number of objects and remains in Morocco do not allow to 521 fully understand their exploitation and incorporation in the human system and in this region 522 during Neolithic and Bronze Age. Here, we both show archeological and biological evidence 523 of human site occupation and of pastoralism 4,371 years ago, while elephants were present at 524 Iffer site. Although we cannot presume human-elephant interactions, we can assume that these 525 animals were indeed part of the anthropological system and could even be exploited. Their 526 importance is attested, for example, by the existence, since the Chalcolithic, of African ivory 527 exchange networks from the Maghreb to southwestern Spain and Portugal (Schuhmacher,

528 2017). Its disappearance from sample 236 at 3,992 cal. BP, for which the plant biodiversity as 529 well as the number of DNA sequences attributed to each taxon is similar to samples 279 and 530 252, could more likely be linked to both anthropic pressure and climatic variations. Their 531 disappearance followed a period of warmer and drier climate leading to a decline of the forest 532 and an opening of the landscape. Together with a possible exploitation of this species, the 533 relative intensification of pastoralism and a change in land use (even if no agricultural practice 534 was detected), their preferred environment could have been modified affecting their ability to 535 survive.

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5. Conclusion

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Here, for the first time in Northern Western Maghreb, we employed both palynological and paleogenomic to reveal climate changes and human presence at Iffer lake from late Neolithic to Bronze Age. Archeological and biological results demonstrate evidence of pastoralism 4.3 ka years ago on this site. The intensification of anthropic pressure in this region precedes the environment opening between 4.1-3.9 ka BP possibly triggered by a drought episode linked to the so-called "4.2 ka cal. BP event". Both factors likely contributed to the local decline of Elephantidae, whose presence is attested through ancient sedimentary DNA.

547 Our results hold significant importance for the archaeological community. They not only shed 548 light on the processes of anthropization in this region, but also provide valuable evidence of 549 pastoral practices for a period poorly documented. Particularly noteworthy is the detection, for 550 the first time through *seda*DNA, of the presence of Bovidae and Elephantidae. It offers new 551 perspectives for the exploration and study of these Mediterranean sites with scarce 552 archeozoological material. An extensive analysis of the genomic data (including at the 553 microbial level) is currently underway to gain a deeper understanding of the ecological changes 554 of this site. This data will be complemented by a more comprehensive paleoclimatic study to 555 facilitate a better interpretation of biodiversity changes.

556 Extending ancient sedimentary DNA analysis to more recent and arid periods, characterized by 557 intensified anthropization and catchment detriticism, would be valuable in observing the 558 evolution and response of DNA signatures and biodiversity. Additionally, a high-resolution 559 coupled analysis between DNAs and pedosedimentary facies, as opposed to traditional 560 sedimentology and geochemistry, could help discern the connection between the DNA signal 561 and the stability/instability of slopes near Lake Iffer.

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575 **Data availability:** Raw genomic sequence data are available on request.

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577 **Declarations of interest:** The authors declare that they have no known competing financial 578 interests or personal relationships that could have appeared to influence the work reported in 579 this paper.

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