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MULTIPROXY-BASED RECONSTRUCTION OF THE FEEDING HABITS FROM THE LATE MIDDLE PLEISTOCENE STRAIGHT-TUSKED ELEPHANT POPULATION OF POGGETTI VECCHI (SOUTHERN TUSCANY, ITALY)

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ABSTRACT: The palaeodietary preferences from the elephants of Poggetti Vecchi, which radiometrically dated around 171,000 years BP, have been investigated by cross-comparing the results obtained with low-magnification analysis of microwear (LDM), 2D-roughness parameters and surface geometry assessment, mesowear and stable isotope analysis. Proboscideans tend to be opportunists, and this improves their ability to respond to climate impacts and environmental fluctuations. The dietary signal of the last meals suggests the consumption of a great amount of Graminaee or other plants quite rich in phytoliths. Conversely, mesowear data indicate more browse-dominated, mixed dietary behaviour, which probably reflects seasonal variations, and environmental changes.

KEYWORDS: Palaeodietary, early middle Pleistocene, palaeoenvironment, *Palaeoloxodon*

1. INTRODUCTION

Modern elephants are regarded as a particular kind of mixed-feeders with complex behaviour. Depending on the availability of different kinds of plants, as well as on climatic conditions, they show most variable feeding habits. Microwear patterns can therefore change considerably and rapidly both seasonally and geographically (Grube et al., 2010). Despite all the potential interpretative biases (which may derive from morpho-functional variations in the chewing apparatuses, the involvement of contaminants, post-mortem alterations, methodological inconsistencies, the use of inappropriate instruments and analyst's skill) conventional dental microwear analysis is a powerful tool and still preferable to other methodologies, first of all because it is the easiest way for functional morphologists to investigate and obtain relevant information from microwear patterns. Moreover, it permits to analyse large numbers of samples at lower costs using basic equipment. However, a reliable quantification of the smallest scars may exceed the capacities of light microscopy, which would thus impose the use SEM or other instruments capable of investigating three-dimensional surface texture. For the present study we adopted an alternative, multiproxy and comparative approach which is based on a combination of different methods. Our results concern dietary signals of last meals, obtained with low-magnification analysis of microwear (LDM), and carbon and oxygen determination of enamel fraction, which reveals the dietary history of earlier phases of an individual's life. We also used the mesowear method, which reflects specifically the abundance of grasses that are present in the habitats where animals live and not just the openness of the landscape. The mesowear method was recently extended to proboscideans (Saarinen et al., 2015). It records dietary signals over long time spans. Left and right molars of each individual can show different mesowear angles (probably due to one-side chewing habits); changes also occur throughout the life of an individual, including seasonal shifts in dietary preferences (e.g., Sukumar & Ramesh, 1995; Cerling et al., 2006, 2009; Rivals et al., 2012). Mesowear signals can therefore record seasonal variations in an elephant's feeding habits. Any surface can be described as a complex landscape with peculiar geometric form and textural alterations (waviness and roughness). Surface textures that appear smooth at coarse scales can be demonstrably rough at sufficiently fine scales. Worn enamel finds correspondence with industrially manufactured surfaces. All surface measuring systems reconstruct 3D surface models by assembling line profiles. However much more detailed information about the enamel wear is encapsulated in 2D-roughness parameters rather than 3D-models. Hence the 2D-surface data and geometric pattern employed in this study can be used to characterize the physical topography of enamel facets, quantifying the universal attrition–abrasion equilibrium controlling dental wear and to classify individuals into feeding categories.

The results of pooled analysis show that the use of different methods is a potentially more effective way to constructively address the many issues on the feeding adaptations of extinct elephants than is relying on only one method. The methodology used in this study reveals the wealth of information that a multiproxy approach can contribute in studies aimed at palaeodietary and palaeoenvironmental reconstructions. In addition, these results seem to agree with preliminary environment reconstruction based on both palaeobiological and taphonomic investigation, as well as with preliminary

Specimens	Tooth	Side	Age (years)
5500	m3	right	40
5546	m2	right	26
265	M2	left	25-30
1609	M2	right	30
1609	M2	left	30
5547	m2	right	25
5547	m2	left	25
98	m1	right	12
98	m1	left	12
219	d3	right	8
219	d3	left	8
219	d4	right	8
219	d4	left	8
5298	D4	right	8
5298	D4	left	8

Tab. 1 - Description of the specimens used for microwear inspection, mesowear and surface texture assessment.

geoarchaeological insight. The Poggetti Vecchi area (42°49'8.92" N, 11° 4'19.41" E) is located in southern Tuscany (central Italy), some 7 km northwest of Grosseto. An in-depth palaeoenvironmental reconstruction of the site of Poggetti Vecchi, obtained combining archaeo -palaeontological records and sedimentological data, can be found in Benvenuti et al. (2017).

2. MATERIALS AND METHODS

Fifteen upper and lower molars of both sides and of a fairly wide spectrum of ontogenetic ages were used to perform both microwear and mesowear analyses. A list of the selected molars, together with a summary description of them, is reported in Tab. 1. In this study, the capital letters represent upper cheek teeth (e.g., M1), while lower case letters indicate lower teeth (e.g., m3).

Molds and casts were prepared by adapting the methods described by Solounias & Moelleken (1992a) and Solounias & Semprebon (2002). They were then examined under a Zeiss standard light stereomicroscope at 40 and 100x magnification using a unidirectional external oblique light source with a shallow incidence angle. Several microphotographs were taken at both magnifications; the latter gave the best-resolved images of the elephant teeth, providing a visual field of 2 mm^2 (Todd et al., 2007). Qualitative and quantitative investigation of microwear scars was carried out directly on computer using the software ImageJ, focusing on functional plates. We performed the following actions: 1) calculated the total number and frequency of scratches and pits; 2) measured the average length and width of the scars; 3) used width/length ratios to discriminate fine from coarse scratches and small from large pits; 4) expressed microwear density as number of features per analysed area.

We measured the carbon and oxygen isotope composition of the enamel of a second molar (n. 1365). A fraction of the enamel was mechanically cleared of dentine and cement using tungsten-carbide rotary tools and finally ground with an agate mortar. The purity and crystallinity of the fine enamel powder was monitored by X-ray diffraction analysis. A randomly oriented powdered sample was mounted on glass slides and scanned. The diffraction pattern was collected on a Philips PW 1050/37 diffractometer using graphite-monochromatised CuKα radiation, obtained at 40 kV and 20 mA. The sample was scanned from 5° to 60° with a step size of 0.02° 2θ and a counting time of 2s.

Following Chenery et al. (2012), the sample was not subjected to leaching pre-treatment; about 150-200 mg of enamel powder was reacted with 100% orthophosphoric acid (H_3PO_4) at 25°C for three days to produce CO_2, which was then collected by cryogenic separation. Finally, the isotope compositions were measured by Finnigan Mat 252 mass spectrometer. The oxygen isotope value ($\delta^{18}O_{sc}$ versus PDB-1) was obtained from the structural carbonate; using Daux et al.'s (2008) equation it was directly translated into the isotope composition of the environmental water $\delta^{18}O_w$ versus SMOW).

The mesowear angles were measured on detailed profiles obtained using a Burton's steel professional comb. The contour gauges were used to identify the dentine valleys; following Saarinen et al.'s (2015) indication, the mesowear angle measurements were taken using a digital goniometer with 0.1 precision. The average angular values were estimated based on angle measures of the three central lamellae of each molar. The results were then compared with those reported by Saarinen et al. (2015). Finally, following the indications given by Saarinen & Lister (2016), the average mesowear angles were correlated with the percentages of the pollen of non-arboreal species (NAP) and, in particular, of Poaceae, to evaluate how much local vegetation is reflected by the inferred dietary habits.

Two representative specimens (the adult n. 5500 and the calf n. 98) have been selected for topographic assessment based on 2D-roughness parameters and geometric patterns. The casts were scanned by a digital microscope (Hirox KH-7700). The Auto Multi Focus tool enabled the creation of 3D images obtained by composing a set of fifteen planes taken at different focuses with a vertical step ranging from 4 to 8 µm (depending on the topographic features of the sample). On the samples surface, a rectangular area of about 450x600 µm was scanned at 0.381 µm resolution using a 500x objective; almost three ROIs (regions of interest) were selected at the center of the two central enamel loops of the teeth and scanned to inspect if and how the surface texture varies on each sample. The software MountainsMap7® Image (DigitalSurf, France) was used to obtain 2D images of the topography of the acquired areas. Almost 10 roughness profiles along with ISO 4287 (1997) standard parameters, were extracted from each image by applying a Gaussian filters to clean the raw data. 2D-roughness parameters are extensively described in Kaiser & Brinkmann (2006). MountainsMap® also includes tools specifically designed to assess fractal dimension (a statistical index of complexity) and isotropy by processing digital maps. Finally, 3D reconstructions were here obtained by the use of Interactive 3D-Surface Plot plugin of ImageJ software, which creates interactive surface plots (i.e., dots, lines, mesh, filled, display colors, etc.) from all kinds of images.

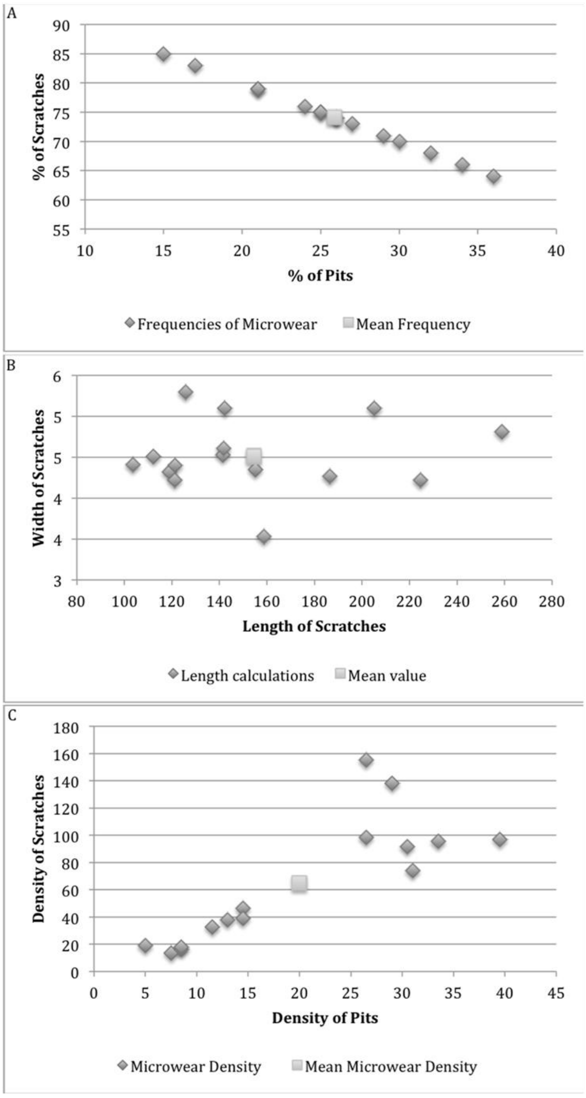


Fig. 1. % of Pits vs % of Scratches scatter diagrams at 100x magnification. A) average percentage of pits plotted against average percentage of scratches; B) average density of pits plotted against average density of scratches; C) average length of scratches plotted against the average width of scratches.

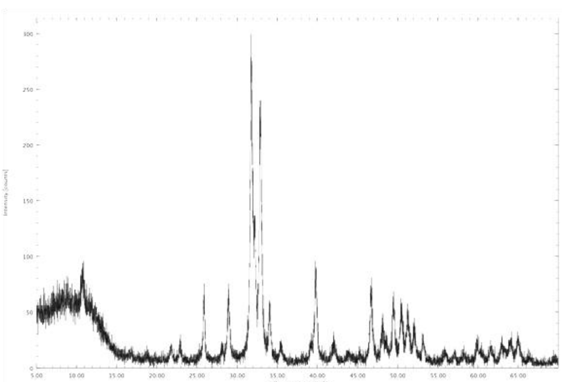


Fig. 2 - XRD pattern of the dental enamel sample analyzed for this study.

3. RESULTS

The qualitative microwear analysis revealed that microwear patterns are similar all over the occlusal surface. Microwear features are most variable on both the lingual and buccal sides of each functional plate, whereas pits and scratches are most variable on distal plates. Qualitative and quantitative analyses and measures confirm that scratches (fine, mixed and coarse) outnumber pits on each of the teeth examined. Furthermore, coarse features are usually more frequent at the centre of the enamel loops, whereas fine ones (which are usually longer, narrower and shallower) are mainly concentrated along their edges. Cross-coarse scratches are particularly abundant in the middle section of molar teeth; also pits (either large, mixed, or small ones) can be abundant locally, especially on buccal and lingual sides. The microwear patterns observed in this analysis are summarized in Fig. 1.

XRD graph plot revealed the absence of impurity phases; the diffraction pattern shows a peak characteristic of apatite (Fig. 2). Its value is rather similar to that of the enamel phase of the teeth of modern and fossil elephants (Ayliffe et al., 1994). The carbon isotopic value of the enamel structural carbonate (-11.05‰ $\delta^{13}C_{sc}$ versus PDB-1) and the corresponding calculated palaeodiet (-25.14‰ $\delta^{13}C_{diet}$ versus PDB-1) suggests that the straight-tusked elephant population of Poggetti Vecchi falls in the ''C3-dominated diet'' field. In tab. 2 are reported the oxygen isotope value or structural carbonate and the environmental water isotope calculations.

The mean acute mesowear angles vary considerably in each specimen, ranging from 90.95° to 119.82°: in the population as whole, the angle measurements vary from 102.63° to 119.82°. The regression analysis that is shown in Fig. 3, as well as the comparison of the mean mesowear angles with the relative Graminae pollen frequencies (Fig. 4) calculated at Poggetti Vecchi (M. Mariotti Lippi personal communication) indicate that our population had browse-dominated mixed diet with relatively flexible feeding behaviors.

Only a small subset of 2D-roughness parameters seems to characterize more efficiently the enamel texture and dietary categories, which are listed in tab. 3, where geometry calculations are also included. The fractal dimension (Fig. 5) was found to be rather similar in both the Poggetti Vecchi specimens ranging from 2.59 and 2.74. The orientation of roughness features (isotropy/anisotropy texture), which is expressed as the tree main angular values and isotropy percentage, is shown in Fig. 6. The latter are more heterogeneous in the specimen 5500, varying from 18.6 to 40.9%, whereas the average value in the specimen 98 is 31.50%. 3D-reconstructions of the enamel samples are shown in Fig.7.

$\delta^{13}C_{sc}$ ‰ (PBB)	$\delta^{13}C_{diet}$‰ (PBB)	$\delta^{18}O_{sc}$‰ (PDB)	$\delta^{18}O_{sc}$‰ (SMOW)	$\delta^{18}O_{p}$‰ (SMOW)	$\delta^{18}O_{w}$‰ (SMOW)
-11.05	-25.14	-4.08	26.70	17.89	-6.32

Tab. 2 - Carbon and oxygen isotope enamel determination and palaeodiet calculation.

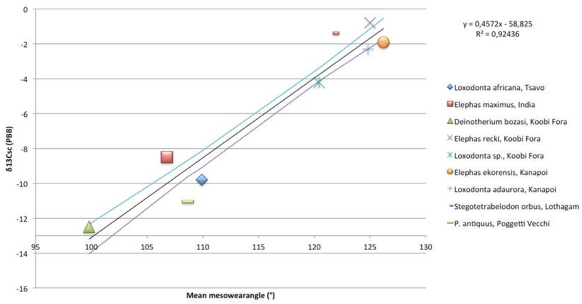
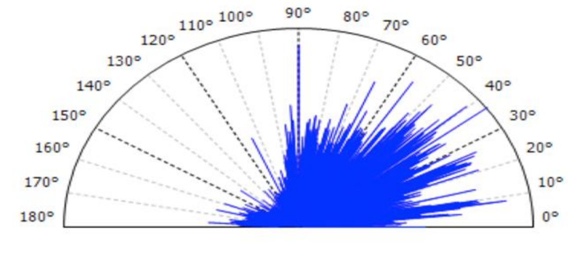


Fig. 3 - Linear regressions of $\delta^{13}C$ values from tooth enamel and mean mesowear angles. Value obtained from the Poggetti Vecchi molar compared with data drawn from Saarinen et al. (2015). The 95% confidence limits are shown as dashed lines (Coefficient R^2 = 0.92, Student's t-tests $P < 0.001$).



Parameters	Value	Unit
Isotropy	28.5	%
First direction	36.7	°
Second direction	90.0	°
Third direction	8.52	°

Fig. 5 - Example of fractal dimension analysis calculation for one the selected enamel ROIs studied for the molar n. 98 following the enclosing boxes method [with the slope and correlation coefficient (R^2) of regression line].

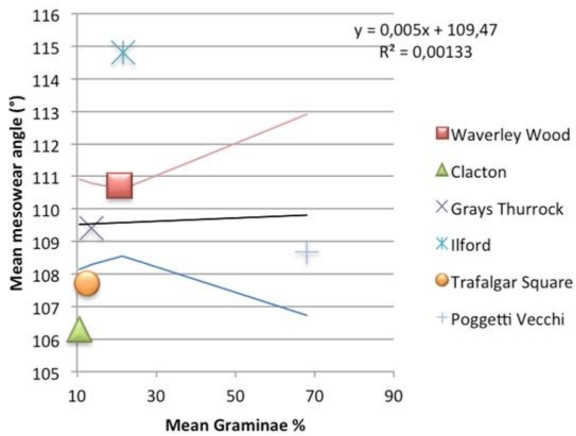
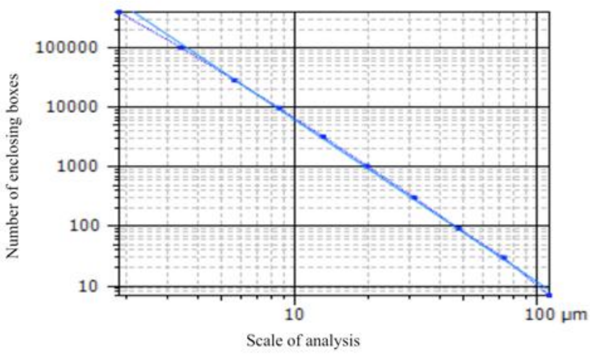


Fig. 4 - Regressions between mean mesowear angles and mean Poaceae (grass) %. Values obtained from Poggetti Vecchi molars compared with data drawn from Saarinen and Lister (2016). (Coefficient R^2 = 0,00133. Student's t-tests P = 0.001).



Information	
Method	Enclosing boxes

Parameters	Value
Fractal dimension	2.72
R²(1)	0.999

Fig. 6 - Polar diagrams showing texture direction and related parameters of the three selected enamel ROIs studied for the molar n. 98.

Parameters	98	5500	Unit
RTp	0.21	0.31	%
RHTp	11.46	9.85	µm
RS	4.09	6.77	µm
Rz	39.22	30.36	µm
RSm	7.00	12.32	µm
RLq	29.97	26.29	µm
Rp	24.98	16.87	µm
Ra	5.57	4.51	µm
Isotropy	31.50	26.83	%
Fractal Dimension	2.74	2.66	No unit

Tab. 3 - A selection of 2D-roughness texture parameters and geometric patterns employed in this study and corresponding average values.

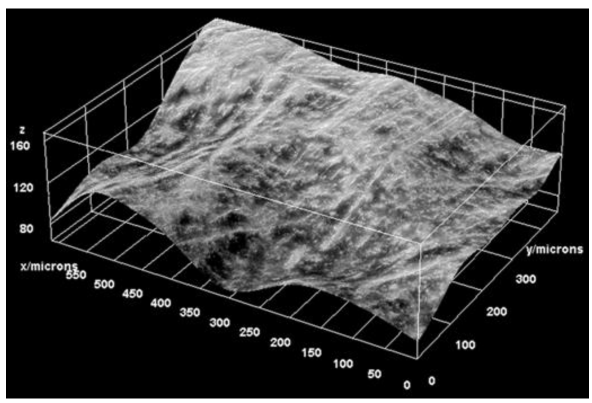


Fig. 7 - Example of 3D-surface models obtained by assembling roughness line profiles.

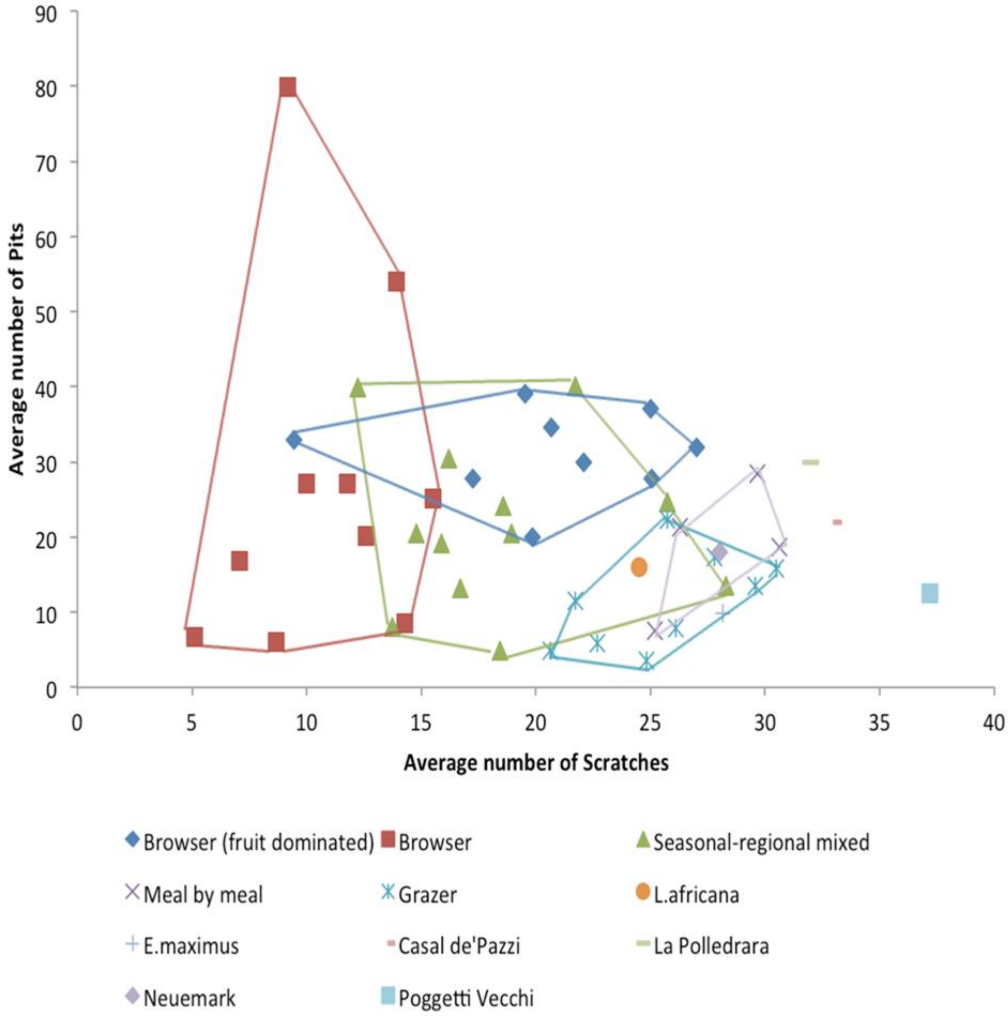


Fig. 8 - Bivariate plot of the average number of scratches versus the average number of pits and corresponding feeding morphospace envelopes (leaf browsing, fruit browsing, grazing, seasonal and meal by meal mixed-feeders taxa). Data for extant ungulates and elephants were drawn from Solounias and Semprebon (2002) and those of palaeoloxodonts specimens from Neumark Nord 1, La Polledrara di Cecanibbio and Casal de'Pazzi were drawn from Grube et al. (2010).

4. DISCUSSION AND CONCLUSION

Our results were compared with the average numbers of pits and scratches observed in modern and extinct taxa, as they are reported in the literature; the corresponding dietary morphospace are shown in Fig. 8. The high percentage of mixed and fine scratches associated with abraded micro-areas observed on the teeth from Poggetti Vecchi indicates feeding on abrasive food, containing high amounts of opal phytoliths, but also the ingestion of soil particles picked up with grasses. Our elephants cluster within the grazer dietary morphospace. Differences between the various Italian palaeoloxodonts may indicate different dietary preferences, as well as a high plasticity to adapt to local environmental and microclimatic conditions. Modern elephants increase grass intake during more humid periods (Sukumar & Ramesh, 1995); if the Poggetti Vecchi elephants behaved similarly, the microwear pattern here obtained is indicative of grass consumption under rather cold and wet climatic conditions.

We could determine the isotopic concentration of only a single sample, and hence the range of variability is not known. Consequently, the results on the palaeodietary habits of these Italian Middle Pleistocene straight-tusked elephants and on the palaeoenvironmental conditions under which they lived are essentially inferred based on comparisons with published data on straight-tusked elephants from other sites, namely La Polledrara and Casal de'Pazzi, in Italy (Palombo et al., 2005), and Neumark Nord 1, in Germany (Grube et al., 2010). The carbon isotope of the structural carbonate in the enamel fraction from Poggetti Vecchi reaches -11.05 ‰, and the corresponding calculated $\delta^{13}C$ value of the diet thus is -25.14‰ and falls into the field of C3 plants. The overall $\delta^{13}C$ values of the diet in comparative samples vary from -23.1‰ to -28.1 (Grube et al., 2010). The collective data drawn from the literature indicate that the average climatic and environmental conditions inferred for the elephants from the so-called "Campagna Romana" and from Neumark Nord 1 were similar to those that existed at Poggetti Vecchi. The water $\delta^{18}O_w$ at Poggetti Vecchi (-6.18 ‰) is slightly more positive than those calculated for the comparative samples (Palombo et al. 2005; Grube et al., 2010); in addition, it seems in equilibrium with the isotopic values of modern local precipitations (which range from -6‰ to -7‰; Giustini et al., 2016). We can therefore conclude that the Poggetti Vecchi elephants likely relied on two sources of drinking water, i.e., ponds/lakes (filled with local rainwater) and rivers. Our results are in line with the general environmental scenario for the Middle Pleistocene of central Italy. The isotopic signature of enamel is consistent with a landscape dominated by wooded grasslands, under colder and/or wetter conditions than the present ones. The dietary shift towards more grazing habits in the Poggetti Vecchi population of *Palaeoloxodon antiquus* can be a response to more humid conditions; in other words, the Poggetti Vecchi elephants had to turn to grass-eating, as living elephants do today in special seasonal circumstances.

The results here presented confirm that microroughness features can be effectively described using 2D-roughness criteria, as previously inferred by Kaiser & Brinkmann (2006), as well as using geometry calculations. The selected subset of parameters is capable to measure the scale of surface alterations recorded by the system and, consequently, capture conventional microwear signals. In this study roughness parameter calculations fall within the thresholds of grazer-dominated animals with mixed-feeding habits. The polar spectrum shows that the angular data vary widely, which indicates the lack of mainly unidirectional structures and, therefore, the absence of strong anisotropy signals. This is corroborated by the relatively higher average values of the fractal dimension calculations, which clearly indicate that both molars (m3 n.5500 and m1 n. 98) here studied have quite complex surface textures.

Compared to the other methods here considered mesowear is faster, not exposed to the risks of contamination, non-destructive, easy-to-do and widely applicable. It provides a direct indication of the amount of abrasive and nonabrasive plants regardless of the photosynthetic pathways employed by the terrestrial plants, and allows the detection of C3-grazing, more directly than using $\delta^{13}C$ values. Based on the mesowear results, the Poggeti Vecchi straight-tusked elephants had a browse-dominated, mixed diet with relatively flexible feeding behaviors. The weak positive correlation between the mean angle measure and the percentage of Poaceae pollen confirms this indication. This mismatch could be essentially due to differences in the temporal resolution of microwear and mesowear methods. In addition, considering that palaeoloxodonts shifted from more browsing or mixed-feeding to more grazing in accordance with local context, mesowear is probably more effective than microwear in capturing the minimum seasonal variations in diet.

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