Analysis of 13C and 15N isotopes from Eurasian Quaternary fossils: Insights in diet, climate and ecology

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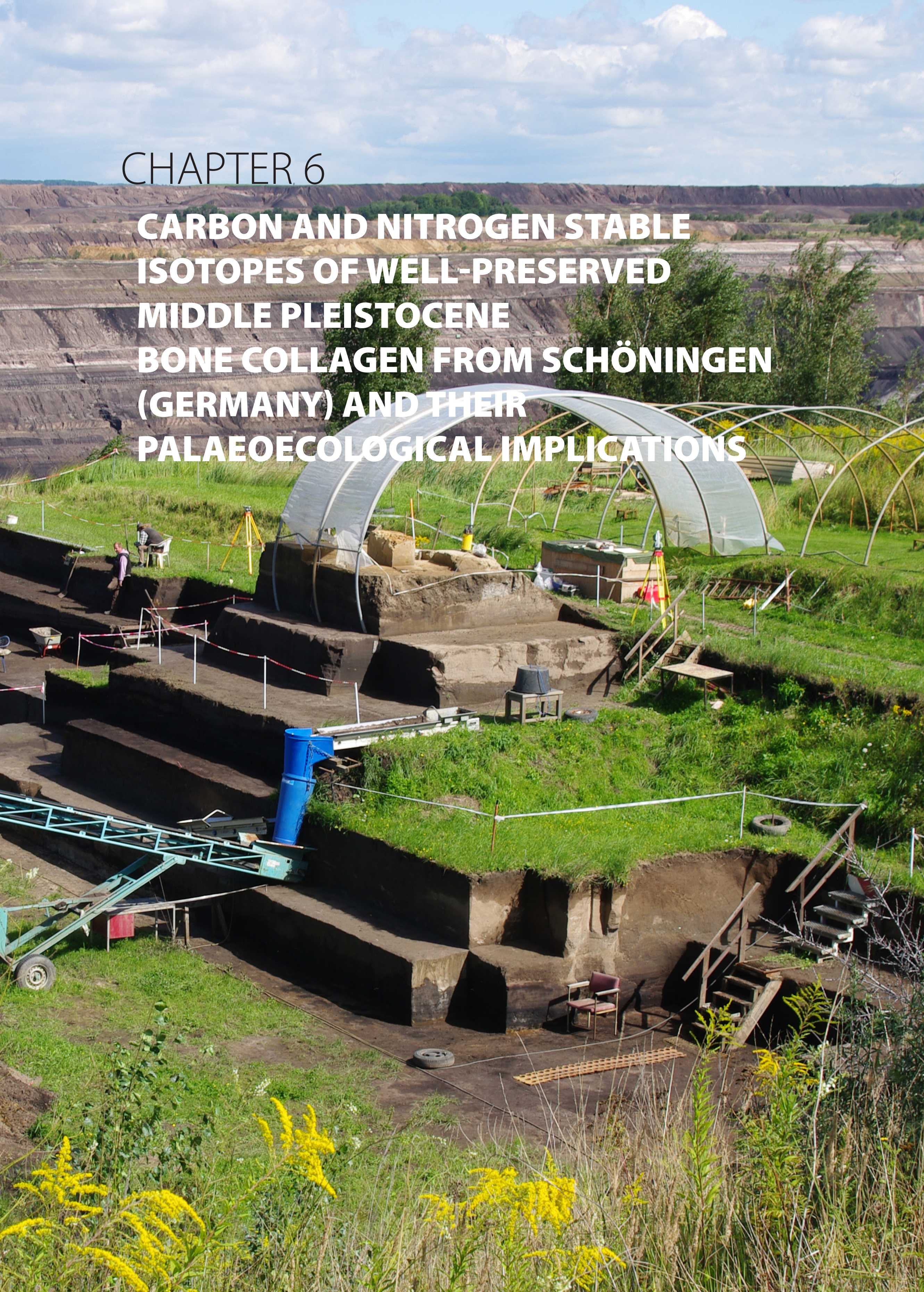
CHAPTER 6

CARBON AND NITROGEN STABLE ISOTOPES OF WELL-PRESERVED MIDDLE PLEISTOCENE BONE COLLAGEN FROM SCHÖNINGEN (GERMANY) AND THEIR PALAEOECOLOGICAL IMPLICATIONS



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6.1 Introduction

Most of the Middle Pleistocene fossils from Schöningen are macroscopically very well preserved, while the preservation at the microscopic level needed further investigation. To determine if the bone collagen was well preserved enough to retrieve reliable $\delta^{13}C$ and $\delta^{15}N$ data to decipher the environmental conditions hominins experienced at Schöningen, a pilot study was executed in 2009 at Leiden University. Based on the very promising results (van der Plicht *et al.*, 2011), further isotopic research on material from the site of Schöningen was conducted in a joint project with the University of Tübingen and published (Kuitems *et al.*, 2015a).

6.1.1 The Schöningen sites and the stratigraphic position of the different assemblages

The Quaternary deposits exposed in the lignite quarry east of the village of Schöningen (Fig. 6.1) during the past two decades, have yielded a large amount of Palaeolithic material, such as artefacts made out of flint and wood, stone and bone tools, and a large number of vertebrate skeletal remains. The exposed Quaternary deposits cover the late Middle Pleistocene up to the Holocene (Mania, 1995; Lang *et al.*, 2012). The Elsterian till forms the base of the Quaternary sedimentary sequence (Fig. 6.1C). On top of the Elsterian till lies a series of so-called channels (Mania, 1995; Thieme, 1999) (Fig. 6.1B). Channels I-III date from the period between the Elsterian and the Saalian (Drenthe) glaciations. The majority of the fossil material studied for this paper is from deposits of the second channel. Channel II contains five depositional levels of organic muds and peats with loess deposits on top, dating from the second half of the Reinsdorf Interglacial and the ensuing Fuhne cold stage. The Reinsdorf Interglacial is generally correlated with MIS 9 with an age of around 300,000 years (Lang *et al.*, 2012; van Kolfschoten, 2012, 2014). For more detailed information about the Reinsdorf sedimentary sequence the reader is referred to Urban *et al.* (2011) and Lang *et al.* (2012). Within Channel II, there are two spatially separated concentrations of sites: Schö 12 and Schö 13, each with a number of sites/depositional levels. The notation of the different sites and depositional levels (for example, Schö 13 II-4) refers to a specific geographical position in the investigated area (Schö 13), to a particular channel (II), and to a specific depositional level within the sedimentary sequence (4; see for example Serangeli *et al.*, 2012b). Most of the larger mammal material was collected from the Schö 13 II site, in particular Schö 13 II-4: more than 12,000 large mammal remains are recorded from the Schö 13 II-4 site representing a variety of species. The famous wooden spears are also from the Schö 13 II-4 site.

The botanical (Urban, 2007a,b) and malacological (Mania, 2007) record from the successive depositional levels in Channel II levels 1-5 indicate an environmental and/or climatic shift from an interglacial climatic optimum at the base of the sequence (level 1) with vegetation that is characterized by the occurrence of warm deciduous forest to the beginning of the following cold stage at the top of the sequence with a dominance of boreal forest and steppe vegetation (Urban, 2007a). However, the changes in the

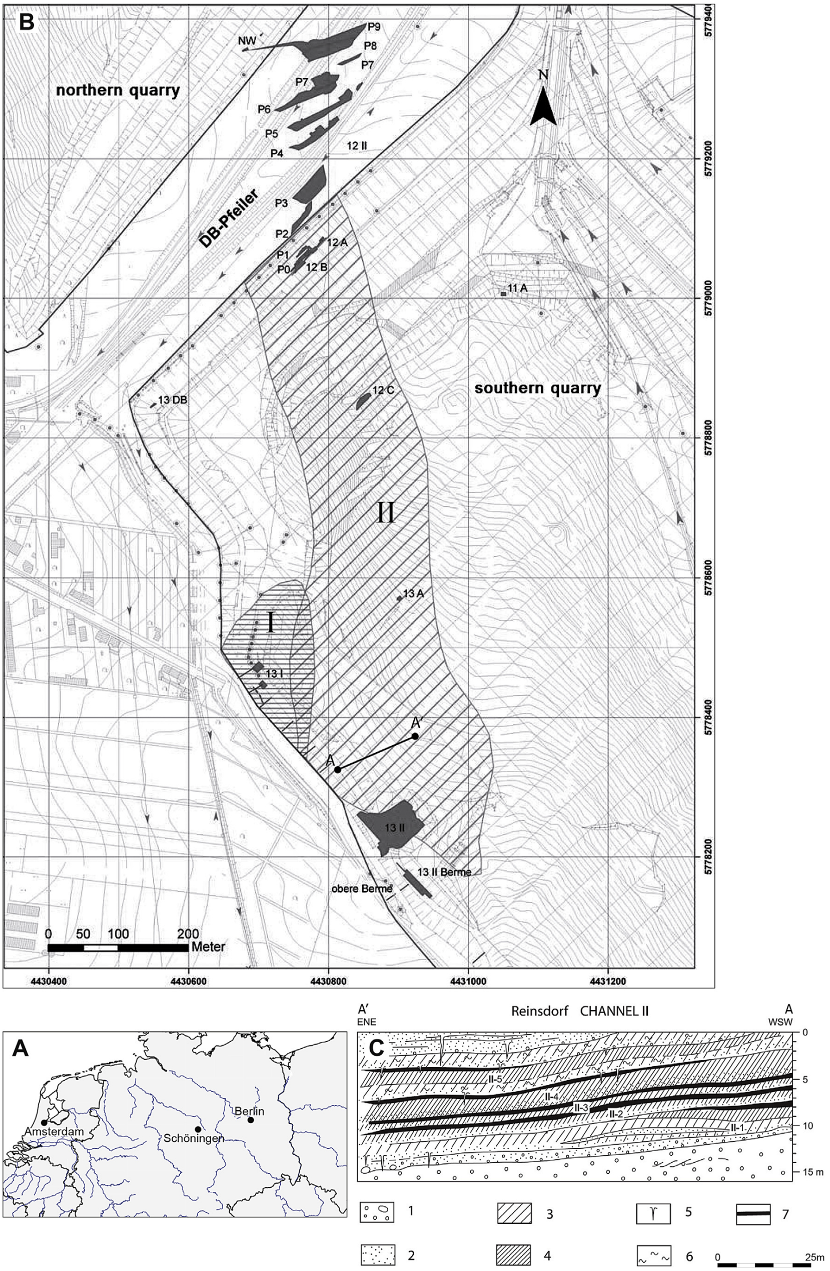

Figure 6.1 Origin of the samples discussed in this chapter. A: Geographical position of the locality Schöningen. B: Horizontal distribution of the sites mentioned in the text (after Serangeli *et al.*, 2012b: 14). C: General lithology of the Reinsdorf stratigraphic sequence, discordantly overlying Elsterian glacial sediments. Key: 1) Elsterian till, gravel and fluvioglacial sediments, 2) sand, 3) basin silt, 4) silty and calcareous mud, 5) ice wedges, 6) solifluction layers, 7) fen peat (Urban *et al.*, 2011: 130).

mammalian record are not as obvious as one would expect (van Kolfschoten, 2014). The Channel II sequence did not yield mammal assemblages that clearly indicate climatic deterioration and the occurrence of glacial conditions. Several assumed forest dwellers (for example *S. kirchbergensis*, *Sus scrofa*) that occur in Channel II level 1 are also represented in the faunal assemblage of Channel II level 4 (van Kolfschoten *et al.*, 2007; van Kolfschoten, 2014). The faunal assemblages from level 1 indicate interglacial conditions and a forested environment alternating with areas of more open, steppe vegetation, while the mammalian fauna from Schö 13 II-4 also suggests the occurrence of forested areas in a steppe landscape.

The environmental context of hominin activities at Schöningen is still a matter of debate, in particular the proportion of forest versus open landscape during the deposition of different levels in Schöningen. Therefore, the aim of this study was to investigate the ecological preference of the different herbivores represented in the Schöningen assemblages using stable isotope data. This isotopic study was preceded by a thorough examination of the preservation of the collagen from such an old context.

6.2 Materials and methods

6.2.1 Material

For this study skeletal material of 69 specimens including bones and antlers has been selected. The specimens are from Schöningen 12 and 13 and belonged to different taxa of large herbivorous mammals: Elephantidae, Rhinocerotidae, Equidae, Cervidae, and Bovidae (Table 6.1).

The Elephantidae were represented by eight samples of the straight-tusked elephant (*Palaeoloxodon antiquus*). Samples of Rhinocerotidae (n = 18) included Merck's rhinoceros (*Stephanorhinus kirchbergensis*), narrow-nosed rhinoceros (*S. hemitoechus*), and *Stephanorhinus* sp. Skeletal material of 20 horses (*Equus mosbachensis*) was sampled; 10

Table 6.1 Overview of the number of samples per taxonomic group and per site and layer/level.

	Level 1		**Level 2**		**Level 3**		**Level 4**		
	Schö 13 II-1	**Schö 12B**	**Schö 12 II-1**	**Schö 13 II-2**	**Schö 12A**	**Schö 13 II-3**	**Schö 13 II-4**	**Schö 13 II A**	
Elephantidae	4	1					1		6
Rhinocerotidae	1	4				1	1		7
Equidae	1	1			1	1	10	1	15
Cervidae	1	2	1	1		4	3		12
Bovidae				1		3	3		7
	7	8	1	2	1	9	18	1	47

bone samples originated from Schö 13 II-4. Among the 16 Cervidae samples, red deer (*Cervus elaphus*/cf. *C. elaphus*) is most frequent. Furthermore, four samples of giant deer (*Megaloceros giganteus*) were taken. The seven Bovidae samples were represented by bison (*Bison priscus*) and a number of samples that could not be determined as either aurochs or bison (hereafter designated as *Bos*/*Bison*).

6.2.2 Methods

Collagen extraction and isotopic analysis was undertaken, and the stable isotope concentrations were measured by IRMS at the CIO in Groningen following the procedure as described in Chapter 2 and at the Department of Geosciences of Tübingen University following the method published by Bocherens *et al.* (1997a).

All 'collagen' samples with atomic C:N ratios outside the widely accepted range of 2.9-3.6 were excluded from further analyses. Besides examining the C% and N% and the atomic C:N ratio with the aim of assessing the quality of the collagen, five representative samples were ^{14}C dated by AMS in order to confirm the antiquity of the collagen samples and, in particular, determine if the samples have been contaminated by geologically young material. The ^{14}C activities are reported in conventional activities ($^{14}a_N$), that is, they are measured relative to oxalic acid standard and corrected (normalised) for isotopic fractionation using the stable isotope ratio $^{13}C/^{12}C$ to $\delta^{13}C$ = -25‰, using a half-life value of 5568 years (Mook and van der Plicht, 1999). From the normalised activities, the conventional ^{14}C ages (in BP) are calculated. In the following, the normalised activities will be indicated by ^{14}a.

6.3 Results and discussion

6.3.1 Collagen preservation

A number of preliminary chemical composition determinations following the approach of Bocherens *et al.* (2005), measuring the percentage of nitrogen in whole bones, were performed and yielded values up to 2-3%, close to the 4% nitrogen found in fresh bones. Collagen was successfully extracted for 72% of the samples, a very high percentage given the geological age of the fossils. However, not all of these samples meet the collagen quality criteria and to determine which samples have good collagen and thus can be considered to produce reliable stable isotope ratios for ecological interpretation, the %C, %N, and C:N ratios were examined.

Figure 6.2 presents the carbon and nitrogen content of the extracted bone collagen and shows that 47 samples have %C between 31.1 and 47.0, %N between 10.2 and 16.3, and an atomic C:N ratio between 3.1 and 3.6. These 47 samples are considered to have good quality collagen, with collagen yield varying between 0.5% and 7.8% (mean = 2.9%). The collagen of the other three samples does not meet the quality criteria, and the stable isotope ratios of these samples are therefore disregarded in the final

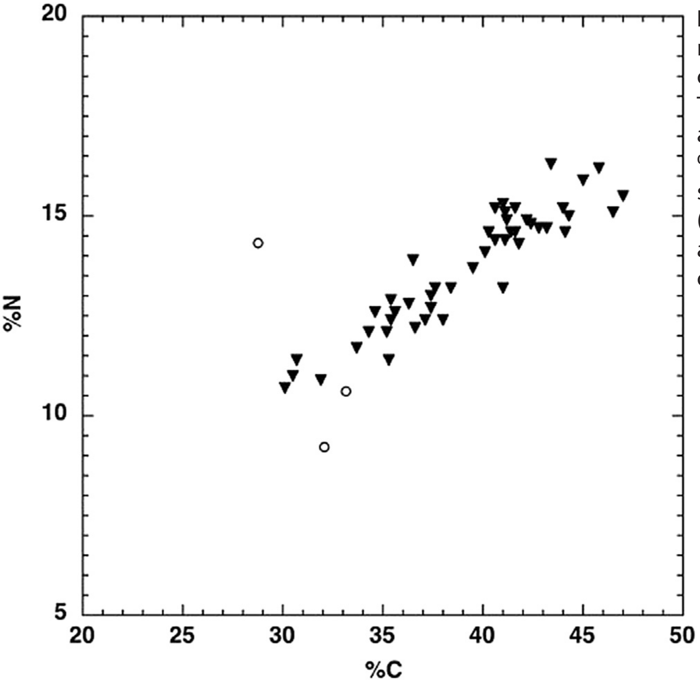

Figure 6.2 Carbon content relative to nitrogen content in the samples. The samples with both acceptable C:N ratios and %C and %N values are shown in black triangles (▼), while rejected samples are represented by open circles (○).

palaeobiological interpretation. The final result is that 68% of the Schöningen samples yielded reliable collagen.

The ^{14}C activities measured are shown in Table 6.2. The background for this batch showed an activity of ^{14}a = 0.20%, corresponding with a ^{14}C age of > 50,000 yr BP. Based on laboratory experience, the background for fossil bone is determined as 45,000 yr BP. There is one sample (GrA-49107) showing a finite age of 47,000 yr BP when using anthracite as a background; we report this age as > 45,000 yr BP. It appears impossible to have proper blanks (that is, infinite age and the same quality or degradation properties as the bone sample) for fossil bone of this age. All results are at the ^{14}C background level, which means they are reported as older than 45,000 yr BP (van der Plicht and Palstra, 2016). This convincingly shows that the Schöningen bones are not contaminated with younger carbon.

Table 6.2 Results of ^{14}C dating.

species	site layer	laboratory nr	^{14}a (%)	^{14}C age (yr BP)
Palaeoloxodon antiquus	Schö 12B	GrA-49111	0.20 ± 0.03	>45,000
Palaeoloxodon antiquus	Schö 13 II-4	GrA-49249	0.00 ± 0.02	>45,000
Stephanorhinus kirchbergensis	Schö 12B	GrA-49107	0.27 ± 0.03	>45,000
Equus mosbachensis	Schö 13A	GrA-49110	0.03 ± 0.03	>45,000
Cervus elaphus	Schö 13 II-3	GrA-49112	-0.02 ± 0.02	>45,000

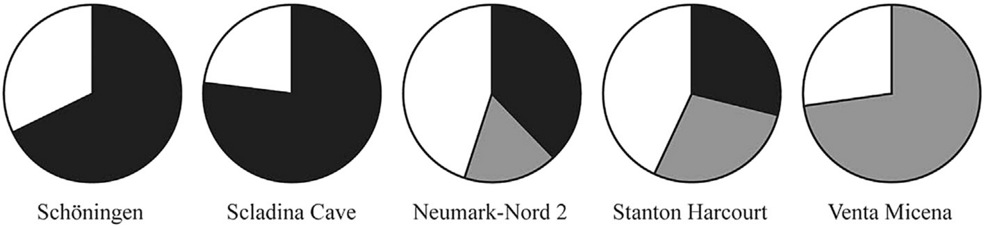

Figure 6.3 Proportions of extracted collagen in bones older than 50,000 yr in different archaeological sites. Reliable collagen samples (collagen with %C and %N values that are within the range of fresh collagen) are shown in black. Collagen samples classified as reliable in other studies but that are or may be outside the range of fresh collagen are shown in grey. Samples that did not yield collagen are shown in white.

The occurrence of good quality collagen in bones that are older than 50,000 years is remarkable but not unique. Stable isotope data have also been recorded from archaeological sites such as Scladina Cave in Belgium (ca. 120,000 years old, Eemian interglacial [MIS 5e], n = 36/47 [77%]: Bocherens *et al.*, 1999), Stanton Harcourt in southern Britain (ca. 200,000 years old [MIS 7], n = 4/7 [57%]: Jones *et al.*, 2001), Neumark-Nord 2 in Germany (ca. 120,000 years old, Eemian interglacial [MIS 5e], n = 23/42 [55%]: Britton *et al.*, 2012), and Venta Micena in southeastern Spain (ca. 1.500,000 years old, n = 77/105 [73%]: Palmqvist *et al.*, 2003, 2008).

The quality of the collagen of skeletal remains from Stanton Harcourt was estimated by analysing the amino acid profiles of the mammoth and elephant remains and by considering the atomic C:N ratio. However, two out of four samples that have been considered to yield qualitatively good collagen had low %C and %N values (*B. priscus*: %C = 23.2 and %N = 8.5; *P. antiquus*: %C = 23.9 and %N = 8.2). For Neumark-Nord, Britton and colleagues (2012) also included samples (n = 7) in their isotope analysis with collagen having low %C (12.6-27.0) and low %N (4.2-9.5), values that are outside of the range of values that are considered in the present study to correspond to good quality collagen. For Venta Micena, Palmqvist and colleagues (2003) performed amino acid analysis on a small number of samples (n = 4) and only considered samples with C:N ratios between 2.9 and 3.6 to constitute good quality collagen. However, the authors did not report the %C and %N for the samples. For the analyses of the Schöningen data we restrict ourselves to collagen with %C and %N values that are within the range of fresh collagen. Despite the fact that such restrictive conditions are applied, quite a high proportion (68%; Fig. 6.3) of the Schöningen fossils fulfil those criteria.

The data indicate that a large part of the Schöningen vertebrate remains is well preserved, not only at a macroscopic level but also at a molecular level. An explanation for the excellent conservation could be the high groundwater level (Lang *et al.*, 2012; Serangeli *et al.*, 2012a). Until very recently the groundwater table at many Schöningen sites was above the find horizons, and the fossil remains were located in waterlogged sediments. This buffered depositional setting offered favourable conditions for the preservation of faunal and botanical remains (Hedges and Millard, 1995; Bocherens *et al.*, 1997b; Prummel and Niekus, 2011; Lang *et al.*, 2012; Serangeli *et al.*, 2012a). Furthermore, the ground water that partly originates from springs in the Elm ridge is rich in calcium carbonate (Huckriede, 1967; Lang *et al.*, 2015), which also has a positive effect on the preservation of organic material.

6.3.2 Stable isotopic values

The results of the stable isotope measurements are shown in Table 6.3. The data set of good quality collagen samples consists of 47 fossil bones (the black down pointing triangle data points in Fig. 6.2), representing different mammal species and different sites and/or stratigraphical levels. More than a third of these samples are derived from the famous spear horizon (Schö 13 II-4, n = 18). In Table 6.3 the samples that have not been accepted (the open circles in Fig. 6.2) are eliminated.

The $\delta^{15}N$ values from all collagen samples ranged from +2.5‰ to +8.7‰, with an average of +4.8‰ and a standard deviation of 1.6. The $\delta^{13}C$ values from all collagen samples ranged from -23.6‰ to -19.8‰, with an average of -21.4‰ and a standard deviation of 0.9. Although the $\delta^{13}C$ and $\delta^{15}N$ ranges of several mammal species show some overlap, certain trends among species are visible. For example, the $\delta^{13}C$ values of Elephantidae (-22.9‰ to -21.4‰) are all lower than those of Bovidae (-21.2‰ to -19.8‰), whereas the $\delta^{15}N$ values of Elephantidae (+6.6‰ to +8.7‰) exceed the $\delta^{15}N$ values of all other analysed herbivores, except for some Bovidae (+3.6‰ to +7.1‰). The significance of the differences observed in $\delta^{13}C$ and $\delta^{15}N$ values between the families was determined with the Mann-Whitney U test, where H0 = not significantly different and H1 = significantly different (Shennan, 1997). As shown in Table 6.4, the $\delta^{13}C$ values were significantly different for Elephantidae compared to Bovidae, Elephantidae compared to Cervidae, Equidae compared to Cervidae, and Equidae compared to Bovidae. Between all families, the $\delta^{15}N$ values were significantly different, except for these of Rhinocerotidae compared to Equidae, and Rhinocerotidae compared to Bovidae.

The data show interesting patterns in respect to the environmental conditions (forest versus open landscape) experienced by hominins at Schöningen. In Fig. 6.4, the $\delta^{15}N$ and $\delta^{13}C$ values, respectively, are shown for the different taxonomic families during depositional level 1 (indicated with black circles; samples derived from Schö 12B, Schö 12 II-1, and Schö 13 II-1) and depositional level 4 (indicated with open circles; samples derived from Schö 13 II A and Schö 13 II-4). Based on, for example, botanical and malacological data, depositional level 1 is assumed to represent the Reinsdorf Interglacial climatic optimum during which the environment was quite closed with forest dominated by oak, ash, and linden, whereas depositional level 4 is characterized by a boreal continental climate and an open steppe-like landscape with some pine, spruce, larch, and birch (Urban, 2007b). As Fig. 6.4 shows, no divergent pattern in $\delta^{15}N$ values exists between the two depositional levels. The $\delta^{15}N$ values of rhinoceroses, horses, and cervids seem to alternate with each other in the different periods. The highest $\delta^{15}N$

Table 6.3 Stable isotope ratios $\delta^{13}C$ and $\delta^{15}N$ for fossil bones from Schöningen with accepted collagen and rejected collagen (crossed out).

species	layer	material	%C	%N	C:N	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Elephantidae							
Palaeoloxodon antiquus	Schö 12 B	bone	40.6	14.4	3.3	-22.0	7.3
Palaeoloxodon antiquus	Schö 13 II-1	bone	36.6	12.2	3.5	-22.4	7.8
Palaeoloxodon antiquus	Schö 13 II-1	bone	30.1	10.7	3.3	-22.9	6.6
Palaeoloxodon antiquus	Schö 13 II-1	bone	35.3	11.4	3.6	-22.5	7.0
Palaeoloxodon antiquus	Schö 13 II-1	bone	37.5	13.2	3.3	-21.8	7.2
Palaeoloxodon antiquus	Schö 13 II-4	bone	41.6	15.2	3.2	-21.4	8.7
					mean	-22.2	7.4
					st dev	0.5	0.7
Rhinocerotidae							
Stephanorhinus kirchbergensis	Schö 12 B	molar root	43.2	14.7	3.4	-22.1	6.4
Stephanorhinus kirchbergensis	Schö 12 B	molar root	40.6	15.2	3.1	-22.4	5.7
cf. *Stephanorhinus kirchbergensis*	Schö 12 B	bone	36.3	12.8	3.3	-22.7	6.5
cf. *Stephanorhinus kirchbergensis*	Schö 12 B	bone	40.3	14.6	3.2	-22.9	3.7
Stephanorhinus species	Schö 13 II-1	bone	35.6	12.6	3.3	-23.6	3.4
Stephanorhinus species	Schö 13 II-3	bone	42.4	14.8	3.3	-20.4	3.1
Stephanorhinus species	Schö 13 II-4	bone	44.0	15.2	3.4	-20.4	3.8
~~*Stephanorhinus species*~~	~~Schö 13 II-3~~	~~bone~~	~~32.1~~	~~9.2~~	~~4.1~~	~~-21.2~~	~~4.3~~
~~*Stephanorhinus species*~~	~~Schö 13 II-3~~	~~bone~~	~~33.2~~	~~10.6~~	~~3.7~~	~~-22.2~~	~~2.8~~
					mean	-22.1	4.7
					st dev	1.2	1.5
Equidae							
Equus mosbachensis	Schö 12 A	bone	38.4	13.2	3.4	-22.7	3.6
Equus mosbachensis	Schö 12 B	bone	34.3	12.1	3.3	-23.2	4.6
Equus mosbachensis	Schö 13 A	bone	37.1	12.4	3.5	-21.9	3.2
Equus mosbachensis	Schö 13 II-1	bone	35.4	12.4	3.3	-20.9	2.5
Equus mosbachensis	Schö 13 II-3	bone	33.7	11.7	3.4	-21.5	3.6
Equus mosbachensis	Schö 13 II-4	bone	46.5	15.1	3.6	-21.2	3.1
Equus mosbachensis	Schö 13 II-4	bone	44.3	15.0	3.4	-21.4	3.1
Equus mosbachensis	Schö 13 II-4	bone	44.1	14.6	3.5	-21.4	3.0
Equus mosbachensis	Schö 13 II-4	bone	34.6	12.6	3.2	-21.6	4.2
Equus mosbachensis	Schö 13 II-4	bone	47.0	15.5	3.5	-21.9	2.7

Equus mosbachensis	Schö 13 II-4	bone	41.1	15.1	3.2	-21.1	2.8
Equus mosbachensis	Schö 13 II-4	bone	45.8	16.2	3.3	-22.1	2.8
Equus mosbachensis	Schö 13 II-4	bone	41.0	13.2	3.6	-20.0	4.5
Equus mosbachensis	Schö 13 II-4	bone	37.4	13.0	3.4	-21.8	3.2
Equus mosbachensis	Schö 13 II-4	bone	39.5	13.7	3.4	-21.4	2.9
~~*Equus mosbachensis*~~	~~Schö 13 B~~	~~bone~~	~~28.8~~	~~14.3~~	~~2.3~~	~~-25.7~~	~~3.7~~
					mean	-21.6	3.3
					st dev	0.8	0.7
Cervidae							
Cervus elaphus	Schö 12 B	antler	30.5	11.0	3.2	-23.0	4.8
Cervus elaphus	Schö 12 B	bone	36.5	13.9	3.1	-20.7	3.9
Megaloceros giganteus	Schö 12 II-1	bone	42.2	14.9	3.3	-21.5	5.4
Cervus elaphus	Schö 13 II-1	antler	30.7	11.4	3.2	-20.5	3.5
Cervus elaphus	Schö 13 II-2	bone	38.0	12.4	3.6	-20.9	4.9
Cervus elaphus	Schö 13 II-3	antler	41.8	14.3	3.4	-20.1	5.2
Cervus elaphus	Schö 13 II-3	antler	42.8	14.7	3.4	-20.6	4.8
Cervus elaphus	Schö 13 II-3	antler	41.6	14.6	3.3	-20.5	4.9
Megaloceros giganteus	Schö 13 II-3	bone	37.4	12.7	3.4	-20.5	5.1
cf. *Cervus elaphus*	Schö 13 II-4	antler	35.4	12.9	3.2	-21.2	4.3
cf. *Cervus elaphus*	Schö 13 II-4	antler	35.2	12.1	3.4	-21.8	3.7
Megaloceros giganteus	Schö 13 II-4	molar root	31.9	10.9	3.4	-20.6	5.5
					mean	-21.0	4.7
					st dev	0.8	0.7
Bovidae							
Bos/Bison	Schö 13 II-2	bone	45.0	15.9	3.3	-20.6	3.6
Bison species	Schö 13 II-3	bone	40.1	14.1	3.3	-20.4	6.5
Bos/Bison	Schö 13 II-3	bone	41.1	14.4	3.3	-19.8	6.6
Bos/Bison	Schö 13 II-3	bone	41.4	14.6	3.3	-20.2	6.4
Bos/Bison	Schö 13 II-4	bone	41.0	15.3	3.1	-21.1	5.5
Bos/Bison	Schö 13 II-4	bone	41.2	14.9	3.2	-20.9	5.2
Bison species	Schö 13 II-4	bone	43.4	16.3	3.1	-21.2	7.1
					mean	-20.6	5.8
					st dev	0.5	1.2

value (+8.7‰) was measured from the sample of a young straight-tusked elephant from level 4 and might reflect the effect of suckling of a not yet weaned individual, as has been observed in woolly mammoths (Metcalfe *et al.*, 2010; Bocherens *et al.*, 2013). The three bovids in Fig. 6.4 are all derived from level 4, and no samples were taken from Bovidae living during the Reinsdorf Interglacial optimum. The Rhinocerotidae and Elephantidae have low $\delta^{13}C$ values (-23.6‰ to -22.1‰ and -22.9‰ to -21.8‰, respectively) during the Reinsdorf Interglacial optimum and higher values in level 4 (-20.4‰ and -21.4‰, respectively; Fig. 6.4). Since only one measurement is available for each of these species from level Schö 13 II-4, a conclusion that we are dealing with a significant difference in $\delta^{13}C$ values between the two periods is not justified. The $\delta^{13}C$ values of the horses and cervids from level 1 overlap with those from level 4.

Since the $\delta^{15}N$ and $\delta^{13}C$ values in bone collagen are related to the food consumed by the animal, the variation in plant $\delta^{15}N$ and $\delta^{13}C$ values is reflected in the tissues of the herbivore eating the plants. For example, fungi and grasses/sedges have relatively higher $\delta^{15}N$ values than lichens, mosses, and shrubs/trees (see summary in Bocherens [2003] and Drucker *et al.* [2010]), and understory vegetation in dense forest usually has more negative $\delta^{13}C$ values (that is, the so-called 'canopy effect') than plants growing in open environments (for example, Drucker *et al.*, 2008). The fact that the $\delta^{15}N$ values do not seem to diverge between depositional level 1 and 4 points to a quite similar dietary plant preference for these mammal species during both periods. The $\delta^{13}C$ values indicate relative stability in the diet despite climatic change but also point to an environmental change. As shown in Fig. 6.4, seven samples have $\delta^{13}C$ values of -22.5‰ and lower, which might suggest that they reflect the canopy effect. This canopy effect is only seen in samples from level 1, the Reinsdorf Interglacial optimum. The $\delta^{13}C$ values thus indicate that the landscape was more densely forested during depositional level 1 and more open during level 4. However, the majority of samples from level 1 have $\delta^{13}C$ values between

Table 6.3 Results of Mann-Whitney U test for $\delta^{13}C$ and $\delta^{15}N$ values between the largest taxonomical families.

Families	$\delta^{13}C$	$\delta^{15}N$
Elephantidae-Rhinocerotidae	H0	H1
Elephantidae-Equidae	H0	H1
Elephantidae-Cervidae	H1	H1
Elephantidae-Bovidae	H1	H1
Rhinocerotidae-Equidae	H0	H1
Rhinocerotidae-Cervidae	H0	H0
Rhinocerotidae-Bovidae	H0	H0
Equidae-Cervidae	H1	H1
Equidae-Bovidae	H1	H1
Cervidae-Bovidae	H0	H1

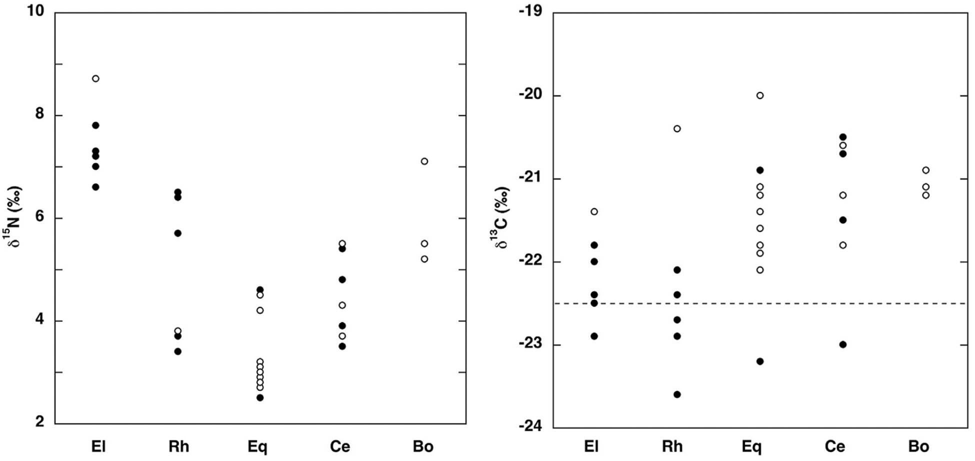

Figure 6.4 Left: δ^{15}N values for Elephantidae (El), Rhinocerotidae (Rh), Equidae (Eq), Cervidae (Ce), and Bovidae (Bo) for the Reinsdorf Interglacial optimum (closed circles) and the colder, fourth depositional phase (open circles). More grass in the diet leads to higher δ^{15}N values, while a browsing diet results in lower δ^{15}N values. Right: δ^{13}C values for Elephantidae (El), Rhinocerotidae (Rh), Equidae (Eq), Cervidae (Ce), and Bovidae (Bo) for the Reinsdorf Interglacial optimum (closed circles) and the colder, fourth depositional level (open circles). Below the dotted line (at -22.5‰), values are expected to reflect the canopy effect.

-22.4‰ and -20.5‰, indicating that these animals mainly fed in an environment that was not densely forested. Thus, the δ^{13}C values suggest that during level 1 at least part of the landscape was forested and alternated with a substantial part that was more open, whereas during level 4 the landscape was more open. However, although the δ^{13}C values of several species point to a relatively open landscape, these δ^{13}C values seem to be too low to reflect a steppe landscape similar to the mammoth steppe-tundra (Bocherens, 2003). This is supported by the zoological data, since remains of typical steppe dwellers such as ground squirrels and hamsters have so far not been recorded in the Schöningen mammalian record.

The isotope data suggest that the Schöningen landscape was inhabited by herbivorous species with different ecological niches, which is reflected, for example, in habitat choice (densely forested versus open landscape) and dietary preferences (browsing, grazing, and mixed feeding). The horses from Schöningen have the lowest δ^{15}N values of all species. The dental wear pattern observed in the horse molars diverges from that seen in horses from other periods and sites, with evidence that browsing formed a substantial part of the horse diet at Schöningen (see Rivals *et al.* [2015] for a microwear and mesowear analysis on the horse molars from Schö 13 II-4). The low δ^{15}N values also support a browsing diet for the Schöningen horses. This browsing role is remarkable, as horses are generally considered to be typical obligate grazers.

The δ^{15}N values of the straight-tusked elephants are remarkably higher than those of the other species, except for one bovid. Several studies (for example Bocherens *et al.*, 1994; Iacumin *et al.*, 2000) demonstrated that the extinct woolly mammoth (*Mammuthus primigenius*) had remarkably high $\delta^{15}N$ values in comparison with other contemporary living herbivores. The results of this study suggest a similar pattern for the stable nitrogen values of straight-tusked elephants from Schöningen. The driving force behind the discrepancy of $\delta^{15}N$ values between woolly mammoth and contemporaneous herbivores is not well understood but may be induced by dietary selection, physiology, or coprophagy (for more detailed information see Bocherens, 2003; Kuitems *et al.*, 2015c). These factors might also explain the elevation of the straight-tusked elephant $\delta^{15}N$ values in this study. In contrast to the woolly mammoth, the straight-tusked elephant is generally assumed to be a browser preferring wooded environments (Stewart, 2004; Stuart, 2005). Usually, browsers tend to have lower $\delta^{15}N$ values than grazers (and lower $\delta^{13}C$ values; for example, Drucker *et al.*, 2010). However, recent investigations of microwear patterns on the teeth of Pleistocene proboscideans showed that *Palaeoloxodon antiquus* exhibits variable dietary preferences similar to those of *Mammuthus primigenius* (Rivals *et al.*, 2012). Therefore, it seems possible that the high $\delta^{15}N$ values of the Schöningen elephants might be linked to a high proportion of ^{15}N-enriched grass in their diet.

Merck's rhinoceros (*Stephanorhinus kirchbergensis*) is also believed to have been a browser supplementing its diet by grazing, dwelling in both forest and open landscape (Loose, 1975). In contrast, the narrow-nosed rhinoceros (*S. hemitoechus*) was a true grazer (Loose, 1975). Fossils of both species have been found in Schöningen. Unfortunately, it was not possible to identify all skeletal elements in the sample to the species level. The rhinoceroses from Schöningen have quite a large range in $\delta^{15}N$ values. Within this spread, it looks as if two groups exist: four lower values (+3.1‰ to +3.8‰) and three values that are more enriched (+5.7‰ to +6.5‰). Of course, the number of data is limited and there is the risk of over interpretation. However, since the samples with a $\delta^{15}N$ value of +3.4‰ and +6.4‰ both belong to Merck's rhinoceros, it is unlikely that the difference in values should (only) be explained by grazing versus browsing. The $\delta^{13}C$ values of the Schöningen rhinoceroses diverge in another way than the $\delta^{15}N$ values, having five lower values (-23.6‰ to -22.1‰) and two (*Stephanorhinus* sp.) higher values of both -20.4‰.

The cervids have quite low $\delta^{15}N$ values. One should take into account, however, that seven out of the nine red deer samples have been derived from antler instead of bone. Since antlers re-grow every year, the isotope values in antler reflect a shorter time period than the isotope values in bone material (which reflect the mean isotope signal of several years). Based on stomach content analysis, the red deer is known to be a mixed feeder (Gebert and Verheyden-Tixier, 2001) able to incorporate both browsing and grazing in its diet. This is supported by the isotope data of this study. While the $\delta^{13}C$ value of one red deer (Schö 12B) is low and seems to reflect the canopy effect (-23.0‰, measured in antler), the eleven other values of red deer (n = 8) and giant deer remains (n = 3) from different sites/levels (including another red deer sample from Schö 12B: -20.7‰) are considerably higher (-21.8‰ to -20.1‰). Among the cervids, giant deer seem to have higher $\delta^{15}N$ values than most red deer. This could indicate that the diet of giant deer included grass in a higher proportion than that of red deer.

Six of the seven bovids (from Schö 13 II-3 and Schö 13 II-4) have both high $\delta^{15}N$ (+5.2‰ to +7.1‰) and $\delta^{13}C$ (-21.2‰ to -20.5‰) values compared with the other species. Both $\delta^{15}N$ and $\delta^{13}C$ values indicate a grazing diet in an open environment. One bovid (from Schö 13 II-2) has a $\delta^{15}N$ value of +3.6‰ and $\delta^{13}C$ value of -20.6‰ and may have supplemented its diet by browsing.

The results of the stable isotope investigations are an important contribution to the debate on the interpretation of the large concentration of fossil horse remains in Schö 13 II-4. It was hypothesized that the Schö 13 II-4 horse assemblage may be the result of a single event during which Palaeolithic hominins killed a group of horses. However, the large variation in the isotope values of the horse bones does not support this hypothesis. The ten horse samples from Schö 13 II-4, the famous spear horizon, have $\delta^{13}C$ values ranging from -22.1‰ to -20.0‰ and $\delta^{15}N$ values ranging from +2.7‰ to +4.5‰. These ranges of isotopic values seem too large for a homogenous population (Lovell *et al.*, 1986), indicating that more than one horse population is represented in the Schö 13 II-4 assemblage. This conclusion supports the suggestion that we are dealing with multiple events and that, in turn, suggests a scenario in which there is a large concentration of horse remains in a landscape with scattered vertebrate remains from a variety of large mammal species including horses (van Kolfschoten *et al.*, 2015a).

6.4 Conclusions

This chapter presents the study of remarkably well-preserved bone collagen of skeletal remains from Schöningen, Germany with an age of 300,000-400,000 years. Our investigation indicates that a large part of the vertebrate remains exhibit excellent preservation, not only at a macroscopic level but also at a molecular level. Stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) of material derived from five taxonomic groups (Elephantidae, Rhinocerotidae, Equidae, Cervidae, and Bovidae) have been measured. The isotopic data indicate that during the Reinsdorf Interglacial the hominins at Schöningen lived in a mosaic-like landscape: during the Reinsdorf Interglacial climatic optimum the landscape seems to have been forested, alternating with open areas, whereas during depositional level 4 the isotopic data give the impression that the landscape was more open, although not steppe-like. These mosaic landscapes were inhabited by herbivorous species with different ecological niches, which is reflected in habitat choice (forest versus open landscape) and diet (browsing, grazing, and mixed feeding). The stable isotope values of the horses from the spear horizon Schö 13 II-4 seem too variable to represent one homogenous population and suggest multiple accumulation events.