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Middle Pleistocene ecology in central Italy. New isotopic insights from fauna tooth enamel of Casal de' Pazzi (Rome, Italy)

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ABSTRACT - The late Middle Pleistocene site of Casal de' Pazzi is one of the numerous archaeological and paleontological sites found in central Italy. It yielded a rich fossil collection, including Middle Paleolithic stone tools and a single retouched bone fragment, faunal remains, a parietal fragment attributed to *Homo heidelbergensis* (or likely ascribed to a Neanderthal hominin), and leaf fossil impressions of *Zelkova* sp., *Laurus nobilis*, and *Cercis siliquastrum*. We investigated stable carbon and oxygen isotopes ($\delta^{13}C$ and $\delta^{18}O$) of tooth enamel from the mammal assemblage of Casal de' Pazzi to reconstruct their diet and habitat and to contribute to the understanding of the Middle Pleistocene environment in central Italy. We analyzed the isotopic composition of tooth enamel of several *taxa*, including *Palaeoloxodon antiquus*, *Bos primigenius*, *Stephanorhinus kirchbergensis*, *Equus ferus*, *Dama dama* and, for the first time in Europe, *Hippopotamus amphibius*. Isotopic analyses indicate that mammals exploited different habitats within a C_3 mosaic landscape with closed and open environments. This is consistent with leaf fossil impressions found at Casal de' Pazzi, and pollen data from the nearby lake of Valle di Castiglione, which indicated that the Casal de' Pazzi area was characterized by riparian habitats and diversified wooded/forested vegetation with extended grasslands. Comparisons with published isotopic data from central Italy between ~600 ka and ~171 ka provided evidence for considerable environmental differences through time and space, according to trends in the local vegetation and general climate.

Keywords: Casal de' Pazzi; Middle Pleistocene; stable isotopes; paleodiet; paleohabitat; paleoenvironment; paleoecology.

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1. INTRODUCTION

A large complex of the Italian Peninsula archaeological archives has been found in central Italy at several Middle Pleistocene sites. The Roman Basin yielded abundant geological, paleontological, and anthropological evidence, much of which were identified between the end of the 19th century and the beginning of the 20th century during the urban expansion of the city of Rome (Buzi et al., 2021; Ceruleo et al., 2021; Romano et al., 2021). Most of the fossil deposits were discovered in alluvial contexts along the Tiber River and its tributaries (e.g., Aniene River) and yielded relevant information on local sedimentary evolution, faunal biochronology, and human occupation in Italy and Europe (Conato et al., 1980; Anzidei, 1983; Petronio and Sardella, 1998; Anzidei et al., 1999; Anzidei and Cerilli, 2001; Manzi et al., 2001, 2010, 2011; Milli et al., 2004; Milli and Palombo, 2005; Funicello et al., 2006; Marra et al., 2014; Manzi, 2016; Palombo, 2018; Iannucci et al., 2021; Strani et al., 2021; Sardella and Strani, 2023).

The Casal de' Pazzi site is located along the lower Aniene Valley, in the suburbs to the northeast of Rome, 33 m above sea level (41°55'42.09"N; 12°33'49.83"E) (Fig. 1a). It was discovered in 1981 and an area approximately 1.200 m^2 was excavated (Fig. 1b), revealing part of a paleo-river channel, probably a small tributary of the Aniene River, according to the last excavation that took place in 2013 (Anzidei and Ruffo, 1985; Anzidei et al., 1999; Gioia et al., 2014). Part of the paleosurface (~300 m^2) is still preserved and musealized (Fig. 1c) (Gioia, 2012, 2015, 2019; Gioia et al., 2005). The chronology of the site has not been firmly assessed and is still under discussion. However, the fluvial sequence overlies a pyroclastic deposit known as Pozzolane Superiori, dated by Villa (1992) at 355±1 ka and by Karner and Renne (1998) at 357±2 ka (Karner and Marra, 1998; Karner et al., 2001). In addition, the archaeological and paleontological complex is correlated to the Vitinia Faunal Unit and to Marine

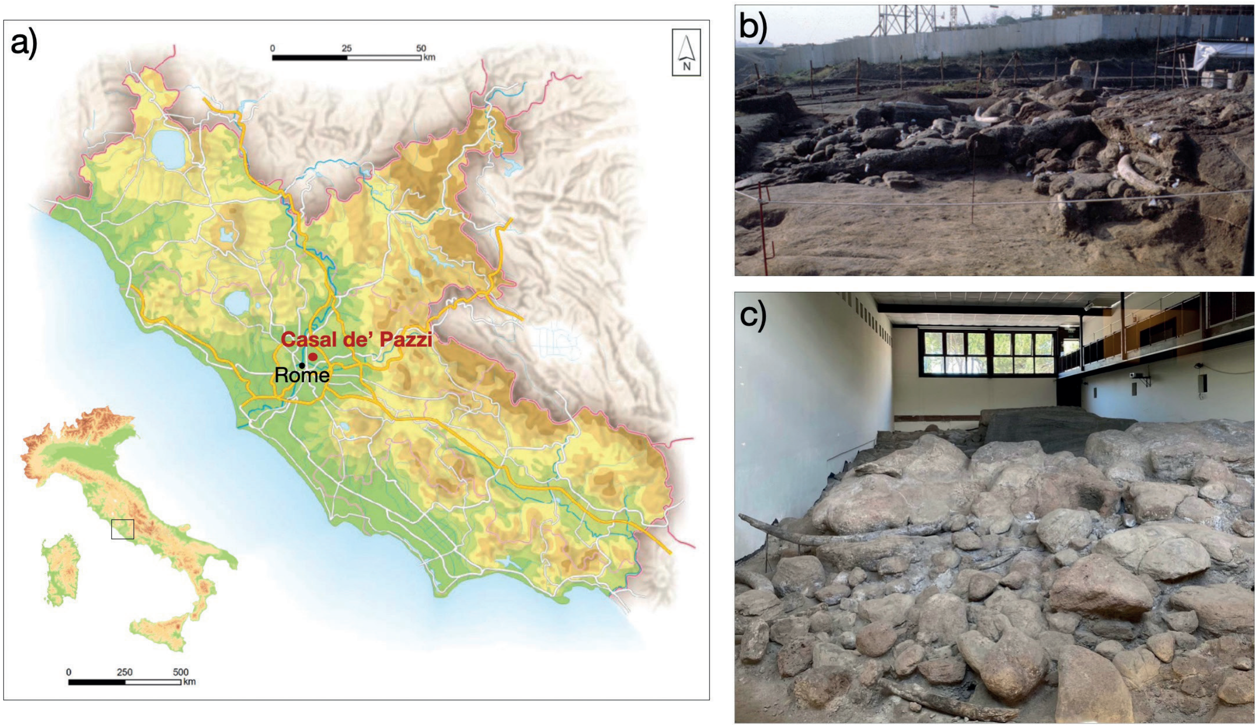

Fig. 1 - a) Map of Latium region in central Italy and location of the Casal de' Pazzi site; b) part of the archaeological deposit during the excavation in 1982; c) the paleosurface still available as part of the Museum of Casal de' Pazzi (https://www.museocasaldepazzi.it/en).

Isotope Stage 7 (MIS 7), with a suggested age of ~240-200 ka (late Middle Pleistocene) (Conato et al., 1980; De Rita et al., 1991; Gliozzi et al., 1997; Anzidei and Cerilli, 2001; Choudhury et al., 2020). A large number of Middle Paleolithic stone tools (>1500) and a single retouched bone fragment have been unearthed (Anzidei and Ruffo, 1985; Anzidei and Gioia, 1992). The faunal remains (>2000) include large herbivores, such as *Palaeoloxodon antiquus*, *Bos primigenius*, *Stephanorhinus kirchbergensis*, *Hippopotamus amphibius*, but also other mammals (*Cervus elaphus*, *Equus* sp., *Dama dama*, *Sus scrofa*, *Canis lupus*, *Crocuta crocuta*), aquatic birds (*Anser albifrons*, *Anas Penelope*, *Anas streptera*, *Anas crecca*), and tortoises (*Emys orbicularis*) (Gioia, 2020). The only human bone found at the site is a parietal fragment attributed to *Homo heidelbergensis* or is likely ascribed to a Neanderthal hominin (Manzi and Passarello, 1989, 1991; Passarello et al., 1987; Manzi et al., 1990; Gioia, 2020). Layers of silt and clay have preserved fragile and exceptional paleobotanic evidence, such as leaf fossil impressions of *Zelkova* sp., *Laurus nobilis*, and *Cercis siliquastrum* (Magri, 2020).

In order to contribute to the understanding of the environmental conditions in central Italy during the late Middle Pleistocene, we used stable isotope analysis ($\delta^{13}C$ and $\delta^{18}O$) of tooth enamel from the mammal assemblage of Casal de' Pazzi to investigate their diet and habitat, as well as to infer the ecological features of the area. Previous isotopic studies of Middle Pleistocene fauna from several archaeological and paleontological sites in central Italy have been limited to single species (Filippi et al., 2001; Palombo et al., 2005; Capalbo, 2018; Strani et al., 2019; Zanazzi et al., 2022). In contrast, this study provided the isotopic analysis of six herbivore families (Elephantidae, Hippopotamidae, Rhinocerotidae, Bovidae, Equidae, and Cervidae) to explore the past vegetation available in the paleoenvironment and selected by the fauna according to their feeding habits. Furthermore, we compared our results with published isotopic data in central Italy to assess whether the same species experienced different dietary adaptations based on local environmental conditions. Finally, we crosschecked our results with other ecological evidence to integrate their outcomes and suggest a broader interpretation. For the first time, we provided stable carbon and oxygen isotope results for hippopotamids from a Middle Pleistocene locality in Europe.

2. STABLE CARBON AND OXYGEN ISOTOPES IN PALEOECOLOGY

Stable carbon and oxygen ($\delta^{13}C$ and $\delta^{18}O$) isotopic abundances in the tooth enamel of herbivores are powerful proxies for reconstructing the dietary preferences of fossil species, as well as past terrestrial habitats and environments. This method is based on the principle that the isotopic composition of the carbonate fraction of teeth and bones reflects that of the food and water ingested by an organism (Kohn, 1999). Tooth enamel is almost entirely inorganic, less susceptible to alteration due to diagenesis, and is not remodeled throughout the lifetime of mammals (Lee-Thorp and Sponheimer, 2003; Bocherens and Drucker, 2013). Therefore, it is more likely to preserve the original isotopic signal (Kohn and Cerling, 2002). Carbon isotopic ($\delta^{13}C$) composition in herbivores reflects the average of an individual diet according to the three photosynthetic pathways used by plants (C_3, C_4, and CAM) (Cerling and Harris, 1999). In Europe, the C_3 photosynthetic pathway is typically found in most vegetation types, whereas C_4 plants are not widespread in natural landscapes. Furthermore, according to modern observations (Janis and Ehrhardt, 1988), very few ungulates consume succulent plants with CAM photosynthesis. The C_3 photosynthetic pathway (Calvin-Benson cycle) occurs in most trees, bushes, herbs, temperate shrubs, and grasses in regions with cool growing seasons. C_3 plants have an average $\delta^{13}C$ value of -27‰ (ranging from -34‰ to -22‰) with relatively enriched light carbon isotopes (^{12}C) (Smith and Epstein, 1971; O'Leary, 1988; Farquhar et al., 1989). C_3 plants growing in a closed and forested environment have lower $\delta^{13}C$ values ("canopy effect") due to poor ventilation, light attenuation, recycling of CO_2 with low $\delta^{13}C$ values from plant respiration and decomposition, and relatively high-water availability in closed-canopy habitats (van der Merwe and Medina, 1991; Bocherens et al., 1999; Bocherens and Drucker, 2013). In contrast, higher $\delta^{13}C$ values indicate open and dry habitats (Edwards and Walker, 1983; Farquhar et al., 1989; Bocherens, 2003). The $\delta^{13}C$ values of large herbivore enamel have an enrichment factor of + 14 ‰ compared to their diet ($\delta^{13}C_{enamel} = \delta^{13}C_{diet} + 14$ ‰; Cerling et al., 1999). Tooth enamel $\delta^{13}C$ values higher than -13‰ indicate an open environment, whereas tooth enamel $\delta^{13}C$ values lower than -13‰ suggest an environment dominated by forests and woodlands (Cerling et al., 1999; Bocherens, 2014). Oxygen isotopic ($\delta^{18}O$) composition in herbivores reflects the source and amount of body water, which is determined by the consumption of meteoric water and water from ingested food. Meteoric water is influenced by precipitation sources and the effects of latitude, continentality, altitude, and humidity. $\delta^{18}O$ values of meteoric water are typically more positive in warmer and dryer environments and more negative in colder habitats (Fricke and O'Neil, 1996; Kohn and Welker, 2005). Generally, *taxa* that feed in an open environment have higher $\delta^{18}O$ values than those from a forested, cooler, and humid habitat. Non-obligate drinkers obtain most of their water from the leaves they eat and occupy warm environments. Therefore, they usually have higher $\delta^{18}O$ values than obligate drinkers. Obligate drinkers that obtain water from rivers or lakes have lower $\delta^{18}O$ values, close to the $\delta^{18}O$ of meteoric water (Sponheimer and Lee-Thorp, 1999). In addition, it is typically observed that semi-aquatic mammals, such as hippos, show lower $\delta^{18}O$ values than those of terrestrial herbivores (Bocherens et al., 1996; Clementz and Koch, 2001; Clementz et al., 2008; Harris et al., 2008).

3. MATERIALS AND METHODS

We selected well-preserved molars, premolars, and incisors (n=22) of *Palaeoloxodon antiquus*, *Hippopotamus amphibius*, *Stephanorhinus kirchbergensis*, *Bos primigenius*, *Equus ferus*, and *Dama dama* from the faunal assemblage of Casal de' Pazzi to measure the $^{13}C/^{12}C$ and $^{18}O/^{16}O$ isotopic ratios. The enamel samples were collected at the Casal de' Pazzi Museum and then pretreated and analyzed at the Biogeology Research Group of the University of Tübingen (Germany), following the internal lab protocol (Bocherens et al., 1996; Koch et al., 1997). The uppermost surface of the teeth was cleaned using a diamond drill bit (diameter > 2.0 mm) to remove potential contaminants attached to the enamel surface. Bulk enamel samples were collected along the tooth length to obtain a mean estimate of the individual's diet during the dental growth period (Fricke and O'Neil, 1996; Sharp and Cerling, 1998). Approximately 12-15 mg of enamel powder was obtained using a drilling device equipped with a diamond-tipped bit (<2.0 mm). After each sample, the tools were cleaned using ethanol to prevent contamination. Subsequently, the powdered enamel was soaked in 2-3% NaOCl for 24 hours at 20 °C to oxidize organic residues and then rinsed three times with Millipore water (Milli-Q H_2O) to remove all NaOCl. The remaining samples were treated with 0.1 M acetic acid-calcium acetate buffer (pH=4.66) for 24 hours at 20 °C to remove exogenous carbonate. The samples were rinsed three times with Milli-Q H_2O and placed in an oven to dry at 40 °C for 72 hours. This pretreatment is the most commonly used method for cleaning bioapatite samples and effectively removes organic matter and exogenous carbonate (Koch et al., 1997; Snoeck and Pellegrini, 2015; Pellegrini and Snoeck, 2016). Only 2.5-3 mg of structural carbonate was reacted with 99% H_3PO_4 for 4 hours at 70 °C using continuous-flow Isotopic Ratio Mass Spectrometry (IRMS). The lyticOS 5.1 software by Elementar was used to carry out multi-point standard isotope calibration by generating a trend line (y=mx+c) that mapped the measured and expected isotopic results of standards, which was then used to calibrate the sample results. Two internal enamel samples used as secondary reference material (SRM) (Elephant and Hippo) were processed with every set of samples following the same protocol. Two international standards (IAEA-603, NBS-18) and one internal standard (LM = Laaser Marmor) standard were used. Stable isotopic results are typically reported in the common δ-notation: $X=[(R_{sample}/R_{standard})-1]\cdot 1000$, where X is referred to as $\delta^{13}C$ and $\delta^{18}O$ values, and R represents $^{13}C/^{12}C$ or $^{18}O/^{16}O$, respectively. The carbon and oxygen isotope compositions were expressed relative to Vienna PeeDee Belemnite (VPDB) and Vienna Standard Mean Ocean Water (VSMOW) standards, respectively.

4. RESULTS

The $\delta^{13}C$ values ranged from -15.8‰ to -9.4‰ (Tab. 1), indicating that the diet was composed entirely of

Tab. 1 - List of the isotopic results obtained on fauna tooth enamel from Casal de' Pazzi.

	Fauna ID	**Sample ID**	***Taxon***	**$\delta^{13}C$ (VPDB)**	**$\delta^{18}O$ (SMOW)**
1	CdP 108	CDP 1	*Hippopotamus amphibius*	-14.4	+22.0
2	CdP 107	CDP 2	*Hippopotamus amphibius*	-14.9	+21.3
3	CdP 110	CDP 3	*Hippopotamus amphibius*	-13.7	+22.8
4	CdP 109	CDP 4	*Hippopotamus amphibius*	-15.8	+23.2
5	CdP 105	CDP 5	*Stephanorhinus kirchbergensis*	-12.6	+24.3
6	CdP 104	CDP 6	*Stephanorhinus kirchbergensis*	-12.5	+24.2
7	CdP 106	CDP 7	*Stephanorhinus kirchbergensis*	-12.6	+22.8
8	CdP 82	CDP 8	*Bos primigenius*	-11.6	+25.8
9	CdP 84	CDP 9	*Bos primigenius*	-10.7	+25.7
10	CdP 83	CDP 10	*Bos primigenius*	-11.4	+25.3
11	CdP 124	CDP 11	*Dama dama*	-12.9	+24.2
12	CdP 125	CDP 14	*Dama dama*	-14.0	+26.9
13	CdP 113	CDP 15	*Equus ferus*	-12.0	+26.0
14	CdP 103	CDP 12	*Palaeoloxodon antiquus*	-13.2	+23.9
15	CdP 101	CDP 13	*Palaeoloxodon antiquus*	-13.3	+24.6
16	CdP 82 bis	CDP 16	*Palaeoloxodon antiquus*	-11.5	+23.5
17	CdP 95	CDP 17	*Palaeoloxodon antiquus*	-14.6	+24.9
18	CdP 96	CDP 18	*Palaeoloxodon antiquus*	-14.7	+24.7
19	CdP 97	CDP 19	*Palaeoloxodon antiquus*	-12.9	+23.9
20	CdP 98	CDP 20	*Palaeoloxodon antiquus*	-10.9	+25.2
21	CdP 99	CDP 21	*Palaeoloxodon antiquus*	-12.6	+22.8
22	CdP 100	CDP 22	*Palaeoloxodon antiquus*	-9.4	+24.8

C_3 plants. Lower $\delta^{13}C$ values of deer (n=2; median: -13.5‰), rhinos (n=3; median: -12.6‰), and elephants (n=9; median: -12.9‰) indicated the consumption of C_3 vegetation grown in a closed canopy environment. Instead, lower $\delta^{13}C$ values of hippopotamids (n=4; median: -14.6‰) could be interpreted with the consumption of C_3 wetland plants, C_3 browse, fruits, aquatic vegetation, or a combination of them. In contrast, bovids (n=3; median: -11.4‰) and equids (n=1; -12‰) fed on C_3 plants in slightly more open environments. Elephant is the only *taxon* that showed a wide range of $\delta^{13}C$ values (from -14.7‰ to -9.4‰), pointing to a mixed C_3 diet in both closed and open environments (Fig. 2). The $\delta^{18}O$ values ranged between +21.3‰ and +26.9‰ (Tab. 1), with lower $\delta^{18}O$ values for hippos (median: +22.4‰) than those of fully terrestrial animals, such as rhinos (median: +24.9‰), bovids (median: +25.7‰), deer (median: +25.6‰), equid (+26‰), and elephants (median: +24.6‰) (Fig. 3).

5. DISCUSSION

5.1 PALEOECOLOGY OF CASAL DE' PAZZI AND SURROUNDINGS

Stable carbon isotope analysis suggests that mammals from Casal de' Pazzi fed and inhabited a diverse C_3 ecosystem with closed and open spaces during the late Middle Pleistocene. There is no isotopic evidence of the consumption of C_4 plants during this time. Deer and rhinos consumed C_3 plants grown in a relatively closed canopy environment, whereas *Palaeoloxodon antiquus* exploited mixed C_3 vegetation in both closed and open landscapes. This mixed-feeder behavior has already been demonstrated by previous isotopic studies on tooth enamel of other *Palaeoloxodon antiquus* individuals from Casal de' Pazzi (Filippi et al., 2001; Palombo et al., 2005). In contrast, bovids and equids occupied a more open environment. Hippopotamids showed lower $\delta^{13}C$ values that we interpreted as consuming C_3 wetland plants, C_3 browse, fruits, aquatic vegetation, or a combination of

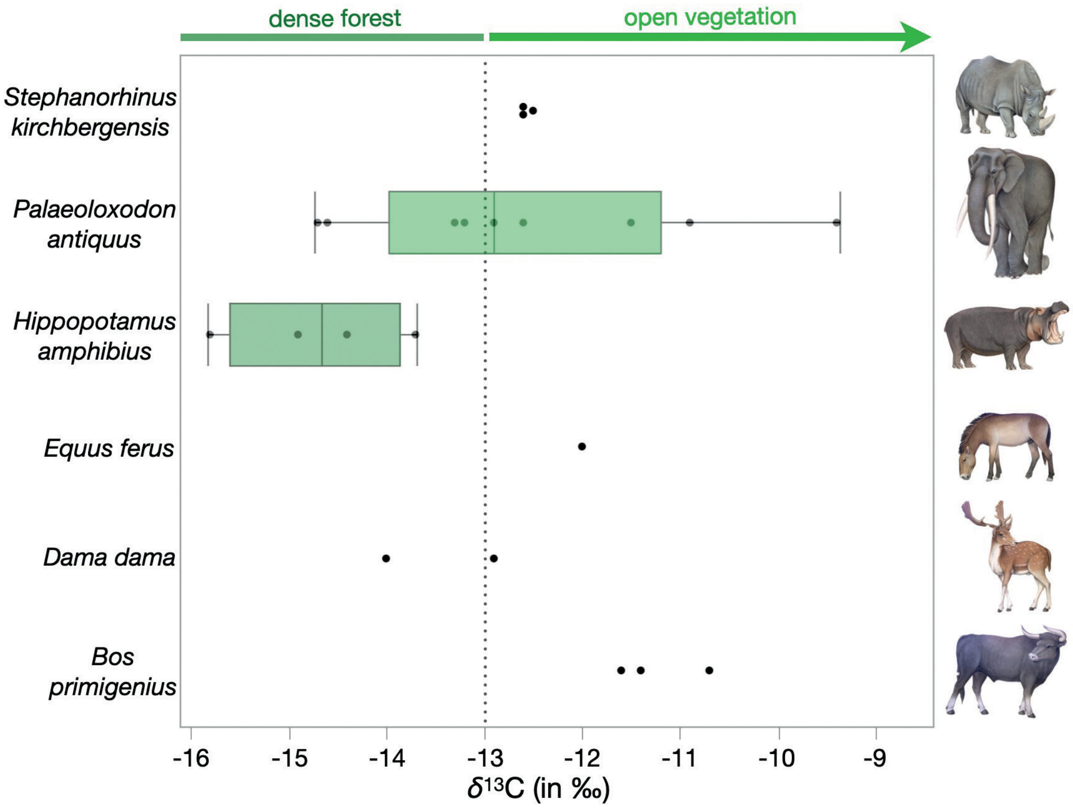

Fig. 2 - The $\delta^{13}C$ values of faunal tooth enamel from Casal de' Pazzi. The $\delta^{13}C$ value -13‰ (vertical dotted line) is fixed to distinguish closed vs. open vegetation, following Bocherens (2014) (animal illustrations by T. D'Este: https://www.museocasaldepazzi.it/it/collezioni/tutte_le_opere).

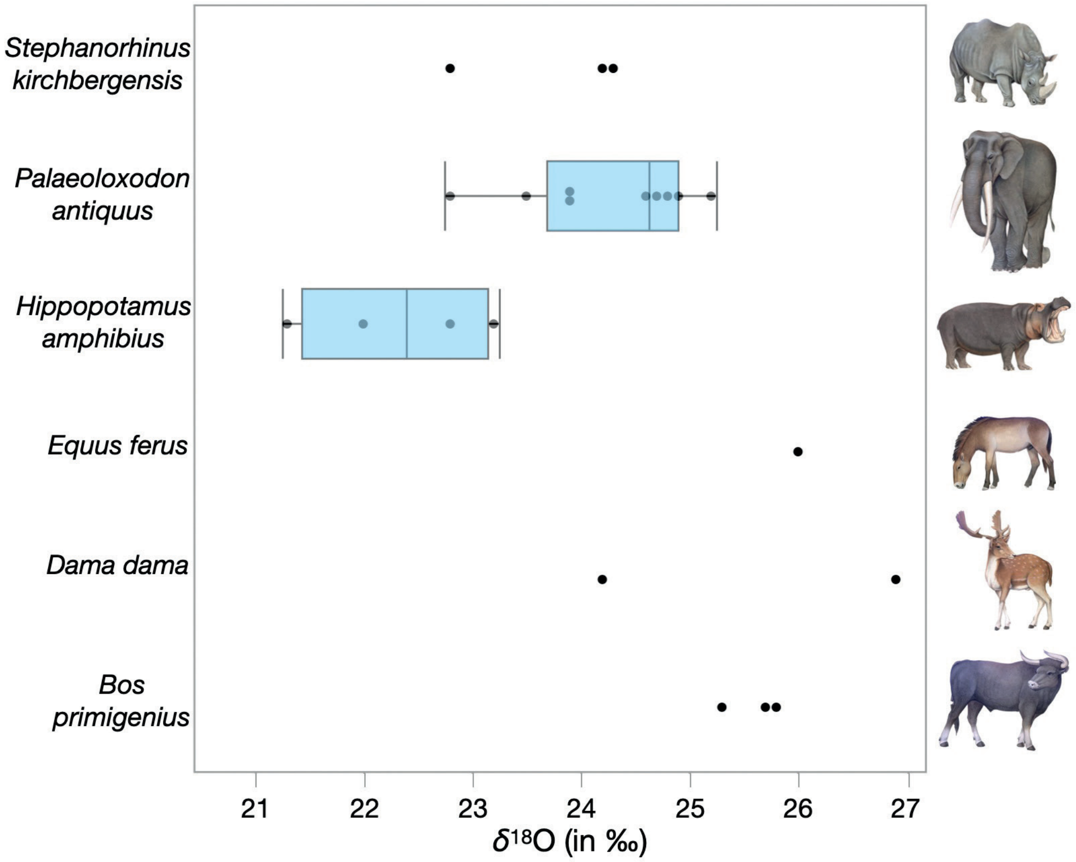

Fig. 3 - The $\delta^{18}O$ values of faunal tooth enamel from Casal de' Pazzi (animal illustrations by T. D'Este: https://www.museocasaldepazzi.it/it/collezioni/tutte_le_opere).

them. These hippo isotopic data are the only data available from Middle Pleistocene archaeological sites in Europe. As a result, comparisons are possible only with fossil and extant hippo species from African contexts (Ansell, 1965; Mugangu and Hunter, 1992; Boisserie et al., 2005; Cerling et al., 2008; Souron et al., 2012).

Habitat preferences are also provided by $\delta^{18}O$ values that clearly discriminate the semiaquatic hippo from terrestrial herbivores. Deer show higher $\delta^{18}O$ values than other taxa that obtain water from rivers or lakes that usually have $\delta^{18}O$ values close to the isotopic signal of meteoric water (Bocherens et al., 1996; Sponheimer and Lee-Thorp, 1999; Clementz et al., 2008).

Our isotopic results can be better understood by combining them with macro-botanical and palynological evidence. Exceptional and fragile leaf fossil impressions of *Zelkova* sp., *Laurus nobilis*, and *Cercis siliquastrum* found at Casal de' Pazzi indicate riparian vegetation with a possibly temperate and humid climate (Magri, 2020). Unfortunately, the rich archaeological complex of Casal de' Pazzi did not provide pollen evidence. Therefore, we integrated our paleoenvironmental discussion with pollen data from the nearby lake of Valle di Castiglione, located ~12.5 km east of the Casal de' Pazzi site. For the late Middle Pleistocene, which according to current knowledge, is the chronological attribution of Casal de' Pazzi, pollen data showed variations of many evergreen and deciduous forest tree species (*Fagus*, *Abies*, *Zelkova*, *Quercus*, and *Olea*), indicating the development of diversified wooded/forested landscapes with extended grasslands, according to the high percentage of xeric vegetation in the pollen assemblage (Follieri et al., 1988). Stable isotopes, leaf fossil impressions, and pollen data are complementary paleoecological proxies that provide different outcomes and diverse temporal and spatial resolutions. Fossil impressions provide snapshot information of the context at the time of deposition; stable isotopes of tooth enamel record an averaged diet during tooth formation (several months to years) based on the feeding strategies and behavior (including selecting the preferred vegetation) of each individual; and, pollen data record local flora located at some distance from the deposit. Furthermore, archaeological remains of Casal de' Pazzi have been found in a fluvial context, where the paleo-Aniene River (or its tributary) transported, naturally selected, and accumulated remains along the water stream. Accordingly, isotopic data on tooth enamel in such contexts likely reflect environments close to the site area, as well as environments located several kilometers apart along the course of the paleo-river.

5.2 PALEOECOLOGY OF CENTRAL ITALY

We compared our $\delta^{13}C$ and $\delta^{18}O$ values with previous isotopic data on tooth enamel (n=66) from Italian Middle Pleistocene localities (Casal de' Pazzi, La Polledrara, Poggetti Vecchi, Isernia La Pineta, Guado San Nicola, and Fontana Ranuccio) dated between ~600 ka and ~171 ka. We selected Elephantidae, Equidae, and Rhinocerotidae (Tables S1-S3) (Palombo et al., 2005; Capalbo, 2018; Strani et al., 2019; Zanazzi et al., 2022), which are all represented in the isotopic dataset of the above-mentioned archaeological sites. In contrast, we did not consider Hippopotamidae, Cervidae, and Bovidae since no comparable isotopic data were available within this chronological time frame.

The $\delta^{13}C$ values of *Palaeoloxodon antiquus* (n=33) from La Polledrara, Casal de' Pazzi, and Poggetti Vecchi indicated different dietary patterns in the C_3 vegetation range (Table S1, Fig. 4). As previously discussed, elephants from Casal de' Pazzi showed a wide range of $\delta^{13}C$ values, suggesting mixed-C_3 feeding in both closed and open habitats. In contrast, elephants from La Polledrara and Poggetti Vecchi showed higher $\delta^{13}C$ values than those from Casal de' Pazzi, indicating that they inhabited or at least fed exclusively in C_3 open environments (Palombo et al., 2005; Capalbo, 2018). However, microwear analysis showed that elephants from La Polledrara had a browse and mixed diet, whereas elephants from Casal de' Pazzi and Poggetti Vecchi consumed graminaceous plants or vegetation with a large amount of phytoliths (Filippi et al., 2001; Palombo et al., 2005; Capalbo, 2018). The variability of $\delta^{18}O$ values (Fig. 4) could indicate cooler climatic conditions at Casal de' Pazzi (lower $\delta^{18}O$ values) than those at La Polledrara and Poggetti Vecchi (higher $\delta^{18}O$ values). This seems to be consistent with the fact that Casal de' Pazzi was attributed to a humid and forested climate during MIS 7, whereas La Polledrara was related to an arid climate during MIS 9 (Palombo et al., 2005).

The equid isotopic data (n=4) of *Equus mosbachensis* and *Equus ferus* from Fontana Ranuccio and Casal de' Pazzi indicated the consumption of C_3 plants in a more closed environment at Fontana Ranuccio (Strani et al., 2019) than at Casal de' Pazzi, where $\delta^{13}C$ values indicated a C_3 diet in a slightly open landscape, such as a light forest or grassland (Tab. S2, Fig. 5). Lower $\delta^{18}O$ values were observed for the equids from Fontana Ranaccio (Fig. 5), probably indicating cooler climatic conditions than those in Casal de' Pazzi. However, the restricted number of samples limits this interpretation.

The comparison of $\delta^{13}C$ values (n=29) for two species of rhino (*Stephanorhinus kirchbergensis* and *Stephanorhinus hundsheimensis*) from Isernia La Pineta, Guado San Nicola, and Casal de' Pazzi (Table S3, Fig. 6) indicated that they exploited different C_3 environments characterized by woodlands at Isernia La Pineta and more open C_3 grasslands at Casal de' Pazzi and Guado San Nicola. The $\delta^{18}O$ values (Fig. 6) could reflect a cooler climate and more humid conditions at Casal de' Pazzi and Isernia La Pineta than at Guardo San Nicola (Zanazzi et al., 2022). Generally speaking, the variability of stable oxygen isotope compositions should be considered cautiously because it can reflect several factors, such as animal physiology, seasonal variations, and water sources at different elevations.

Further paleoecological data from Fontana Ranuccio and Cava di Breccia - Casal Selce 2 (~750 ka; MIS 11) were

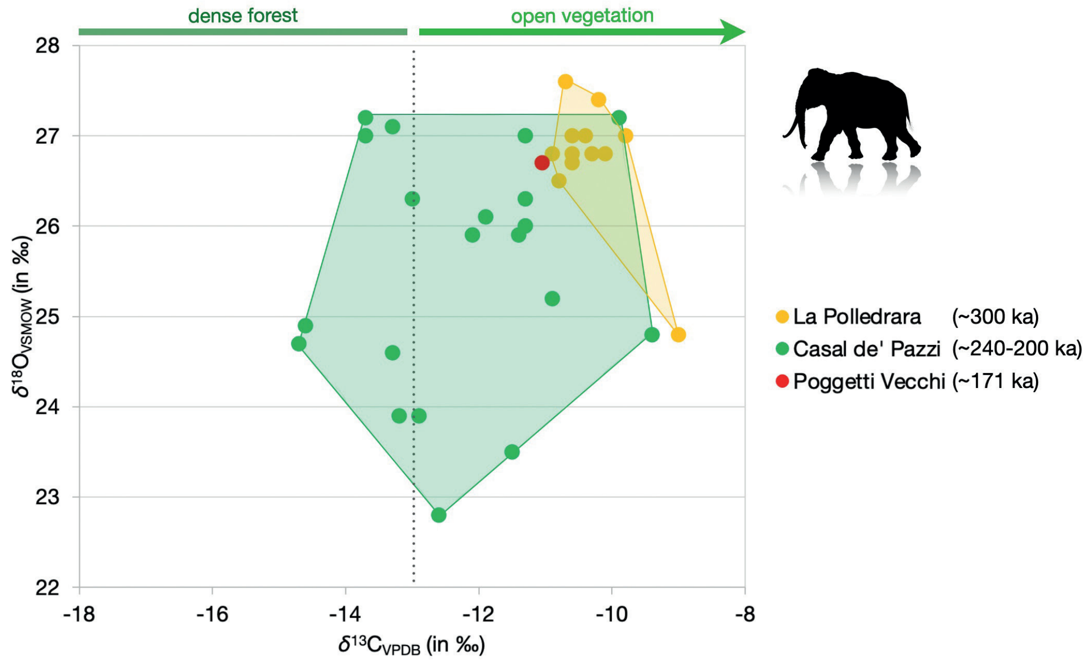

Fig. 4 - Scatter plots of δ^{13}C and δ^{18}O values (tooth enamel) of elephants from Italian Middle Pleistocene sites (animal silhouettes: http://phylopic.org). The δ^{13}C value -3‰ (vertical dotted line) is fixed to distinguish closed vs open vegetation, following Bocherens (2014).

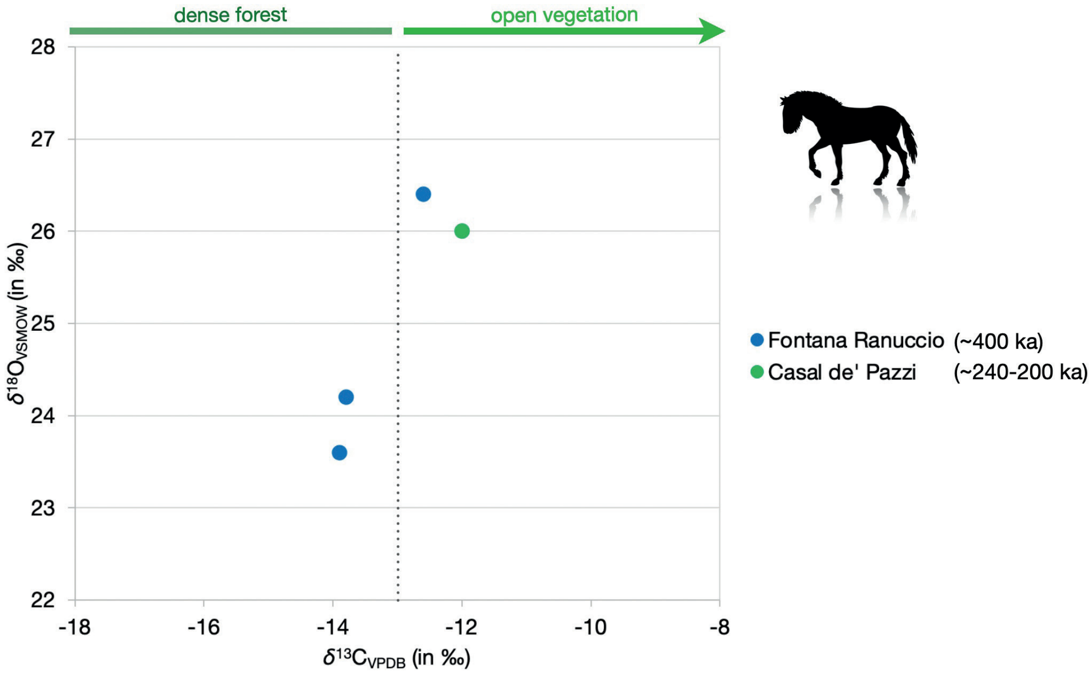

Fig. 5 - Scatter plots of δ^{13}C and δ^{18}O values (tooth enamel) of equids from Italian Middle Pleistocene sites (animal silhouettes: http://phylopic.org). The δ^{13}C value 13‰ (vertical dotted line) is fixed to distinguish closed vs open vegetation, following Bocherens (2014).

assessed by combining hypsodonty, meso- and micro-wear analysis on several ungulates, indicating wooded landscapes at Fontana Ranuccio and open habitats with scattered woodlands at Cava di Breccia - Casal Selce 2 (Strani et al., 2018, 2022). In addition, the pollen record of Fontana Ranuccio recorded a temperate phase characterized by dense forests dominated by *Pterocarya* (>50%) and *Alnus*, whereas *Picea*, *Tsuga*, and *Cedrus* were poorly represented (Corrado and Magri, 2011).

Overall, this paleoecological evidence showed general and considerable differences through time and space for the diet and habitat of the Middle Pleistocene mammals

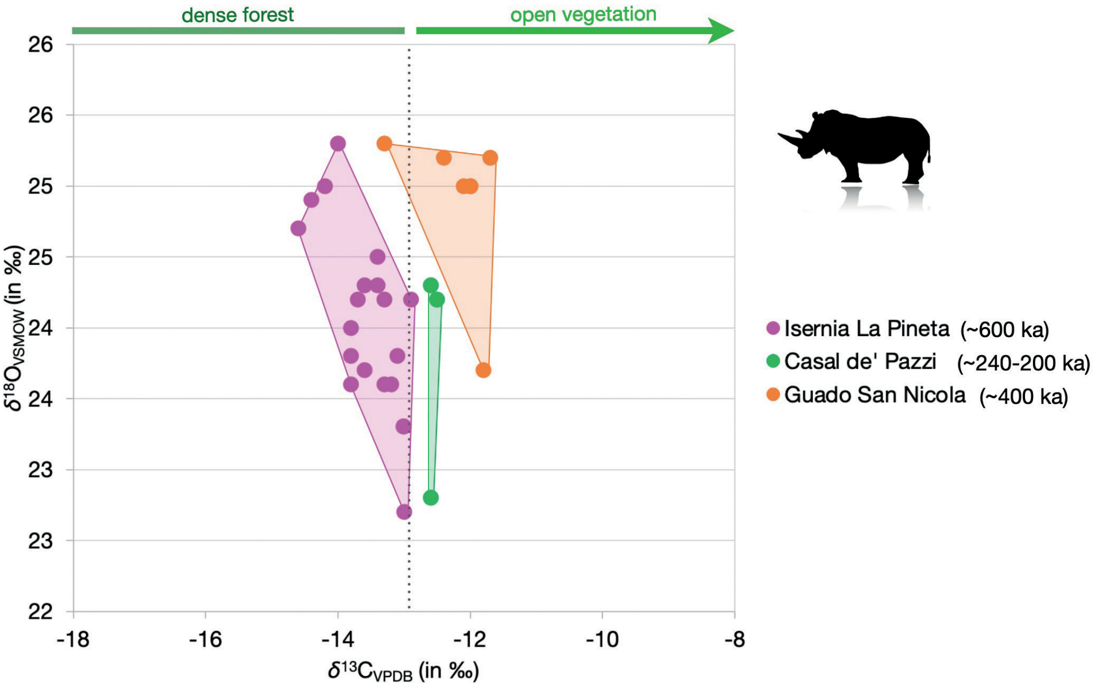

Fig. 6 - Scatter plots of $\delta^{13}C$ and $\delta^{18}O$ values (tooth enamel) of rhinos from Italian Middle Pleistocene sites (animal silhouettes: http://phylopic.org). The $\delta^{13}C$ value -13‰ (vertical dotted line) is fixed to distinguish closed vs open vegetation, following Bocherens (2014).

in central Italy, according to the local vegetation and climate. This is not surprising, given the different ages for the compared archaeological localities. The Middle Pleistocene (Chibanian, ~774-129 ka; MIS 19-MIS 5e) was a climatic phase characterized by an increase in the amplitude of glacial-interglacial climate cycles from ~41 ka to ~100 ka, with several faunal turnover events and vegetational alterations followed global climatic patterns (Maslin and Ridgwell, 2015; Manzi et al., 2011; Magri and Palombo, 2013; Head and Gibbard, 2005; Strani et al., 2021; Suganuma et al., 2021; Sardella and Strani, 2023). With respect to this and accepting the suggested age of Casal de' Pazzi (~240-200 ka) within MIS 7, we interpret our stable isotope results and comparisons among other sites to support the attribution of the Casal de' Pazzi area to a humid climate phase with forested/wooded environments and extensive grasslands.

6. CONCLUSION

We provided isotopic results ($\delta^{13}C$ and $\delta^{18}O$) from the tooth enamel of six herbivore families (Elephantidae, Hippopotamidae, Rhinocerotidae, Bovidae, Equidae, and Cervidae) from the late Middle Pleistocene site of Casal de' Pazzi. These data indicate that elephants, hippos, equids, bovids, cervids, and rhinos occupied different environments in a C_3 ecosystem, spanning from forest to woodland and grassland habitats. *Stephanorhinus kirchbergensis* and *Dama dama* inhabited closed-canopy environments. In contrast, *Bos primigenius* and *Equus ferus* occupied slightly open landscapes. *Hippopotamus amphibius* probably consumed C_3 cool-season grasses, C_3 browse, fruits, aquatic and wetland plants, or a combination of them. *Palaeoloxodon antiquus* was the only *taxon* feeding in a mixed-C_3 environment, ranging from closed to open habitats. Our data interpretation is consistent with leaf fossil impressions of *Zelkova* sp., *Laurus nobilis*, and *Cercis siliquastrum* found at Casal de' Pazzi and pollen data from the nearby lake of Valle di Castiglione, which indicated that the Casal de' Pazzi area was characterized by riparian habitats and diversified wooded/forested vegetation with extended grasslands. The comparison with published isotopic data from other Middle Pleistocene localities in central Italy between ~600 ka and ~171 ka provided evidence of considerable environmental differences through time and space, according to the local vegetation and general climate trends.

At Casal de' Pazzi, future analyses should be focused on microwear and intra-tooth isotopic profiles in order to provide a more accurate understanding of different C_3 plant contributions to the mammalian diets, and to reconstruct the effects of possible seasonal variations on environmental composition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data generated and analyzed during the study are available in this published article and its Supplementary Information file.

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