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# Neanderthal subsistence, taphonomy and chronology at Salzgitter-Lebenstedt (Germany): a multifaceted analysis of morphologically unidentifiable bone

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**ABSTRACT:** Pleistocene faunal assemblages are often highly fragmented, hindering taxonomic identifications and interpretive potentials. In this paper, we apply four different methodologies to morphologically unidentifiable bone fragments from the Neanderthal open-air site of Salzgitter-Lebenstedt (Germany). First, we recorded zooarchaeological attributes for all 1362 unidentifiable bones recovered in 1977. Second, we applied zooarchaeology by mass spectrometry (ZooMS) to 761 fragments, and calculated glutamine deamidation values. Third, we assessed the collagen preservation of 30 fragments by near-infrared spectroscopy (NIR) and, finally, we pretreated 10 bones with high predicted collagen values for radiocarbon dating. All returned dates at, or beyond, the limit of radiocarbon dating, indicating an age of older than 51 000 years ago. The ZooMS faunal spectrum confirms a cold environment, dominated by reindeer, alongside mammoth, horse and bison. The low occurrence of carnivore modifications (1%) contrasts with an abundance of human modifications (23%). Cut marks and marrow fractures were observed across reindeer, horse and bison. The mammoth remains are less well preserved and show a lower degree of human modifications, indicating, perhaps, a different taphonomic history. Overall, this study illustrates the importance of retaining, studying and incorporating the unidentifiable bone fraction to optimize interpretations of site formation and subsistence behaviour at Palaeolithic sites.

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**KEYWORDS:** Middle Palaeolithic; Neanderthal subsistence; near-infrared spectroscopy; radiocarbon dating; zooarchaeology by mass spectrometry

## Introduction

Neanderthal subsistence was predominantly focused on the acquisition and processing of large and medium ungulates (equids, bovids and cervids), as repeatedly demonstrated through zooarchaeological studies (Gaudzinski, 1995; Marean & Kim, 1998; Rendu, 2010; Niven *et al.*, 2012; Smith, 2015; Daujeard *et al.*, 2019) and isotope analyses (Richards *et al.*, 2001; Bocherens *et al.*, 2001, 2005; Richards & Trinkaus, 2009; Bocherens, 2011; Britton *et al.*, 2011; Jaouen *et al.*, 2019; Jaouen *et al.*, 2022). The Middle Palaeolithic open-air site of Salzgitter-Lebenstedt (northwest Germany) played a prominent role in shaping current understanding of Neanderthals as proficient and habitual hunters (Gaudzinski & Roebroeks, 2000). Excellent organic preservation at the site has resulted in a wealth of environmental and archaeological data, including a large faunal collection (>8700 bone fragments) (Tode *et al.*, 1953; Todo, 1982; Grote, 1978; Grote and Preul, 1978; Krönneck & Staesche, 2017). Salzgitter-Lebenstedt has provided one of the best examples of systematic and routinized processing of game by Neanderthals, with a heavy dominance of reindeer (c. 80% of the identifiable fauna; see Supporting Information Tables S1

and S2; Krönneck & Staesche, 2017) and a strong focus on the adult reindeer carcasses (Gaudzinski & Roebroeks, 2000). However, the timing of the Neanderthal occupation at the site remains unclear, with suggestions ranging from Marine Isotope Stage 6 (MIS-6) to MIS-3 (Bosinski, 1963; Selle, 1991; Pfaffenberg, 1991; Pastoors 2001, 2009). Also, potential differences in taphonomic history between the reindeer and the other animal species (mammoth, horse, bison and rhinoceros) (Gaudzinski, 2000; Gaudzinski & Roebroeks, 2000, 2003; Munson and Marean, 2003; White *et al.*, 2016) require further contextualization.

In general, Pleistocene faunal assemblages are often highly fragmented, meaning that 70–90% of the bones cannot be identified taxonomically. Consequently, there are important limitations in the ways these morphologically unidentifiable bones can contribute to further analyses and interpretations of site formation and subsistence behaviour (but also see Villa *et al.*, 2004; Sinet-Mathiot *et al.*, 2019; Gallo *et al.*, 2021). Recent advances in biomolecular archaeology have now developed a suite of methodologies applicable to fragmented bone to maximize its data retrieval and interpretive potential. Collagen peptide mass fingerprinting, known as zooarchaeology by mass spectrometry (ZooMS, Buckley *et al.* 2009; Richter *et al.*, 2022), can provide taxonomic identifications of morphologically unidentifiable bone fragments, usually up to

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subfamily or genus level. This greatly increases the number of taxonomically identified bones at a site, facilitating a larger dataset to investigate links between taphonomic agents, species representation, and broader patterns of hunting and processing behaviour. ZooMS analysis also allows for the calculation of glutamine deamidation values, which are indicative of protein preservation (van Doorn *et al.*, 2012). This has been integrated with ZooMS studies at various Pleistocene sites (Welker *et al.*, 2017; Sinet-Mathiot *et al.*, 2019; Brown *et al.*, 2021b; Ruebens *et al.*, 2022; Silvestrini *et al.*, 2022) and has the potential to provide additional insights into differing patterns of diagenesis, especially when combined with detailed recording of taphonomic attributes such as weathering, abrasion and bone surface modifications.

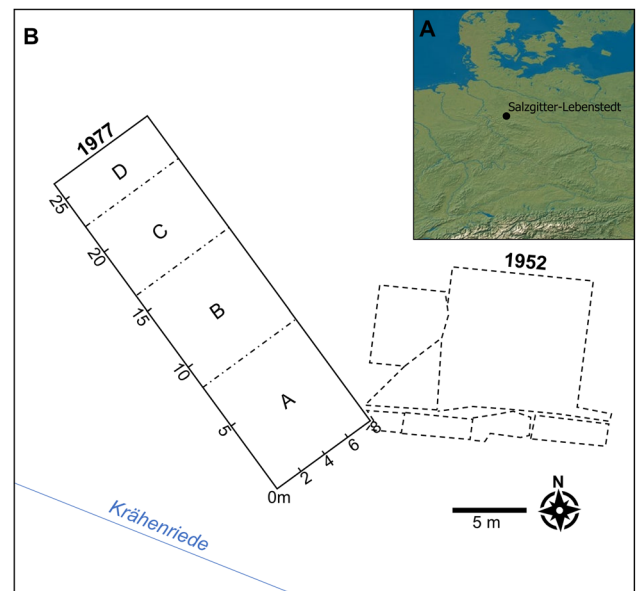
In addition, the precision and accuracy of radiocarbon dating continues to improve, with sample pre-treatment methods for Pleistocene material refined over the past several decades (e.g. Higham, 2011; Bird *et al.*, 2014; Deviese *et al.*, 2018; Korlević *et al.*, 2018; Talamo *et al.*, 2021a), including significant reductions to the size of required bone samples (Fewlass *et al.*, 2017, 2019a), and the latest version of the calibration curve extended back to 55 000 years ago (Reimer *et al.*, 2020; Bard *et al.*, 2020). Recently, near-infrared spectroscopy (NIR) has also been applied successfully to Pleistocene bone fragments as a fast, non-destructive method to predict their collagen preservation (Sponheimer *et al.* 2019), and as such is a useful screening method prior to  $^{14}\text{C}$  dating and isotopic analyses (Fewlass *et al.*, 2019b; Talamo *et al.*, 2021b).

This paper presents an application of these latest methods to the unidentifiable portion of the faunal material recovered at Salzgitter-Lebenstedt during the excavations of the 1970s. It is aimed at providing additional data on faunal diversity, taphonomic histories, Neanderthal subsistence practices and their chronology. First, a series of taphonomic and zooarchaeological attributes were recorded for all 1362 morphologically unidentifiable bone fragments. Second, the ZooMS method was applied to 761 bone fragments (excluding burnt fragments and fragments smaller than 25 mm) and deamidation values were obtained for 489 bones. This represents the first large-scale proteomic screening of an open-air Palaeolithic site. Third, the collagen preservation of 30 bone fragments was assessed by NIR and, finally, based on this collagen screening, ten bones were pretreated for radiocarbon dating. Through this multifaceted approach, we also aim to exemplify the added value and interpretive potential of this type of in-depth study of morphologically unidentifiable bones, which are widely available in archival faunal collections.

## Background

### Site location, excavation and archaeology

Archaeological material was recovered at the open-air locality Salzgitter-Lebenstedt (Lower Saxony, Germany,  $52^{\circ}10'26''\text{N}$ ,  $10^{\circ}19'42''\text{E}$ ) during the construction of a wastewater treatment plant in 1952 (195 m<sup>2</sup>, Tode *et al.* 1953) and during its expansion in 1977 (221 m<sup>2</sup>; Grote and Preul, 1978) (Fig. 1). The site is located in the valley of the small Krähenriede stream, close to its confluence with the river Fuhse. The find horizon is up to two metres thick and located at a depth of 4.5–5.5 metres under the current ground surface (Preul, 1991, 2017). The finds were originally deposited into geological unit B1 (which is the equivalent of the *untere Brodeleinheit* of the 1952 excavation). This unit contains gravels, humic sands and silt, which represent differing



**Figure 1.** A: map with the location of Salzgitter-Lebenstedt; B: schematic representation of the extent of the two excavation campaigns: 1952 (c. 14 × 14 m) and 1977 (c. 8 × 26 m) (after Pastoors, 2001 and Preul, 2017). The zoning of the 1977 excavation (A–D) follows Pastoors (2001). [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.com)]

depositional contexts, with sandy sediments and gravels indicating running water and humic peat lenses reflecting backwater bodies. Subsequently these deposits were covered by geological unit g1/g2 (*obere Brodeleinheit*). Intense frost and cryoturbation moved some of the finds from B1 into g1/g2 (Preul, 2017). Subsequently, a stream cut through the sediments in the southern part of the 1977 excavation area. This stream altered its course several times and part of the B1 finds were redeposited in the stream deposits. Finally, the flow intensity of the stream increased and the stream covered almost the entire 1977 excavation area, further incorporating the find material into younger geological units (G and i3; for section drawings see Preul, 2017).

Around 4196 lithics and over 8774 faunal remains were recovered from both excavation campaigns (Table 1; Pastoors 2001; Gaudzinski and Roebroeks, 2000; Krönneck & Staesche, 2017). Five Neanderthal remains were identified from the 1952 excavation area, including one occipital, two conjoining fragments of a right parietal (Hublin, 1984) and two fragmentary left femurs (one adult and one infant; Gaudzinski, 1998). The Salzgitter-Lebenstedt stone tools are characterized by the production of Levallois flakes, blades, scrapers and bifacial tools (Pastoors, 2001, 2009) and have been classified as belonging to the Middle Palaeolithic Keilmessergruppe (KMG; Jöris, 2004; Ruebens, 2013; Jöris *et al.*, 2022). Further, the dominance of reindeer remains has been linked to autumn hunting of reindeer by Neanderthals, followed by the butchery of their carcasses and extraction of the marrow, with a preference for the adult reindeer carcasses (Gaudzinski and Roebroeks, 2000). The other animal species (mammoth, rhinoceros, bison, horse and giant deer) have a less clear signature of human involvement (Gaudzinski, 1999; Staesche, 2017) and could, at least partly, represent a natural background fauna (Gaudzinski and Roebroeks, 2000).

All previous researchers have treated the recovered archaeological material as one entity, making no distinction between various find units, but acknowledging the presence of vertical and horizontal refits (Kleinschmidt, 1953; Tode, 1982; Schäfer, 1993; Gaudzinski, 1998; Pastoors, 2001; Staesche, 2017). Despite this general merging of the finds, there are some clear

**Table 1.** Overview of the lithic and faunal material recovered during the two excavation campaigns at Salzgitter-Lebenstedt (lithic data based on Pastoors 2001; faunal data from Krönneck and Staesche 2017, and this study).

Excavation		Lithics				Fauna			
Year	Area (m <sup>2</sup> )	≤20 mm	≥20 mm	Total	Lithic density	Identifiable	Unidentifiable	Total	Faunal density
1952	195	27	813	840	4.3	>3000	?	?	?
1977	221	1576	1780	3356	15.2	>2000	>1362	?	?
Total	416	1603	2593	4196	10.09	5444	3330	8774	21.09

**Table 2.** Overview of past radiometric dates on archaeological material from Salzgitter-Lebenstedt (based on Pastoors, 2001, 2009; Preul, 2017). All radiocarbon dates are uncalibrated (<sup>14</sup>C a BP).

Lab number	Year	Method	Dated material	Unit	Date ( <sup>14</sup> C a BP or a)
<b>1952 excavations</b>					
GrN 2083	1980	C14	Peat	Main find units (Obere Brodeleinheit)	55 600 ± 900 <sup>14</sup> C a BP
GrN 1219	1980	C14			48 500 ± 2000 <sup>14</sup> C a BP
KIA 34483	2008	C14 AMS	Reindeer humerus	Main find units (Obere Brodeleinheit)	45 280 + 1270/–1090 <sup>14</sup> C a BP
KIA 34484			Reindeer humerus		43 110 + 1010/–900 <sup>14</sup> C a BP
KIA 34481			Mammoth rib		33 970 + 540/–500 <sup>14</sup> C a BP
KIA 34482			Mammoth rib		37 950 + 540/–500 <sup>14</sup> C a BP
<b>1977 excavations</b>					
Hv 8397	1978	C14	Peaty silt	Upper find unit (I)	19 700 ± 140 <sup>14</sup> C a BP
Hv 8843	1978	C14	Humus silt	Upper find unit (G)	33 100 ± 1130 <sup>14</sup> C a BP
Hv 8842	1978	C14			28 500 + 3520/–2440 <sup>14</sup> C a BP
Hv 8642	1978	C14	Humus silt	Upper find unit (F2)	22 400 ± 410 <sup>14</sup> C a BP
Hv 9378					22 600 ± 165 <sup>14</sup> C a BP
Kn 2449	n/a	C14	Peat	Middle find unit (C2)	>47 500 <sup>14</sup> C a BP
GrN 10702	1980s	C14	Peat	Lower find unit (B1, main find horizons)	52 700 ± 600 <sup>14</sup> C a BP
GrN 9254					48 780 ± 260 <sup>14</sup> C a BP
GrN 9188					>49 500 <sup>14</sup> C a BP
GrN 9372					36 000 ± 550 <sup>14</sup> C a BP
GrN 9894					39 300 ± 800 <sup>14</sup> C a BP
BKY 90 007	?	U/Th	Bone fragment	Unknown	43 300 ± 7800 a
BKY 90 009	?	U/Th	Bone fragment	Unknown	76 300 ± 7700 a

differences between the material recovered from the 1952 and 1977 excavation campaigns (Table 1). During the 1952 excavation two rich find concentrations were recovered from two chronologically successive backwater bodies. At the location of the 1977 excavation the finds are more dispersed and the bone material more fragmented. However, note that in 1977 part of the sediments were screened to recover small fragments, while in 1952 the small fraction was not recovered systematically (Grote and Preul, 1978; Gaudzinski and Roebroeks, 2000; Pastoors, 2001). The faunal material has been studied by various scholars who all report on different sample sizes and different amounts of unidentifiable bones (see Supporting Information Tables S1 and S2), making it difficult to obtain a clear overview of faunal density per excavation campaign (Table 1). Conversely, the lithic density clearly reflects this difference in excavation methods with a very low number of fragments smaller than 2 cm recovered in 1952 (Pastoors, 2001; Table 1).

### Palaeoclimatic reconstruction and chronology

The Salzgitter-Lebenstedt material from both excavation campaigns has been studied by a broad multidisciplinary team, allowing palaeoclimatic reconstructions based on microfauna, beetles, pollen, macro-botanical remains, fungi and molluscs (Selle, 1991; Schüttrumpf, 1991; Pfaffenberg, 1991; Johannes and Schuh-Johannes, 1991; Preul, 1991; van Kolfschoten, 2017). The pollen spectra indicate a shrub tundra with a rich herb flora and bodies of open water during cool, glacial conditions.

Cold-adapted species such as dwarf birch (*Betula nana*) and willow (e.g. *Salix polaris*) could be identified (Selle, 1991; Schüttrumpf, 1991). The presence of isolated trees and the lack of a closed forest finds parallels with several post-Eemian interstadials, but assignment to a specific interstadial is, currently, not possible. Oxygen isotope analyses of reindeer remains confirm these cold conditions, with an estimated mean annual temperature 6–10 °C lower than today (Stephan, 2017).

The exact age of the archaeological material from Salzgitter-Lebenstedt has formed a topic of debate for many years. Suggestions have been made ranging from MIS-6 (Saalian glaciation; Bosinski 1963), Early Weichselian [Brörup Interstadial (c. 100 ka); Selle, 1991; Pfaffenberg, 1991], Oerel Interstadial (58–54k <sup>14</sup>C a BP; Preul, 2017) or to later in MIS-3 (Pastoors 2001, 2009). Radiometric dates, including uranium/thorium on teeth, radiocarbon dating on peat and accelerator mass spectrometer (AMS) dating on worked bone (Table 2), returned results ranging from 76 to 33 ka (Pastoors 2001, 2009; Preul, 2017). The majority of these dates were obtained in the 1970s and 1980s and, therefore, a new series of AMS dates on bone is presented in this study.

### Methodology

#### Contextual, taphonomic and zooarchaeological analysis

In total, 1362 unidentifiable faunal remains from the 1977 excavation were available for study from the

Braunschweigisches Landesmuseum in Wolfenbüttel (Germany). In a first step, the contextual data written on the find labels were recorded for each bone fragment (Fig. 2), including find number, northwest and northeast coordinates, layer and excavation date. Because of problems with collagen preservation, burnt pieces were not sampled. Pieces smaller than 25 mm were also excluded so enough bone remains available for further analyses (e.g. direct radiocarbon dating or ancient DNA analysis). In a second step, the available find numbers were linked to data available from recent faunal analysis [including weight (g), body size class (large, medium-large, medium, medium-small, and small) and bone element (Staesche, 2017)]. A third step involved a zooarchaeological and taphonomic analysis of each bone fragment based upon previous methodologies (Smith, 2015; Sinet-Mathiot *et al.*, 2019, 2023; Smith *et al.*, 2021; Ruebens *et al.*, 2022). Attributes recorded include tissue type (trabecular, cortical or indeterminate), bone element (long bone, flat bone, cranial, rib, indeterminate), bone surface readability (0%, 1–50%, 51–99%, 100%), weathering stage (0–5), abrasion (0%, 1–50%, 51–99%, 100%), break morphology (fresh, dry, indeterminate), non-anthropogenic modifications (carnivore tooth marks, breakage, digestion) and anthropogenic modifications (cut marks, chopping marks, scraping marks, marrow fractures) (Behrensmeyer, 1978; Binford, 1981; Lyman, 1994; Fisher, 1995; Fernandez-Jalvo & Andrews, 2016). Finally, for each fragment maximum length and width were measured with a digital calliper (in mm).

### ZooMS extraction and analysis

ZooMS extraction was conducted at the palaeoproteomics lab at the Max Planck Institute for Evolutionary Anthropology in Leipzig (Germany) following existing protocols (Buckley *et al.*, 2009; van Doorn *et al.*, 2011; Welker *et al.*, 2016; Sinet-Mathiot *et al.*, 2023). To maximize data retrieval, collagen was extracted from all bone samples using two protocols: ammonium-bicarbonate (AmBic) buffer and demineralization with hydrochloric acid (HCl). This strategy allows the maximization of peptide marker recovery, and enhanced taxonomic identification, from a single bone sample. First, to clean and remove any soluble contamination the bone samples were immersed in 100 µl AmBic for 21 h at room temperature. This buffer was then removed and the samples re-immersed in AmBic for 1 h at 65 °C to extract soluble protein through gelatinization (van Doorn *et al.* 2011). Next, the supernatant containing denatured protein was incubated

overnight in trypsin (Promega V5111) at 37 °C allowing for the protein to be digested and cleaved into peptides. After 17–18 h, trifluoroacetic acid (TFA) was added to stop the digestion and the peptides were purified, extracted and spotted onto a 384-spot MALDI plate. Spotted plates were analysed partly at the Fraunhofer IZI in Leipzig (Autoflex speed LRF MALDI-TOF; Bruker) and partly at the Biomolecular Science Facility at the University of Kent (Ultraflex extreme MALDI-TOF; Bruker). The remaining bone samples were immersed in 0.6 M HCl for 21 h, rinsed and then processed again starting with the gelatine extraction step (following Welker *et al.*, 2015, 2016). Empty wells were processed as blanks alongside the bone samples for quality control. All returned empty spectra, illustrating that no contamination was introduced during the lab work.

The triplicate data files obtained from the MALDI were merged in R using the packages MALDIquant and MALDIquantForeign (Gibb and Strimmer, 2012) to smooth the intensity of the peaks (applying a moving average function), remove the baseline (using the TopHat method) and align the spectra (SuperSmoother, signal to noise ratio of 3). The three replicates were then summed into a single spectrum and the baseline removed once more using the TopHat approach. The obtained.msdf files were analysed in the open source MS tool mMass (<http://www.mmass.org/>, Strohalm *et al.*, 2010) with a signal to noise ratio set to 3. The presence of nine peptide markers was assessed and taxonomic identifications were made through comparisons with existing databases (Welker *et al.*, 2016; Brown *et al.*, 2021a). Glutamine deamidation values were calculated using the Betacalc3 package (Wilson *et al.*, 2012).

### NIR screening and radiocarbon dating

NIR was used to assess the collagen preservation of 30 morphologically unidentifiable bone fragments from the 1977 collection using a LabSpec 4 hi-res NIR spectrometer (Malvern Panalytical), following protocols outlined by Sponheimer *et al.* (2019). Subsequently, 10 were selected for dating based on the level of collagen preservation indicated, the ZooMS taxonomic identification and the presence of anthropogenic surface modifications.

Next, each bone was cleaned with a sandblaster and around 400 mg was sampled for collagen extraction at the Department of Human Evolution at the Max Planck Institute for Evolutionary Anthropology (Leipzig, Germany). The samples were pretreated using a modified Longin (1971) plus ultrafiltration



**Figure 2.** A: examples of the unidentifiable bones from the 1977 excavations at Salzgitter-Lebenstedt; B: examples of the find label associated with the bone fragments. [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

(Brock *et al.*, 2007) protocol described in Fewlass *et al.* (2019a). They were graphitized (Wacker *et al.*, 2010a) and AMS dated at the Laboratory for Ion Beam Physics at the Eidgenössische Technische Hochschule (ETH) Zurich, Switzerland (Wacker *et al.*, 2010b). To monitor lab-based contamination, a background bone (>50 000  $^{14}\text{C}$  a BP) was pretreated and dated alongside the samples. The AMS measurements of the collagen backgrounds were highly reproducible (mean  $F^{14}\text{C} = 0.0027$ ,  $1\text{SD} = 0.00018$ ,  $n = 5$ ) and were used in the age correction of the samples, with an additional 1‰ error added, as per standard practise.

To assess the quality of each extract prior to dating, collagen (~0.5 mg) was weighed into a tin cup and analysed on a ThermoFinnigan Flash elemental analyser coupled to a Thermo Delta plus XP isotope ratio mass spectrometer (EA-IRMS). Stable carbon isotope ratios were expressed relative to VPDB (Vienna PeeDee Belemnite), and stable nitrogen isotope ratios were measured relative to AIR (atmospheric  $\text{N}_2$ ), using the delta notation ( $\delta$ ) in parts per thousand (‰). Analysis of internal [in-house collagen standard:  $\delta^{13}\text{C} = -19.77 \pm 0.12\text{‰}$  (1SD) and  $\delta^{15}\text{N} = 5.00 \pm 0.07\text{‰}$  (1SD)] and international standards (IAEA N1:  $\delta^{15}\text{N} = 0.43 \pm 0.10\text{‰}$  (1SD), IAEA N2:  $\delta^{15}\text{N} = 20.41 \pm 0.13\text{‰}$  (1SD), CH<sub>6</sub>:  $\delta^{13}\text{C} = -10.45 \pm 0.07\text{‰}$  (1SD), CH<sub>7</sub>:  $\delta^{13}\text{C} = -32.15 \pm 0.001\text{‰}$  (1SD)] indicates an analytical error of better than 0.2‰ (1 $\sigma$ ).

## Results

### Faunal spectrum

A total of 1362 bone fragments labelled as unidentifiable were present in the 1977 museum collection, of which 601 were excluded from ZooMS analysis (410 smaller than 25 mm and 191 burnt pieces). Of the remaining 761 bone fragments analysed, 57.4% could be identified to either species or genus through the AmBic extraction protocol (Supporting Information Table S3). This identification rate increased to 76.7% after acid demineralization of the bones (Table 3). Six taxa were identified (Table 3). Reindeer is dominant (73.5%), followed by mammoth (10.8%), horse (8.4%) and *Bos/Bison* (2.6%). A few fragments could also be identified as rhinoceros ( $n = 2$ ) and Cervid/Saiga ( $n = 3$ ), the majority of these only after acid demineralization (Table S1). While the grouping Cervid/Saiga includes a number of possible species (Table 3), in the morphologically identifiable fauna only giant deer (*Megaloceros giganteus*) has been identified. No carnivore remains are present in the ZooMS fraction, although remains of both wolf ( $n = 8$ ) and cave lion ( $n = 2$ ) are known from the site (Tables S1 and S2; Krönneck and Staesche, 2017).

**Table 3.** ZooMS taxonomic identification of the morphologically unidentifiable fraction of the 1977 selection of Salzgitter-Lebenstedt.

ZooMS ID	Possible species	N	%
Elephantidae	<i>Mammuthus primigenius</i>	63	10.79
Rhinocerotidae	<i>Coelodonta antiquitatis</i>	2	0.34
Equidae	<i>Equus</i> sp.	49	8.39
Reindeer	<i>Rangifer tarandus</i>	429	73.46
<i>Bos/Bison</i>	<i>Bison</i> sp./ <i>Bos</i> sp.	15	2.57
Cervid/Saiga	<i>Cervus</i> sp./ <i>Alces alces</i> / <i>Megaloceros</i> sp./ <i>Saiga tatarica</i> / <i>Dama</i> sp.	3	0.51
Bovidae/Reindeer		23	3.94
<b>Total identifiable</b>		<b>584</b>	<b>76.74</b>
<b>Total unidentifiable</b>		<b>177</b>	<b>23.26</b>
<b>Total analysed</b>		<b>761</b>	<b>100</b>

### Bone preservation

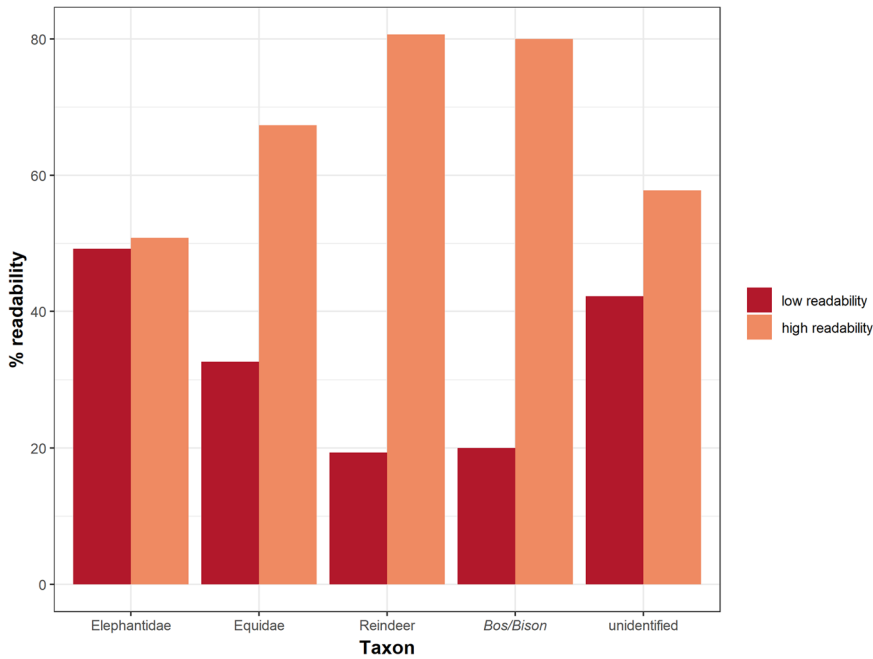
Within the studied sample, bone surfaces are generally well preserved, with most specimens (80–90%) exhibiting low surface abrasion, and only very few heavily abraded fragments. This is further illustrated with the vast proportion (70.7%) of the bone fragments illustrating high readability. The mammoth remains have an even proportion of surface preservation (50:50), while for the other taxa surfaces with high readability dominate (Fig. 3). This difference was tested through the calculation of composite chi-square values and adjusted residuals (Supporting Information Table S4) and illustrates that there is a statistically significant increase in mammoth fragments with low bone surface readability and in reindeer fragments with high readability ( $\chi^2 = 60.89$ ,  $p < 0.001$ ).

Bone weathering data (following the stages of Behrensmeier 1978) exhibit a similar pattern across the major taxa at Salzgitter. While in general bone fragments are mainly in the low (0–1) or medium (2–3) weathering stages (Fig. 4A), mammoths have a higher proportion of bone fragments (15.5%) in the heavy weathering stage (4–5) (Fig. 4B). This change in weathering stages between the main taxa (including unidentifiable remains) was tested through a similar calculation of composite chi-square values and adjusted residuals (Supporting Information Table S5). These illustrate a significant difference between both low and medium ( $\chi^2 = 16.1$ ,  $p = 0.0028$ ) and medium and heavy ( $\chi^2 = 18.4$ ,  $p = 0.001$ ) weathered specimens. This is driven by a significant decrease in medium and heavily weathered reindeer specimens and a comparable significant increase in medium and heavily weathered Elephantidae and unidentifiable specimens (see Table S6). These differences in surface readability and weathering could suggest a different site formation history for the reindeer and mammoth remains.

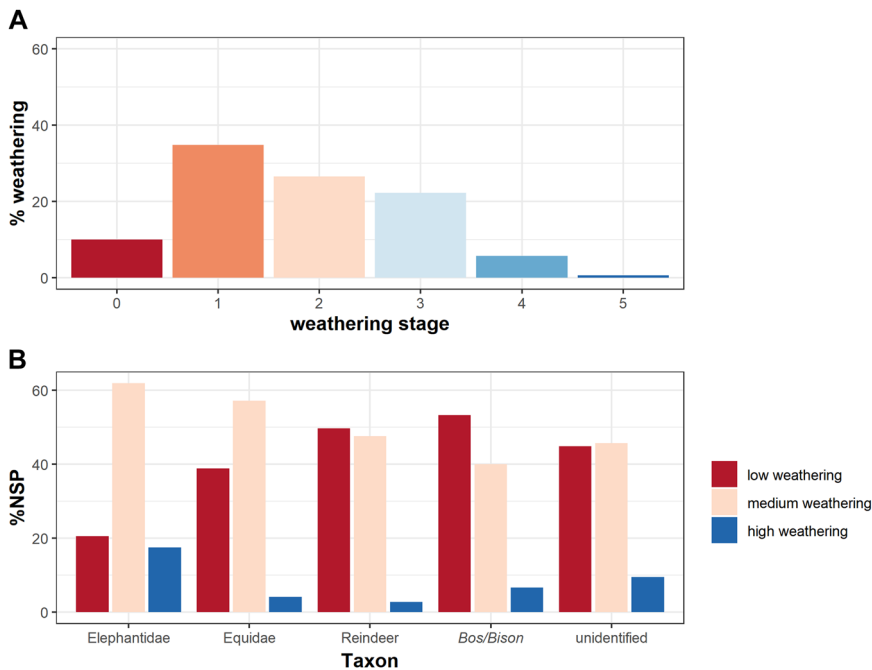
### Biomolecular preservation

There appears to be no one-on-one causal link between bone preservation, as observed through macroscopically recorded taphonomic variables, and the ability to provide a taxonomic identification through ZooMS (Fig. 4). Of the 49 heavily weathered bones, 14 (29%) were identifiable after AmBic extraction and this number increased to 29 (59%) after acid demineralization (for raw data on extraction protocol and weathering stage see Supporting Information Table S6). Of the 341 lightly weathered bones, 76 (22%) remained unidentifiable even after acid demineralization. In general, the acid demineralization increased the identification rate across all weathering stages and taxonomic groups (Table S6), with the highest increase in the groups with the lowest number of bone fragments (e.g. weathering stages 4–5, Cervid/Saiga and Rhinocerotidae).

The biomolecular preservation of the bone fragments was assessed further through calculation of the glutamine deamidation values (following the methodology of Wilson *et al.*, 2012). Because we used two different MALDI instruments (a Bruker Autoflex in Leipzig and a Bruker Ultraflex-treme in Kent), we first cross-compared three control samples between both machines and between all Kent runs. This comparison indicates a low amount of inter- and intra-instrument variation (within the range of 0.05) justifying the direct comparison of all obtained values, regardless of instrument or run (for raw data see Supporting Information Table S7). We calculated a *t*-test value that showed no significant difference in calculated deamidation values for either the AmBic or acid protocol obtained on the MALDI instruments from either Leipzig or Kent (see Supporting Information Tables S7 and S8).



**Figure 3.** Overview of bone surface readability by major taxa identified through ZooMS; low readability means between 0% and 50% of surface remaining; high readability means between 50% and 100% surface remaining. [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1002/jqs.3499)]



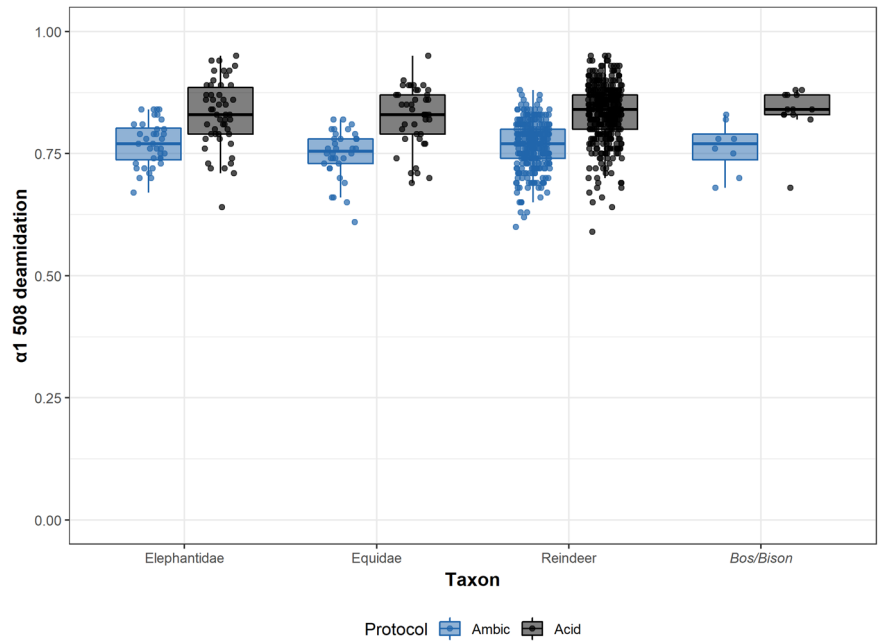
**Figure 4.** Overview of the weathering stages (Behrensmeyer, 1978) of the Salzgitter-Lebenstedt studied bone material (A) and by ZooMS-identified taxon (B). [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1002/jqs.3499)]

Glutamine deamidation values were calculated for both the AmBic and acid extracts, allowing a comparison between the two extraction methods. The obtained deamidation values may have been affected by the specific extraction protocol, with AmBic extracts generally resulting in slightly more advanced deamidation values compared to their acid equivalent (Fig. 5). This can be explained by the fact that the AmBic method extracts peptides from the soluble collagen, which at Salzgitter will have been affected by taphonomic processes, while the acid demineralization releases the insoluble fraction (van Doorn *et al.*, 2011).

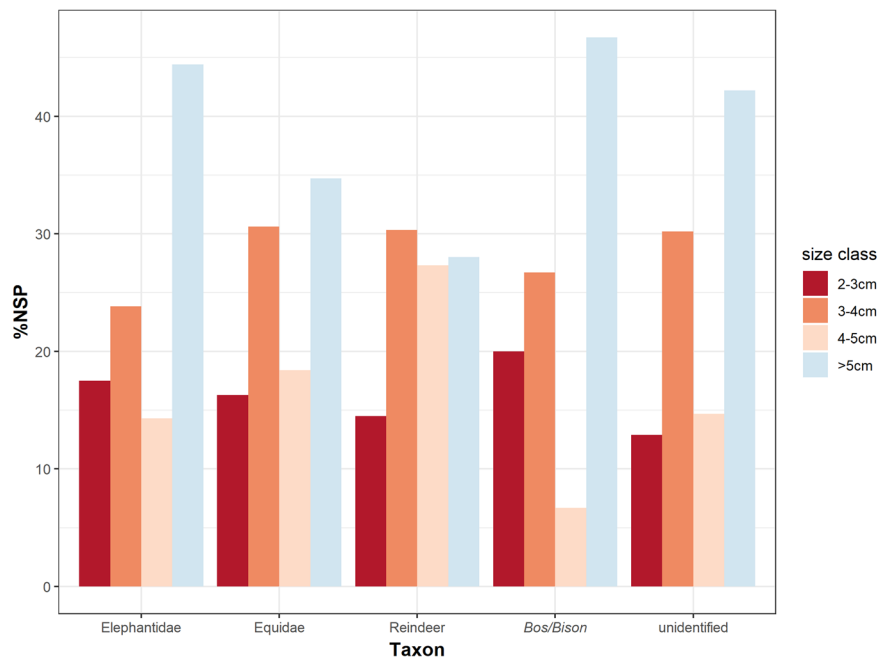
Acid deamidation values for COL1 $\alpha$ 1 508–519 were obtained for 83.7% of the bone fragments that were identifiable through acid demineralization. While 89% of the reindeer provide a deamidation value, this percentage is similar for *Bos/Bison* (86.7%), but lower for the Equidae (83.4%) and the Elephantidae (79.4%). When further compared by taxonomic group, the deamidation values overlap, with values ranging

from about 0.65 to 0.85 across the four main taxa (Fig. 5). A Wilcoxon test illustrates no significant difference between the mean acid COL1 $\alpha$ 1 508 deamidation values for the major taxa from Salzgitter-Lebenstedt (Reindeer vs. Equidae: statistic = 7453,  $p = 0.6$ ; Reindeer vs. Elephantidae: statistic = 9328,  $p = 0.8$ ; Reindeer vs. *Bos/Bison*: statistic = 2674,  $p = 0.6$ ; Elephantidae vs. *Bos/Bison*: statistic = 358,  $p = 0.6$ ; Elephantidae vs. Equidae: statistic = 1065,  $p = 0.8$ ; Equidae vs. *Bos/Bison*: statistic = 291,  $p = 0.6$ ) (see Supporting Information Table S9). Therefore, glutamine deamidation as an indicator of protein degradation does not explain the variation in identified taxa from the site. At Salzgitter-Lebenstedt no extreme outliers are present among the AmBic deamidation values, which could have indicated an intrusion into the archaeological horizon. Overall, the glutamine deamidation values can neither confirm nor disprove patterns of different diagenetic process between the various taxa at Salzgitter-Lebenstedt, but the lower number of deamidation





**Figure 5.** Comparison of the glutamine deamidation values obtained through Ambic and acid extraction method by main taxonomic groupings for COL1 $\alpha$ 1 508–519. Sample sizes for Ambic are Elephantidae ( $n = 36$ ), Equidae ( $n = 34$ ), reindeer ( $n = 298$ ), *Bos/Bison* ( $n = 8$ ) and for acid are Elephantidae ( $n = 50$ ), Equidae ( $n = 41$ ), reindeer ( $n = 380$ ), *Bos/Bison* ( $n = 13$ ). [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]



**Figure 6.** Overview of the occurrence of bone fragments from different size classes across the major taxa identified through ZooMS. Fragments smaller than 2 cm were not sampled. [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]

values obtained for the mammoth remains does indicate a lower preservation of the collagen within this taxon.

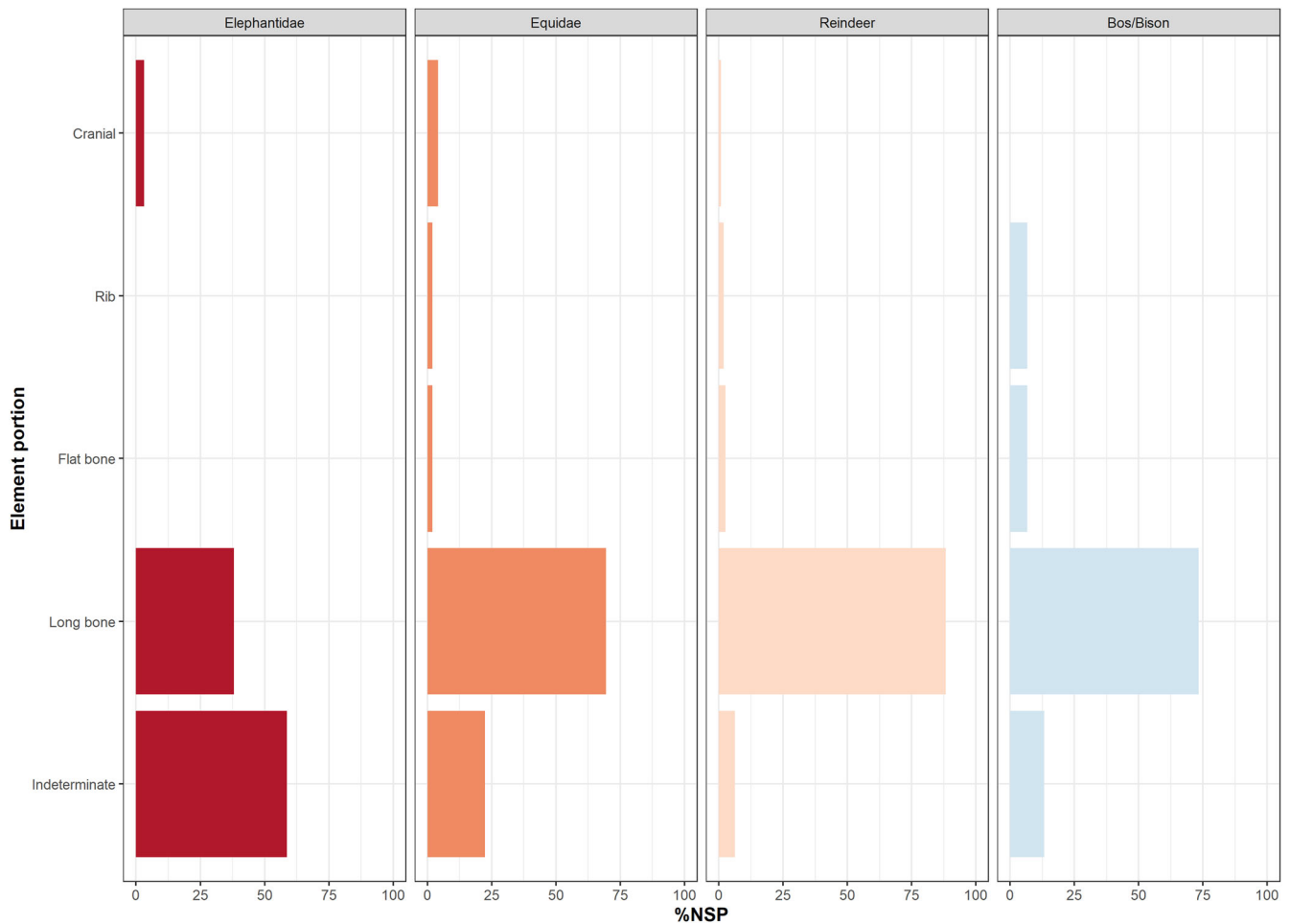
### Fragmentation

The majority of the morphologically unidentifiable bone fragments at Salzgitter-Lebenstedt are less than 50 mm in length. The few pieces larger than 100 mm that were analysed ( $n = 16$ ) were morphologically unidentifiable due to heavy weathering. Of these 16 fragments only three were identifiable and these were all mammoth. In the smaller fraction there does not seem to be a direct correlation between fragment size and identified taxon (Fig. 6). While Elephantidae ( $54.3 \pm 39.3$  mm) have longer specimens compared to *Bos/Bison* ( $46.8 \pm 18.6$  mm), Equidae ( $45.9 \pm 17.6$  mm) and reindeer ( $44.9 \pm 15.1$  mm), there is no significant difference in the overall distribution [*Bos/Bison* vs. Elephantidae:  $t$ -test ( $-0.98$ ),  $df = 49.1$ ,  $p = 0.33$ ; *Bos/Bison* vs. Equidae:  $t$ -test ( $0.34$ ),  $df = 23.5$ ,  $p = 0.73$ ; *Bos/Bison* vs. reindeer:

$t$ -test ( $0.62$ ),  $df = 14.7$ ,  $p = 0.54$ ; Elephantidae vs. Equidae:  $t$ -test ( $1.52$ ),  $df = 92.3$ ,  $p = 0.13$ ; Elephantidae vs. reindeer:  $t$ -test ( $1.92$ ),  $df = 64.9$ ,  $p = 0.06$ ; Equidae vs. reindeer:  $t$ -test ( $0.4$ ),  $df = 56.3$ ,  $p = 0.69$ ] (see Supporting Information Table S10). Almost 20% of the identified mammoth bone fragments are between 2 and 3 cm in length, indicating a high fragmentation of these mammoth carcasses. Fig. 7.

### Skeletal element representation

Bone elements could be identified for 83.6% of the small bone fraction with a dominance of long bone fragments (76.3%), followed by smaller proportions of flat bones (2.6%), ribs (2.6%) and cranial (1.6%) remains. Across all four main taxa different skeletal elements were identified, but with a very low proportion of cranial remains [reindeer ( $n = 4$ ), equids ( $n = 2$ ), mammoth ( $n = 2$ ), bovids ( $n = 0$ )]. These data supplement the patterns of skeletal representation obtained from the



**Figure 7.** Skeletal element representation across the main taxa identified through ZooMS at Salzgitter-Lebenstedt. [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1002/jqs.3499)]

**Table 4.** Overview of the carnivore and human modifications on the bones from Salzgitter-Lebenstedt sampled for ZooMS. For the human modifications the category 'other' relates to chop and scrape marks.

Taxon	N	Carnivore modifications						Human modifications							
		Gnawed		Digested		Total		Cut		Marrow		Other		Total	
		n	%	n	%	n	%	n	%	n	%	n	%	n	%
Elephantidae	63	1	1.7			1	1.7								
Equidae	49							4	8.2	5	10.2			9	18.4
Reindeer	429	1	0.2	4	1.0	5	1.2	64	14.9	44	10.6	19	0.7	127	29.6
<i>Bos/Bison</i>	15							1	6.7	2	15.4			3	20
Cervid/Saiga	3	1	33.3	1	33.3	2	66.7								
Bovidae/Reindeer	23							5	21.7	4	13.8			9	39.1
Rhinocerotidae	2														
Unidentifiable	177							16	9.0	8	4.2	5		29	16.4
<b>Total</b>	<b>761</b>	<b>3</b>	<b>0.4</b>	<b>5</b>	<b>0.7</b>	<b>8</b>	<b>1.1</b>	<b>90</b>	<b>11.8</b>	<b>63</b>	<b>8.3</b>	<b>24</b>	<b>0.4</b>	<b>177</b>	<b>23.3</b>

zoarchaeological studies (Gaudzinski and Roebroeks, 2000). Taken together, because of a broad skeletal element representation (including head elements) for all four taxa, it seems most plausible that the animals died at this locality, rather than being killed elsewhere with only selected body parts transported to the site (see Discussion).

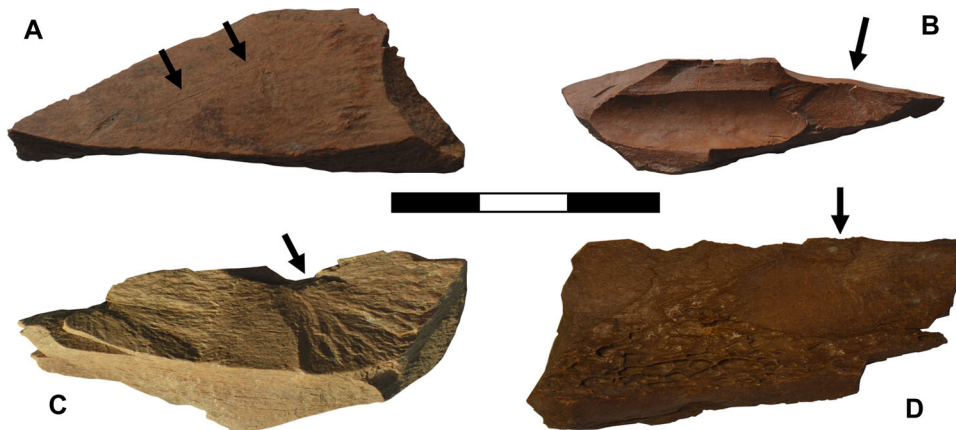
### Bone surface modifications

Carnivore modifications (including tooth pits and traces of digestion) were observed on eight bone fragments (all long bones), representing only 1% of the 761 analysed bones

(Table 4). All eight specimens were identifiable through ZooMS. The majority were recorded on reindeer ( $n = 5$ ) and, interestingly, two of the three identified remains of Cervid/Saiga also display traces of carnivore modification, including both tooth marks and digestion traces (Fig. 8B). Further, there is one mammoth bone that displays carnivore tooth marks (Fig. 8A).

Anthropogenic modifications were recorded across the major taxa in relatively high proportions (ranging from 18 to 29%, Table 4), with cut marks (Fig. 9A) and marrow fractures (Fig. 9B–D) across reindeer, equids and *Bos/Bison*. The majority of the human modifications are concentrated on

**Figure 8.** Carnivore modifications on ZooMS-identified bone fragments from Salzgitter-Lebenstedt. A: carnivore tooth pits (SL-166, Elephantidae); B: carnivore digested piece (SL-213, Cervid/Saiga). [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1002/jqs.3499)]



**Figure 9.** Human modifications on ZooMS-identified bone fragments from Salzgitter-Lebenstedt. A: cut marks (Equid, SL-535); B: marrow fracture (SL-607, Reindeer); C: marrow fracture (SL-564, unidentifiable); D: marrow fracture (SL-290, Equid). [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1002/jqs.3499)]

**Table 5.** Overview of the spatial distribution of the unidentifiable bones selected for ZooMS analysis from the 1977 excavations. The division in sections A–D follows the methodology of Pastoors (2001).

Section	Steg	Schnitt	NW coordinate (m)	ZooMS fragments	
A	I–IV, XXV–XXVII	1, 2, 13–15, 17, 18	0–8.20	462	
B	V–XII	4, 6, 7	8.21–16.20	252	
C	XIII–XIX	5, 8, 9	16.21–22.30	20	
D	XX–XXIV	10, 11	22.31–26.00	6	
					62.40%
					34.10%
					2.70%
					0.80%

reindeer ( $n = 127$ , 71.8%) and include all stages of carcass processing, such as disarticulation, skinning, meat removal and marrow extraction (Table 4). These marks were identified on reindeer long bones, flat bones and ribs, confirming the intense processing of these carcasses by Neanderthals. Both marrow fractures and cut marks are also present on the *Bos/Bison* and horse remains, for the latter including on both skull fragments and flat bones. In general, these bone surface modifications across the ZooMS-identified fragments match the patterns of modifications identified in the morphologically identifiable portion (Staesche, 2017). Interestingly, human modifications are absent on the 63 ZooMS-identified mammoth remains and on the few remains that were identified as belonging to Cervid/Saiga and Rhinocerotidae. This matches the low number of cut marks that was recorded on the morphologically identifiable remains of these taxa. For example, 11 cut marks have been recorded on the 550 morphologically identifiable mammoth remains (equalling 2%, Staesche, 2017). Note that bone surface preservation and readability makes these numbers an underestimation of the actual number of bone surface modifications, across all species, but especially on the more poorly preserved mammoth remains.

### Spatial analysis

During the 1977 excavation campaign the majority of individually labelled finds were plotted recording  $x$ ,  $y$  and  $z$  coordinates on the find label. The recording system of the stratigraphic layers changed throughout the excavation campaign. Bones excavated in September 1977 have an exact depth, while those excavated in October and November have a letter for the layer. We were not able to correlate these layers on the find labels to the published stratigraphy. Due to these complexities we were not able to assess correlations between depth or stratigraphic unit and our ZooMS data, but we were able to assess the horizontal distribution of the faunal remains.

From the 761 bone fragments studied here, 56% ( $n = 429$ ) do not have associated spatial  $x$  and  $y$  coordinates. However, the vast majority have been assigned to an excavation area, with only 21 specimens originating from an unknown location. To assess their spatial distribution, the finds were assigned to four zones based on either their coordinates or their excavation area (Fig. 1 and Table 5). These four zones were divided following the methodology of Pastoors (2001) who applied this zoning system to study the spatial distribution of the lithic assemblage (Table 5). In general, the finds are

concentrated in the southern part of the excavation trench (zone A), within a small stream (Preul, 2017), and closest to the area of the 1950s excavation (Fig. 1). The find distribution then disperses towards the north, with only a few finds recorded above 20 m north.

From the 26 bones from the northern zones C and D, 16 are identifiable (61%), which is below the overall identification rate of 76.7%. Moreover, only four bones could be identified as reindeer (25%) with a larger proportion of mammoth (37.5%) remains. In terms of bone preservation, zones C and D have an increase in the proportion of heavily weathered bones (25 and 33%) compared to zones A and B (c. 5%, Table 6). Similarly, in zones C and D only 35% of the bones provided (acid) deamidation values while in zone B this number increased to 52% and 72% in zone A. All three of these lines of evidence (ZooMS identification rate, bone weathering and deamidation) seem to indicate a higher incidence of poorly preserved non-reindeer bones in these areas. However, the overall low number of recovered bones from this area, alongside the absence of bones smaller than 30 mm due to the lack of systematic screening, make it difficult to interpret this pattern securely. The low number of bones from zones C and D also prevent a more detailed spatial comparison of the obtained deamidation values.

In terms of bone surface modifications, all the bones with carnivore modifications come from zones A and B. Similarly, only two bones with cut marks were recovered from zone C and none from zone D (Table 7). Of the 1362 unidentifiable fragments studied, 191 (14%) were burned and the majority of these were recovered from zones A and B. Similarly, the bone fragments which were smaller than 25 mm and not selected for ZooMS analysis show a similar distribution with a predominance in zones A and B (Table 7). Unfortunately, spatial data are absent for the material from the 1952 excavation, not allowing further comparisons.

### Site chronology

Thirty bones deemed suitable for radiocarbon dating based on macroscopic observations of good surface preservation were assessed for collagen preservation by NIR screening. The bones returned predicted collagen values ranging between 2 and 10% (root mean square error of validation: RMSEv  $\pm$ 3%), indicating the bones had a variable level of preservation but were generally well preserved for a Middle Palaeolithic assemblage (Supporting Information Table S11). Twenty-four bones could be identified to family through ZooMS (acid protocol). There seems to be a general trend whereby the bones with the lowest NIR-predicted collagen percentages could also not be identified through either ZooMS protocol (Table S11).

Ten bones with predicted collagen preservation of 5–10% were selected for dating, covering different species, with a focus on bones with anthropogenic modifications. They include seven reindeer bones with anthropogenic modifications and three fragments from other species (two Equidae and

one Elephantidae) with no modifications (Table 8). For five of the bones spatial coordinates were available and the other five were labelled with their excavation area (Supporting Information Table S12). They cover a vertical distribution of at least 45 cm (76.38–76.83 m) and in line with the overall finds distribution, they all originate from areas A and B (Fig. 1) and mainly from between 7–10 m NW and 2–4 m NE.

The collagen preservation of the 10 analysed bones (6.7–17.5%) was excellent for specimens of this age range, falling well above the ~1% minimum requirement for dating and all C%, N% and C:N ratios (3.0–3.4) fall within the range of modern bones, indicating their high level of preservation and suitability for dating (van Klinken, 1999; Talamo *et al.*, 2021a; see Table 8). The NIR prescreening accurately predicted the collagen content for seven out of the 10 dated bones. In the remaining three cases, the bones had exceptionally high collagen yields (12.6–17.5%) and the NIR screening under-estimated the level of collagen preserved (predicted values 7.8–9.9  $\pm$  3%). As these bones yielded more than enough material for dating following the pretreatment and overall the NIR screening was 100% successful in identifying bones which were sufficiently preserved for dating.

Four specimens returned infinite dates (>56 000  $^{14}\text{C}$  a BP) and the six others other returned finite ages between 51 120  $\pm$  1580 and 56 130  $\pm$  2930  $^{14}\text{C}$  a BP, which fall right at the detectable limit of the  $^{14}\text{C}$  method and beyond the IntCal20 (Reimer *et al.*, 2020) calibration curve (see Table 9). These new  $^{14}\text{C}$  dates demonstrate that the Neanderthal occupation at Salzgitter-Lebenstedt took place before 51 000 cal a BP. These dates are older than previously obtained radiocarbon dates on peat and humic silts (Table 2) and could relate to various interstadials between MIS-5a and MIS-3 (see Discussion).

## Discussion

This study represents the first large-scale, untargeted proteomic screening of an open-air Palaeolithic site. Our focus on the 1977 unidentifiable remains complements existing zooarchaeological studies from Salzgitter-Lebenstedt, which have analysed the 1952 reindeer remains (Gaudzinski and Roebroeks, 2000), and the combined set of 1952 and 1977 identifiable fauna (Staesche, 2017). In this section, we will use our Salzgitter-Lebenstedt data to explore the added value of this type of in-depth analysis of fragmented bone from Pleistocene faunal archives, in terms of both faunal accumulation dynamics and subsistence practices.

### Faunal accumulation dynamics

Animal carcasses, as well as stone artefacts and bone tools made by Neanderthals, accumulated on a flood plain setting at Salzgitter-Lebenstedt during an interstadial more than 51 000 years ago. This chronological position is established by 10 radiocarbon-dated bones which were pre-selected for good collagen preservation by NIR screening. The added value of fast, non-destructive NIR pre-screening has also been

**Table 6.** Bone weathering of the bone fragments sampled for ZooMS across the different spatial zones (A–D) at Salzgitter-Lebenstedt. The division in sections A–D follows the methodology of Pastoors (2001). Weathering data modified from Behrensmeyer (1978); see text for categories.

	A		B		C		D	
Lightly weathered	208	45.02%	124	49.21%	3	15.00%	1	16.67%
Medium weathered	228	49.35%	115	45.63%	12	60.00%	3	50.00%
Heavily weathered	26	5.63%	13	5.16%	5	25.00%	2	33.33%
<b>Total</b>	<b>462</b>	<b>100.00%</b>	<b>252</b>	<b>100.00%</b>	<b>20</b>	<b>100.00%</b>	<b>6</b>	<b>100.00%</b>

**Table 7.** Spatial distribution across spatial sections (A–D) across Salzgitter-Lebenstedt of the unidentifiable bone fragments smaller than 25 mm, fragments with traces of burning, and fragments with carnivore and/or human surface modifications.

Zone	Smaller than 25 mm		Burnt		Surface modifications			
					Carnivore		Human	
A	239	58.29%	102	53.40%	5	62.50%	85	59.03%
B	144	35.12%	83	43.46%	2	25.00%	54	37.50%
C	12	2.93%	2	1.05%			2	1.39%
D	2	0.49%	0	0.00%				
Unknown	13	3.17%	4	2.09%	1	12.50%	3	2.08%
	410	100.00%	191	100.00%	8	100.00%	144	100.00%

**Table 8.** Sample information obtained from radiocarbon-dated bones from Salzgitter-Lebenstedt, including the predicted collagen percentages obtained through near-infrared spectroscopy (NIR).

Finds ID	R-EVA	NIR collagen percentage ( $\pm 3\%$ )	Sample (mg)	Collagen (mg)	Collagen percentage	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	C%	N%	C:N
305	3621	5.1	348	26.7	7.7	-19.7	1.2	48.2	16.5	3.4
315/1	3626	9	397.3	50	12.6	-21.3	2.5	45.2	16.4	3.2
6483/1	3627	7.8	456	79.6	17.5	-22.0	5.7	46.0	16.5	3.3
260	3629	5.4	335.8	24.1	7.2	-19.1	1.5	42.2	15.9	3.1
1711/9	3681	5.5	418.6	30.4	7.3	-19.1	2.0	43.8	16.0	3.2
5636	3682	4.8	460.9	31.1	6.7	-18.8	1.8	44.4	15.7	3.3
6491/2	3683	6.1	346.2	25.5	7.4	-19.5	1.6	41.1	15.2	3.1
5422	3684	9.9	436.6	62.9	14.4	-21.1	2.4	46.0	16.7	3.2
1723/1	3685	6.2	371.5	28.9	7.8	-19.5	1.8	42.3	16.2	3.0
1717/3	3686	4.9	315.1	22	7.0	-19.5	1.7	43.3	15.4	3.3

**Table 9.** Radiocarbon dates obtained from bone collagen from Salzgitter-Lebenstedt alongside their ZooMS taxonomic identifications.  $^{14}\text{C}$  dates were calibrated in OxCal 4.4 (Bronk Ramsey, 2009) but all extend beyond the range of the IntCal20 calibration curve (Reimer *et al.*, 2020). Infinite ages are given where  $F^{14}\text{C} < 2\sigma$ , according to convention (Van der Plicht and Hogg, 2006).

Finds ID	ZooMS ID	ZooMS taxon	Lab number (ETH)	$F^{14}\text{C}$	Error	$^{14}\text{C}$ a BP	Error	68.3% cal a BP		95.4% cal a BP	
								from	to	from	to
305	SL-004	Reindeer	115247	0.0017	0.0003	51 120	1580	...	52 570	...	51 620
315/1	SL-026	Equidae	115248	0.0011	0.0003	55 010	2570	57 570	52 630	61 960	50 980
6483/1	SL-166	Elephantidae	115249	0.0000	0.0003	>58 000			Beyond calibration curve		
260	SL-59	Reindeer	115250	0.0012	0.0003	54 270	2330	...	54 700	...	54 960
1711/9	SL-249	Reindeer	115251	0.0002	0.0003	>57 000			Beyond calibration curve		
5636	SL-522	Reindeer	115252	0.0014	0.0003	52 800	1950	...	52 770	...	54 950
6491/2	SL-302	Reindeer	115253	0.0017	0.0003	51 240	1610	...	52 570	...	51 620
5422	SL-535	Equidae	115254	0.0002	0.0003	>56 000			Beyond calibration curve		
1723/1	SL-361	Reindeer	115255	0.0001	0.0003	>58 000			Beyond calibration curve		
1717/3	SL-403	Reindeer	115256	0.0009	0.0003	56 130	2930	60 220	53 730	66 970	51 920

illustrated at other Palaeolithic sites (Fewlass *et al.*, 2019b; Talamo *et al.*, 2021b) and is an effective tool to apply at sites where collagen preservation is variable (e.g. Ruebens *et al.*, 2022).

A more precise chronological assignment to an interstadial >51 000 years ago, most likely either the Oerel or Glinde interstadial, remains currently not possible. Moreover, it remains debated whether the Oerel (Behre and van der Plicht, 1992) should be placed at the transition of MIS-5a to MIS-4 [Dansgaard/Oeschger (D/O) events 20 and 19, c. 70 ka] or at the end of MIS-4 (D/O events 17 and 16, 58–54k  $^{14}\text{C}$  a BP) (see discussion in Jörjs, 2004). Additional dating techniques [e.g. optically stimulated luminescence (OSL)] are required to provide further chronological resolution. This has been successfully applied to other open-air localities in Germany. For example, at Lichtenberg (Northern Germany), the early MIS-3 (57  $\pm$  6 ka) chronology has now been revised to an early

MIS-4 (71.3  $\pm$  7.3 ka) age through a newly established OSL chronology (Weiss *et al.*, 2022).

Assessing the spatial distribution of the faunal remains at Salzgitter-Lebenstedt remains difficult. The vast majority of the 1977 bones were recovered in the southern part of the site, where a gully occurs. In other areas of the site (1977 northern part and entire 1952 area) the small bone fraction was not systematically recovered through screening, making it difficult to directly assess differing accumulation dynamics across various areas of the 416-m<sup>2</sup> excavation area. While the lithics and bones generally have sharp edges and are not rounded by fluvial action, the presence of vertical (up to 1 m) and horizontal refits (up to 9 m) (Tode, 1982; Pastoors, 2001) indicates a degree of movement of the archaeological material through both fluvial reworking and cryoturbation.

Across the fragments we sampled for ZooMS, we did not identify additional carnivore remains and carnivore

modifications are generally scarce. Both human and carnivore surface modifications have been reported in varying quantities by different scholars (see Supporting Information Tables S1 and S2). For example, for the 1952 fauna a carnivore gnawing mark ratio from 20% up to 70% has been reported for various species, contrasting strongly with 1.7% for reindeer (Gaudzinski, 1999; Gaudzinski and Roebroeks, 2000, 2003). The unidentifiable fraction from the 1977 excavations indicates a low involvement of carnivores in this part of the site, a pattern that is repeated at other open-air Neanderthal localities (Gaudzinski, 1995, 1996, 2004; Smith, 2012; Gaudzinski-Windheuser *et al.*, 2014; Kindler *et al.*, 2014, 2020).

The remains of at least 86 reindeer individuals (potentially 140, when including the 1977 material and the antlers, Krönneck & Staesche, 2017) were recovered, alongside at least 17 mammoths, eight horses and three bovids (Gaudzinski and Roebroeks, 2000). It has been observed that bone preservation is dependent on sedimentary matrix, with fine-grained contexts providing excellent preservation and sandy deposits causing more weathering (Kleinschmidt, 1953; Roebroeks and Gaudzinski, 2000). Our taphonomic analysis and study of biomolecular preservation indicates varying bone preservation across all taxa. However, it also illustrates a larger amount of well-preserved reindeer remains, contrasting with an overall more weathered state of the mammoth remains. The presence of butchery marks, traces of burning and fresh breaks, combined with the scarcity of carnivore involvement, demonstrate a strong human involvement with the carcass accumulation at Salzgitter-Lebenstedt. However, it remains debated to what extent certain species (such as the rhinoceros and mammoth) died naturally at the site, rather than being hunted by Neanderthals (Gaudzinski, 1999, 2000).

### Neanderthal subsistence practices

Our ZooMS study has identified a lower number of taxa (NTAXA = 6) compared to the zooarchaeological analyses (NTAXA = 8). This is a reflection of the fact that NTAXA is generally correlated with sample size (ZooMS: 761 bones, Zooarch: >5000 bones) (Grayson, 1979; Lyman, 2015). In smaller faunal assemblages, ZooMS studies were often able to increase taxonomic richness (Welker *et al.*, 2015; Berto *et al.*, 2021; Ruebens *et al.*, 2022). It can also be hypothesized that open-air localities provide less potential for an increase in the number of identified taxa since this site type is used less by carnivores compared to caves and rock shelters (Dusseldorp, 2013; Morin *et al.*, 2016). Generally, carnivore dens contain a higher species diversity compared to human accumulations (Dusseldorp, 2013), and this seems to be confirmed through ZooMS. For example, at Pin Hole Cave (Derbyshire, UK) 18 different taxa, including eight carnivore species, were identified in a ZooMS sample of 782 bones (Buckley *et al.*, 2017). Conversely, at Salzgitter-Lebenstedt, carnivore involvement is lower and only six taxa were identified in a ZooMS sample of comparable size ( $n = 761$ ).

In terms of species abundance, the ZooMS proportions at Salzgitter-Lebenstedt closely match the morphologically identifiable fauna. This contrasts with other Palaeolithic ZooMS studies, in which abundance differences between the morphologically identifiable and unidentifiable fraction could be observed (e.g. Buckley *et al.*, 2017; Sinet-Mathiot *et al.*, 2019; Bouchard *et al.*, 2020; Ruebens *et al.*, 2022; Sinet-Mathiot *et al.*, 2023). ZooMS-identified specimens from Salzgitter illustrate comparable specimen lengths with no statistically significant difference between the different taxa, suggesting bone fragmentation cannot adequately account for variation in identification. This shows that at Salzgitter-Lebenstedt all main

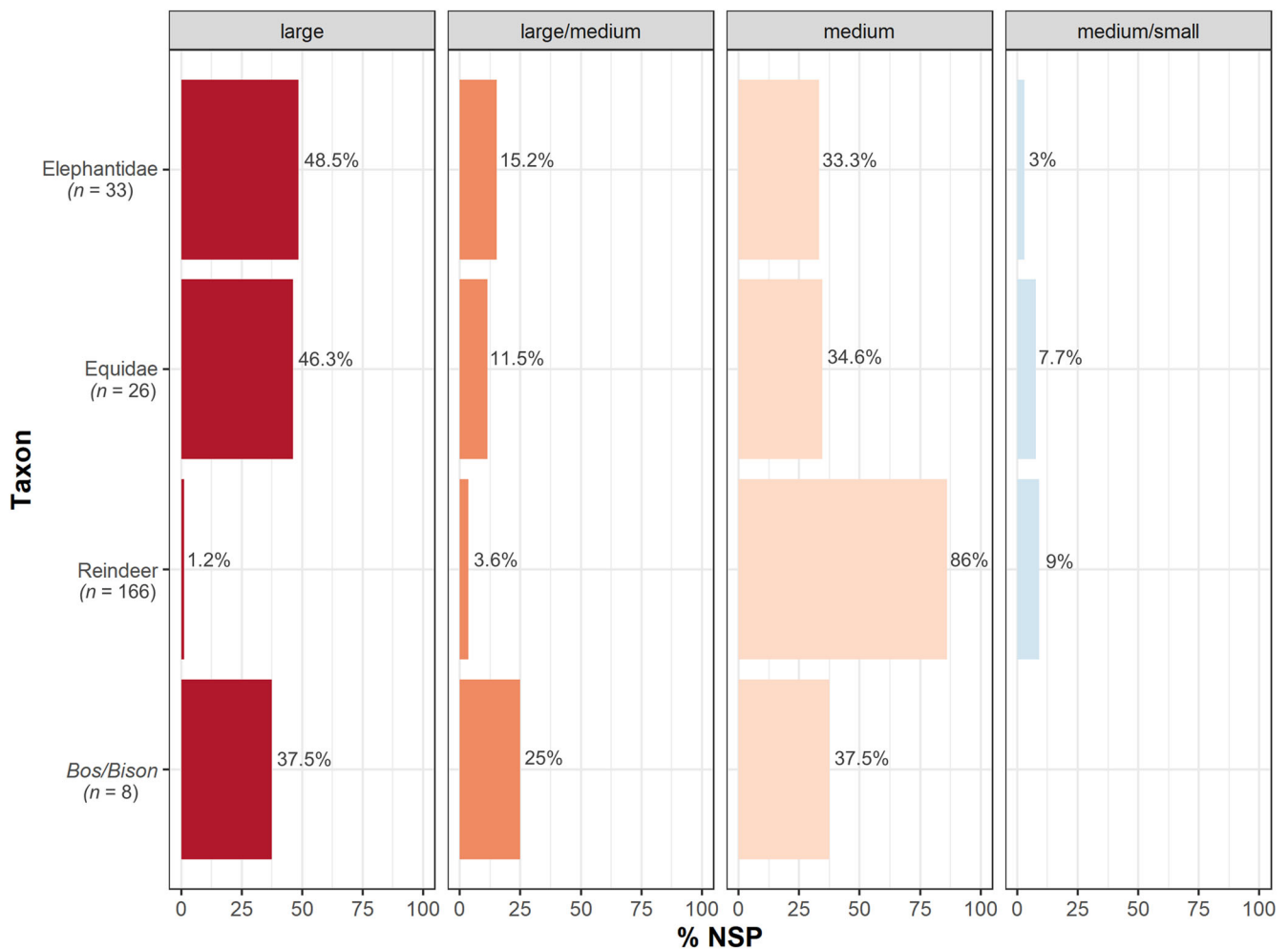
taxa (reindeer, mammoth, horse and *Bos/Bison*) were similarly fragmented, although the mechanisms underlying this fragmentation (natural process, human processing or carnivore fracturing) remain more difficult to reconstruct.

While detailed zooarchaeological and taphonomic analyses have stressed the systematic processing of large quantities of adult reindeer at Salzgitter-Lebenstedt (Gaudzinski and Roebroeks, 2000), based on the weight of the animal carcasses it has been suggested that reindeer were not the main food resource (Staesche, 1983). The latter is based on the presence of at least 17 mammoth individuals, as well as on the corresponding weight distribution of the unidentifiable bone fragments (Krönneck and Staesche, 2017). The unidentifiable fragments from both excavation campaigns were sorted into body size groups, ranging from small to large (based on cortical thickness and spongiosa composition, Krönneck and Staesche, 2017). While this is a common practice in zooarchaeological studies (Morin, 2012; Smith *et al.*, 2021), several ZooMS studies have illustrated such body size classes may not be an accurate predictor of taxonomic identity (Sinet-Mathiot *et al.*, 2019; Brown *et al.*, 2021c). Cross-checking the body size class assignments for the major taxa from Salzgitter, a similar variability between these body size classes and species identity was observed (Fig. 10). While the majority of the bone fragments were assigned to the correct body size class, specimens of all taxa were also placed in the wrong group, especially for the larger animals (Elephantidae, Equidae, *Bos/Bison*) (Fig. 10). For example, 33% of the remains identified through ZooMS as mammoth had been classified in the medium body size group, while only 1.2% of the reindeer remains had been classified as large (Fig. 10). This illustrates that on a coarse-grained level, this method can produce a reliable species composition, but it should not be used to make more detailed interpretations of individual specimens. Reconstructing the precise contribution of each species to the Neanderthal diet at Salzgitter remains complicated, but it is clear that a large number of reindeer were processed.

There are several examples of Middle Palaeolithic reindeer-dominated assemblages (Gaudzinski, 2006; Rendu *et al.*, 2012; Daujeard *et al.*, 2019), and they can be divided into two categories based on patterns of skeletal element representations. Faunal assemblages with many head elements (antlers and skulls) are interpreted as kill sites, while assemblages dominated by long bones are seen as base camps (Binford, 1980; Morin *et al.*, 2016). Salzgitter-Lebenstedt seems to represent a water source that attracted herds of reindeer, which were then killed at this location by Neanderthals (Gaudzinski and Roebroeks, 2000; Staesche, 2017). The fact that Neanderthals were extracting marrow at the site itself, combined with indications for the use of fire (small burnt bones), the *in situ* production of stone tools (applying Levallois and bifacial flaking; Pastors, 2001) and the presence of a series of bone tools made on mammoth long bones (Gaudzinski, 1998, 1999), suggests that the site was used, potentially repeatedly, for longer than just a short stop over.

### The added value of studying Pleistocene fragmented bone

Even though bone fragments 1–3 cm in length dominate the vast majority of Palaeolithic bone assemblages, they are only sporadically used to reconstruct patterns of site formation and hominin behaviour. They are often just counted or weighed, especially if retrieved through screening, and not included in further in-depth analyses. By combining a series of methodologies, including zooarchaeology, taphonomy, proteomics, NIR screening and radiocarbon dating, we illustrate the types of



**Figure 10.** Comparison of the ZooMS taxa with the body size class assignment (large relates to mammoth, rhinoceros, horse, bison and giant deer; medium relates to reindeer and wolf; see Krönneck and Staesche, 2017). [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1002/jqs.3499)]

information that can still be extracted from morphologically unidentifiable bone.

Zooarchaeological studies of these bone fragments, combined with ZooMS taxonomic identifications, can provide additional insights into patterns of skeletal element representation and carcass transport. While in this study we only recorded the specific skeletal part using general categories (e.g. long bone, flat bone, cranial, rib), to fully realize the potential of the ZooMS-identified fauna it is crucial to develop a method of element quantification using standard zooarchaeological metrics such as minimum number of elements (MNE), individuals (MNI) and anatomical units (MAU). Such an approach will allow for further in-depth integration with the morphological dataset in order to investigate aspects of carcass transport or selective destruction at Palaeolithic sites, and is of crucial importance for future integrated zooarchaeological analyses.

Detailed taphonomic studies of the unidentifiable bone fraction can further clarify patterns of movement of material at a site, especially when detailed spatial data are available (cfr. Discamps *et al.*, 2019). Combined with ZooMS taxonomic identifications, it can also help to provide a more detailed picture of carcass processing, as illustrated for example by the lack of cut marks on the ZooMS-identified mammoth remains at Salzgitter. Assessing the presence of bacterial bio-erosion through micro-computed tomography scanning is also an extra tool that can be used in future studies to assess if carcasses were buried fleshed (e.g. burial or natural death) or defleshed (e.g. butchered) (Brönnimann *et al.*, 2018; Smith *et al.*, 2022). Our study of the unidentifiable bones from Salzgitter also

illustrates the common presence of small bones with macroscopic traces of burning ( $n = 191$ ). The use of fire at Salzgitter has not been discussed in detail before (only two lithics are fire cracked, Pastoors, 2001) and provides an interesting avenue for further research (e.g. by applying Fourier-transform infrared spectroscopy to determine burning stages; Legan *et al.*, 2020; Gallo *et al.*, 2021).

In Pleistocene caves and rock shelters, ZooMS collagen fingerprinting usually has a high identification rate. The majority of sites have over 90% (Welker *et al.*, 2015; Sinet-Mathiot *et al.*, 2019; Berto *et al.*, 2021) or around 80% (Brown *et al.*, 2021c; Ruebens *et al.*, 2022) of the bones identifiable, with only a few examples of low success rates [e.g. 36.5% at the rock shelter of Riparo Bombrini (Italy; Bouchard *et al.*, 2020), and 26.4% at Vindija Cave (Croatia; Deviese *et al.*, 2017)]. Here we show that ZooMS, also in more challenging open-air settings, can be used to identify Palaeolithic bone fragments with high success rates. While it is clear that collagen preservation is generally worse in temperate settings, recent studies have also successfully used ZooMS on African Stone Age faunal material (Desmond *et al.*, 2018), illustrating the usefulness of ZooMS as a tool for taxonomic identification on bones from various contexts. In the future, additional proteomic sequencing methods [e.g. SPIN (Species by Proteome INvestigation); Rüter *et al.*, 2022] will also become available to provide more fine-grained taxonomic identifications (e.g. *Bos* or *Bison*) to allow further environmental and behavioural insights.

Finally, this study has illustrated further the added value of assessing bones for collagen preservation through NIR screening

at sites where collagen preservation is variable. At Salzgitter, this prescreening has led to a 100% success rate in radiocarbon dating and prevented bones with low collagen from being pretreated, saving time and resources. It also illustrates the potential to select and directly date ZooMS-identified small bone fragments, which is especially useful in cases where human bones are identified (as has been done at Bacho Kiro Cave, Fewlass *et al.*, 2020).

## Conclusion

This study illustrates how a stepped approach, combining detailed zooarchaeological and taphonomic attributes, ZooMS taxonomic identifications, glutamine deamidation values, NIR screening and radiocarbon dating, can provide additional insights into site formation and subsistence behaviour at Palaeolithic sites, including patterns of prey selection and carcass processing. As a consequence, we would like to stress the importance of screening and retention of even the smallest bone portions during excavations, as well as the ongoing curation of all components of existing faunal collections in museums.

Our study of the 1977 small bone fraction from Salzgitter-Lebenstedt complements existing zooarchaeological studies on the material and indicates the processing of mammoth, horse and bovid remains, alongside the intensive exploitation of large numbers of reindeer carcasses. Our data show that the mammoth remains are less well preserved compared to the reindeer and show a lower degree of cut marks, indicating potentially differential taphonomic histories for both these species. New dates indicate that the Neanderthal activities took place more than 51 000 years ago, potentially during the transition of MIS-5a to MIS-4 or at the end of MIS-4. The identified zooarchaeological and taphonomic patterns remain difficult to disentangle in terms of procurement method (hunting vs. scavenging), number (single or repeated) and duration of events (autumn-specific or year-round), but indicate both specialized behaviour (marrow extraction of reindeer) and behavioural versatility (processing of horse, bovid and mammoth carcasses) among late Neanderthal groups at Salzgitter-Lebenstedt.

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## Data availability statement

ZooMS spectra are available on Zenodo: <https://zenodo.org/record/7390279>. The R code used to generate the figures is available through the Open Science Framework: <https://osf.io/chtvj/>.

**Author contributions**—**Karen Ruebens:** Conceptualization; Investigation; Writing – original draft; Methodology; Writing – review & editing; Formal analysis. **Geoff M Smith:** Conceptualization; Investigation; Writing – original draft; Methodology; Writing – review & editing; Formal analysis. **Helen Fewlass:** Writing—original draft; Methodology; Writing – review & editing; Formal analysis. **Virginie Sinet-Mathiot:** Writing – original draft; Writing – review & editing; Methodology. **Jean-Jacques Hublin:** Funding acquisition; Supervision; Writing – review & editing. **Frido Welker:** Supervision; Conceptualization; Writing – original draft; Writing – review & editing; Methodology; Formal analysis.

## Supporting information

Additional supporting information can be found in the online version of this article.

Supporting information.

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