Supplementary Material for:

Magdalenian environments and ecosystems of the northern Alpine foreland: The case of Gnirshöhle and Petersfels

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Supplementary Text:

S1. ZooMS analyses at Petersfels

S1.1. Materials:

During the primary zooarchaeological analysis of the Petersfels P6 material, we selected 100 morphologically unidentifiable faunal remains for destructive analysis via ZooMS (Buckley et al., 2009) to identify rare taxa to investigate their paleoecological context and contribution to human subsistence. Considering the results of previous studies (e.g., Peters, 1930; Albrecht et al., 1983), a random sample of remains was likely to be predominantly reindeer and horse. Since we hoped to identify rare taxa, we selected specimens that appeared larger or smaller than the average reindeer (i.e., ibex, red deer) and specimens larger than the average horse (i.e., bovines). This was determined using morphological features, such as cortical thickness and bone texture, which provided a rough estimate of body size. Size class categories were defined by average body mass (kg) and generally follow Stiner (2005) and Bunn et al. (1998) with some modifications for Eurasian Pleistocene taxa (Saarinen et al., 2013). The size class categories included: small ungulate (10–50 kg; e.g., roe deer, chamois), medium ungulate (50–100 kg; e.g., fallow deer, ibex), large ungulate (100–350 kg; e.g., reindeer, red deer), extra-large ungulate (>350 kg; e.g., horse, aurochs), small carnivore (<30 kg; e.g., marten, fox), large carnivore (>30 kg; e.g., wolf, bear), small mammal (<10 kg), medium mammal (10–300 kg), and large mammal (>300 kg) (McCartin et al., 2023). It is important to note that size class estimations can be imprecise, as morphological features and animal body sizes overlap and vary between individuals (Driver et al., 2011) and body mass estimations from modern animals may be a poor analog to past animals (Gayford et al., 2024).

After receiving the initial results, we also resampled a subset of remains identified as equids to differentiate between horse (*Equus ferus*) and wild ass (*Equus hemonius*). A large bovine mandible fragment, associated with the tooth sampled for isotopes (PTF-B1), was also sampled to distinguish between musk ox (*Ovibos moschatus*) and aurochs or steppe bison (*Bos primigenius* or *Bison priscus*), as morphological and metric identification measures were inconclusive.

S1.2. Methods

Sampling and analyses were conducted at the Archaeo- and Palaeoproteomics laboratory at the University of Tübingen following an acid-insoluble protocol (Brown et al., 2020; Buckley et al., 2009; Welker, 2015). Bone chips (approximately 20 mg) from each sample, removed with a diamond-studded drill bit, were first demineralized in 0.6 M HCl at 4 °C. Once demineralized, the bone chips were rinsed three times with 200 µl of 50 mM ammonium bicarbonate (AmBic) to obtain a neutral pH and gelatinized for one hour at 65 °C. 50 µl of the resulting supernatant was treated with 0.4 µg of trypsin and incubated overnight at 37 °C. 1 µl of 5% (v/v) TFA was added to stop digestion.

A selection of the equids identified using ZooMS was further analysed to achieve species-level identifications following an established protocol (Paladugu et al., 2023). The demineralised bone chip for each equid was re-demineralised in 50 µl 50 mM Ambic at 65 °C for one hour. The resulting supernatant was reduced in a vacuum centrifuge and resuspended in 50 µl of Tris buffer (100 mM Tris(hydroxymethyl)aminomethane hydrochloride, 10 mM calcium chloride, pH 8.0). The samples were then digested in 0.4 µg of chymotrypsin.

All samples were desalted and concentrated using C18 ZipTips and eluted in 50 µl of conditioning solution. 0.5 µl of the resulting solution was mixed with 0.5 µl of α cyano-4-hydroxycinnamic acid solution (10 mg/mL in 50% acetonitrile and 0.1% trifluoroacetic acid) and allowed to crystallize. Samples were measured using a Bruker Autoflex Matrix-Assisted Laser Desorption/Ionisation Time of Flight mass spectrometer (MALDI-TOF MS).

The resulting spectra were peak picked with a signal-to-noise ratio of 3.5 after baseline correction, smoothing, and deisotoping with the default parameters and analyzed with flexAnalysis 3.4 (Bruker Daltonics) and mMass (Strohalm et al., 2008). For species identification, spectra were compared against a reference library of known peptide markers (Buckley et al., 2009, 2010; Buckley and Kansa, 2011; Welker et al., 2016; Paladugu et al., 2023; Strohalm et al., 2008).

S1.3. Results

Collagen was extracted from 96 out of the 100 bone samples (96% success rate). Of the successful samples, 54 (56%) were identified as reindeer and 42 as Equidae (44%) (Sup. Fig. 1). Species differentiation was attempted for 22 of the equid specimens; however, the marker used to differentiate horse and donkey was only identified in four samples, likely reflecting the antiquity and preservation of collagen within the bone. In all cases, this marker confirmed the equids correspond to horse rather than wild ass (Paladugu et al., 2023). ZooMS also ruled out musk ox for the

bovine mandible fragment, indicating the individual is either an aurochs or a steppe bison. A full table of all measured values is given in the open access repository PANDORA (DOI: https://www.doi.org/10.48493/3qd1-yq09).

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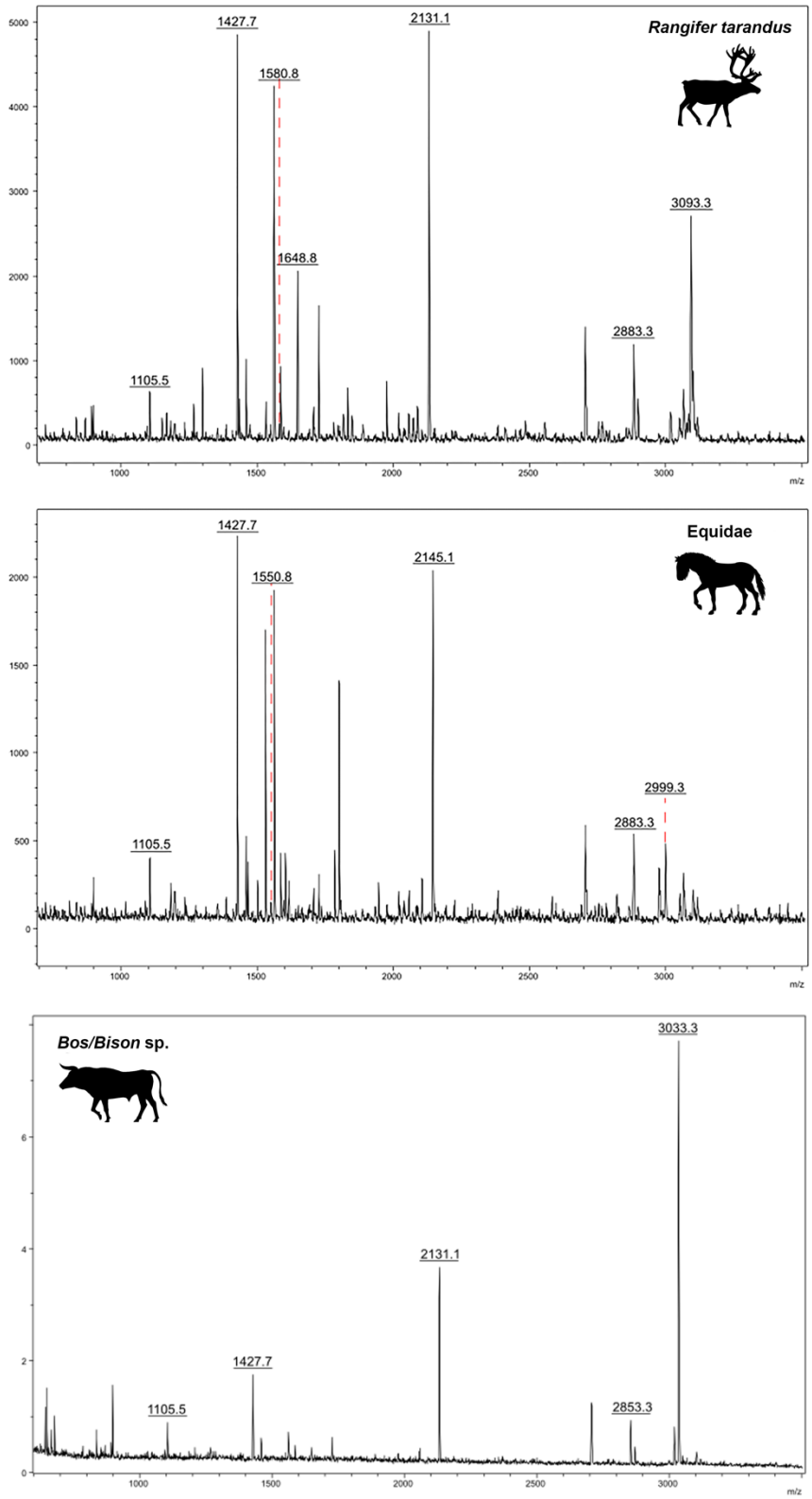
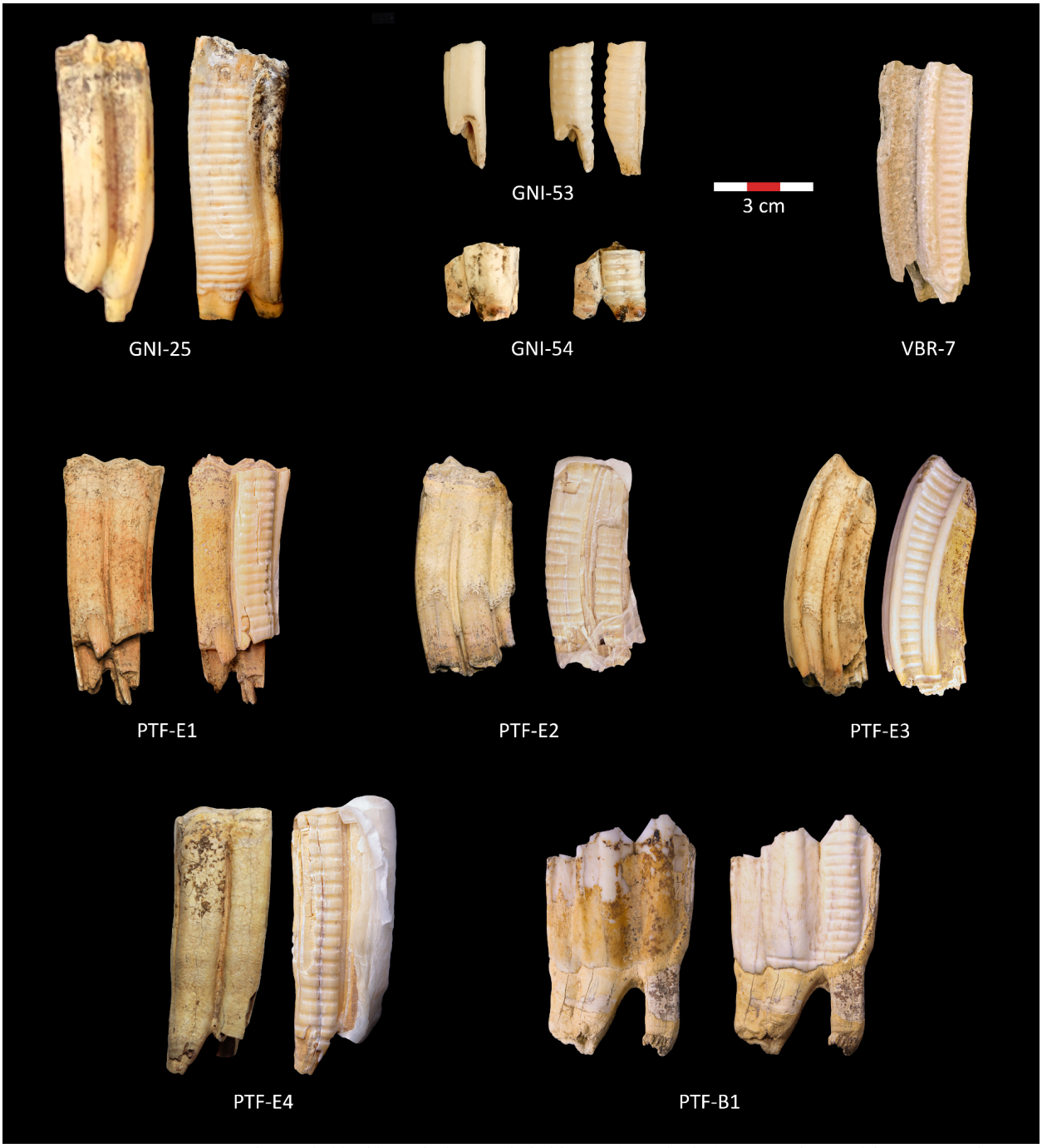
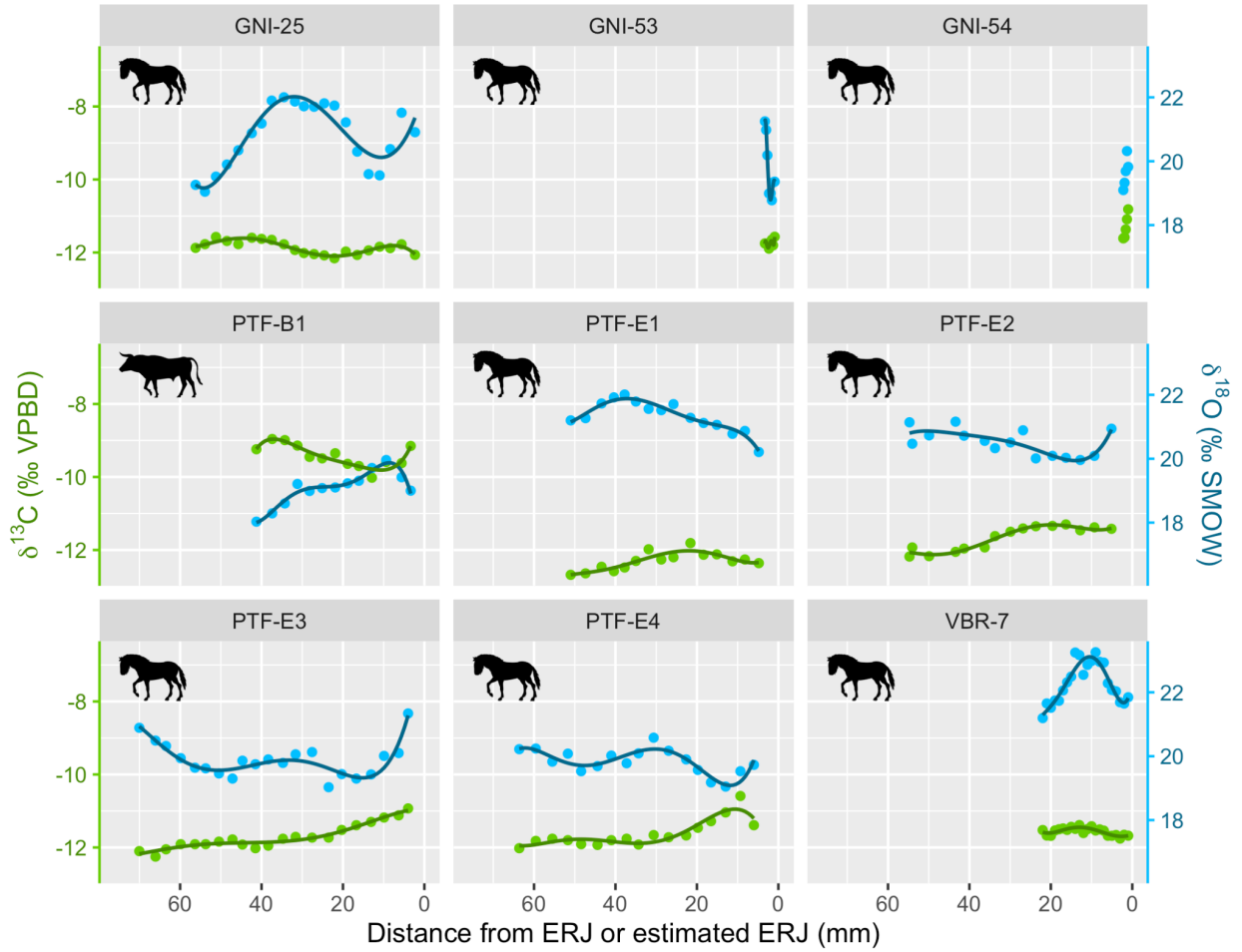
Supplementary Figures:



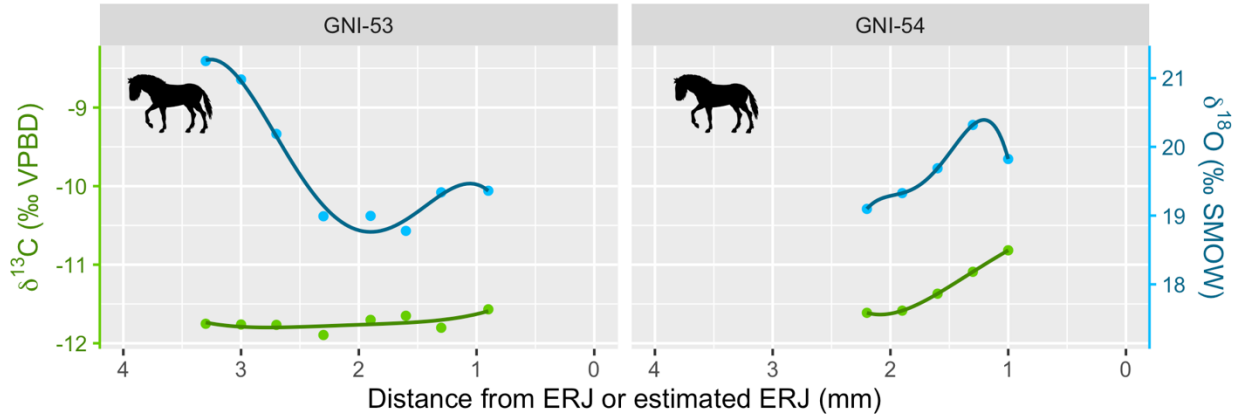
Supplementary Figure 1: Spectra from samples identified as reindeer (*Rangifer tarandus*), Equidae (*Equus* sp.), and Bos/Bison using ZooMS. Peaks typically used in the identification of each taxon are labelled, the red lines indicate the placement of smaller peptide peaks or those which are obscured by other, larger peaks.



Supplementary Figure 2: Teeth sampled in this study from Gnirshöhle (GNI), Verberie (VBR), and Petersfels (PTF). Scale: 3 cm total, each segment is 1 cm. Gnirshöhle photos by TP, Verberie photos by DGD, and Petersfels photos by MJM. Photo of VBR-7 prior to sampling is not available.



Supplementary Figure 3: $\delta^{13}C$ and $\delta^{18}O$ of ungulate (all but PTF-B1 are equids) tooth enamel carbonate from Gnirshöhle (GNI), Petersfels (PTF), and Verberie (VBR) plotted across sampling area. Sampling area is presented as either distance from the enamel root junction (ERJ) or distance from the estimated enamel root junction; the timing of mineralization progresses from left to right.



Supplementary Figure 4: $\delta^{13}C$ and $\delta^{18}O$ of horse tooth enamel carbonate from the two smallest Gnirshöhle (GNI) specimens (GNI-53 and GNI-54) to better illustrate change in isotopic values over the sampling area. Sampling area is presented as distance from the enamel root junction (ERJ); the timing of mineralization progresses from left to right.