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

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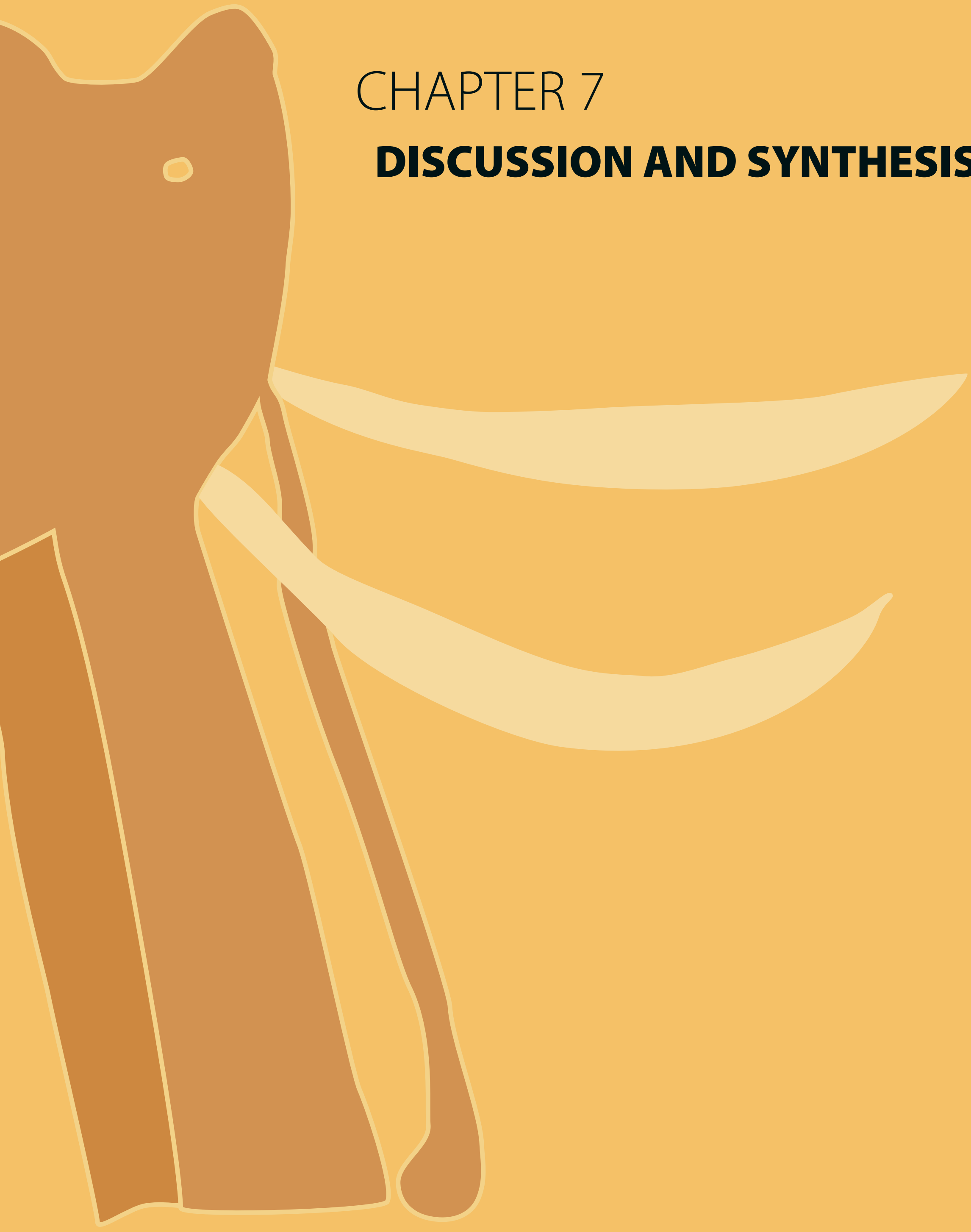
CHAPTER 7

DISCUSSION AND SYNTHESIS



The thesis presents research that is based on unique $\delta^{15}N$ and $\delta^{13}C$ datasets, often in combination with the ^{14}C age of the fossil remains. Unique not only because of the large amount of data, but also because of the species represented in the dataset, the age of the samples and their geographical origin.

These datasets, presented in a number of articles and discussed in chapters 3-6, consists predominantly of isotope data from mammalian fossils. These data give information about diet, environment and climate through time and space for numerous animal species: information that also indirectly gives valuable insights in the living circumstances of coeval living hominins.

Moreover, results raised new questions and data from the individual chapters require mutual comparison. The integrated data offer the possibility to discuss a number of intriguing topics as there are a) unexpected $\delta^{15}N$ results, b) difficulties using appropriate model species, and c) questions concerning the Late Quaternary megafaunal extinction. These topics will be discussed in this chapter.

7.1 High $\delta^{15}N$ values observed in proboscidean tissues

Throughout most of the Late Pleistocene, woolly mammoths (*M. primigenius*) have remarkably high bone collagen $\delta^{15}N$ values compared to other contemporaneous large herbivores. This phenomenon is not the result of a regional effect; a comparable pattern (that is, a discrepancy of roughly the same magnitude between the $\delta^{15}N$ values of mammoths and other coeval herbivores) manifests in Europe (Bocherens *et al.*, 1997a), Siberia (Bocherens *et al.*, 1996) and Alaska (Bocherens *et al.*, 1994; Bocherens, 2003). Some evidence has been found that $\delta^{15}N$ values of mammoths became more comparable to those of coeval living herbivores towards the end of their existence in certain locations, such the Ukraine (Drucker *et al.*, 2014). But in general, the phenomenon remains visible through time and space.

Different factors are suggested to have determined the relatively high $\delta^{15}N$ values in woolly mammoths, such as aspects related to (isotopic) diet composition, digestive physiology and feeding habits (Schoeller, 1999; Sponheimer *et al.*, 2003a; Kuitems *et al.*, 2015c). Diet-related aspects concern, for example, consumption of high amounts of ^{15}N-enriched plant parts and/or species (Bocherens, 2003; Metcalfe *et al.*, 2013). However, considering the bulk amounts of food a mammoth probably consumed on a daily basis to fulfil its nutritional needs, it seems more likely that the mammoth ate all appropriate and preferred plants that it came across rather than selecting specific plant parts. A suggested option is though that the mammoth's tusks would have served as a tool to get access to ^{15}N-enriched partly decayed vegetation under the snow, that would have been inaccessible for other inhabitants of the mammoth steppe biome (Schwartz-Narbonne *et al.*, 2015). However, the amount of snow in major parts of the area they inhabited was limited. Therefore, it is unlikely that the consumption of plants under the snow caused the widely observed pattern of high $\delta^{15}N$ values of woolly mammoths.

Investigations of for example intestinal remains recovered in the Siberian permafrost from woolly mammoth and other hindgut fermenters such as horse and rhinoceros, and the dental wear patterns of these species from Europe and Alaska (Rivals *et al.*, 2010) showed that these animals were feeding predominantly on grasses and other herbaceous plants and -in particular during winter- on some amounts of browse (Olivier, 1982; Vereshchagin and Baryshnikov, 1982; Guthrie, 1990; Ukraintseva, 1993; Putshkov, 2003; Mol *et al.*, 2006; van Geel *et al.*, 2008; Willerslev *et al.*, 2014). Based on these results, a difference in diet between Pleistocene horse, woolly mammoth and woolly rhinoceros does not seem to be the major cause for the discrepancy in $\delta^{15}N$ values between woolly mammoth and the other species. To some extent, however, variation in $\delta^{15}N$ values between ungulates sharing the mammoth steppe biotope must be explained by resource partitioning. As a general rule in ecology, the cohabitation of species leads to niche differentiation in order to avoid direct competition. Such differentiation is often well reflected in a bivariate diagram of inter-specific $\delta^{13}C$ and $\delta^{15}N$ values. Indeed, studies on dental wear patterns show that resource partitioning existed within the mammoth steppe. However, although for instance the amount of browse in the diet differed to some extent between species (Rivals *et al.*, 2010), such studies do not explain why the difference in δ^{15}N values between woolly mammoths and other hindgut fermenters is larger than the variation in δ^{15}N values between other hindgut fermenting species, nor why the δ^{15}N values of specifically woolly mammoths tend to be high.

The higher the protein throughput, the more the body needs to get rid of excess nitrogen, that is, by urea excretion. Due to fractionation, the isotopically lighter ^{14}N is favoured during excretion over the heavier ^{15}N, resulting in the retention of heavier ^{15}N in the body. Therefore, protein rich diets lead to higher δ^{15}N values in body tissues than nutrient poor diets (Ambrose, 1991; Sponheimer *et al.*, 2003a; Fuller *et al.*, 2005). Analysis of cementum from fossil mammoth molars suggests that woolly mammoths faced food deficiency on an annual basis (Guthrie, 1990; Wang *et al.*, 2018). In addition to the beforementioned protein-rich diets, protein shortage has been found to correlate with enrichment of ^{15}N in body tissues (Sealy *et al.*, 1987; Hobson *et al.*, 1993; Adams and Sterner, 2000). However, it seems unlikely that temporal enrichment of ^{15}N in body tissues alone is 'strong' enough to have caused the observed high δ^{15}N values in bone collagen, which has a turnover time of years. Moreover, the mammoth has been a successful species for a time range of hundred-thousands of years. Therefore, a significant part of the population must have had access to sufficient amounts of nutrients on a yearly basis, in order to reproduce and to maintain the population's fitness.

Woolly mammoths may have had specific (metabolic) adaptations to compensate for low digestion efficiency (Kuitems *et al.*, 2015c), or to cope with periods of winter starvation (Bocherens *et al.*, 1996). Results of investigations by van Geel *et al.* (2008, 2011) on frozen mammoth dung from Alaska (ca. 12.300 yr BP) and from intestinal remains from a mammoth carcass from Yakutia (ca. 18.500 ^{14}C yr BP) reveal evidence for coprophagy, and even suggest that this might have been a routine practise amongst woolly mammoths. Dung must have contained considerable amounts of undigested food. Coprophagy may lead to slightly higher δ^{15}N values in body tissues, in particular if it enhances the digestive efficiency substantially (Ugan and Coltrain, 2011). Faecal consumption is observed amongst free ranging modern African elephants, but not as a common practice (van Geel, 2011).

The results of a stable isotope study by the author of nail samples, collected from modern herbivores, showed that the nails of captive elephants, rhinoceroses and horses have similar δ^{15}N values (Kuitems *et al.*, 2015c). These animals lived predominantly in Dutch zoos and never had to cope with food and water stress, competition for resources nor niche partitioning leading to dietary differentiation. Instead, the animals got all the nutritional components they need and all got roughly the same food. It is known that young elephants, living in the zoos where the nail samples came from, occasionally eat dung. It concerns mainly faeces from their mother but also from other elephants of their group. The analysed nail samples came from adult elephants that had never been observed eating any dung at an advanced age.

The obtained results indicate that aspects related to diet and physiology, as well as coprophagy are indeed possible explanations for differentiation in stable isotope values, but they do not explain the observed discrepancy of δ^{15}N values between Pleistocene herbivores. Probably, neither results of the analysis of nail keratin, nor of δ^{15}N values in bulk collagen will ever point to the specific discriminating factor(s) (McMahon and McCarthy, 2016), since equifinality (that is the principle that a specific end state - here the high δ^{15}N values - can be reached via many potential pathways) is inherent to stable isotope values of fossils from free-ranging animals in the past.

Analysis of the δ^{15}N composition of single compounds, that is, individual amino acids in collagen, may be an important and valuable addition to this discussion in future, since it allows discrimination between ^{15}N-enrichment in 'source' amino acids and 'trophic' amino acids (Schwartz-Narbonne *et al.*, 2015; McMahon and McCarthy, 2016). The idea is, that 'source' amino acids have nitrogen isotopic values that are closer related to those of same amino acid in the consumed food than 'trophic' amino acids, whose amino-nitrogen is part of a metabolic amino-nitrogen pool. A study of compound-specific isotopes of different Pleistocene large herbivore and carnivore species carried out by Schwartz-Narbonne *et al.* (2015) pointed towards a dietary (as opposed to a physiological) explanation for the high mammoth δ^{15}N values. However, more study is necessary to get precise insight into the complex cycling of single amino acids through the metabolic network, that is the underlying metabolic drivers for the observed nitrogen isotopic patterning in amino acids (McMahon and McCarthy, 2016; O'Connell, 2017). In support of a dietary explanation, and not a proboscidean-specific physiological adaptation, is the fact that mastodons (*Mammut americanum*) tend to have lower δ^{15}N values than woolly mammoths, which might be explained by a species-specific diet (Metcalfe *et al.*, 2013, 2016).

However, mastodons are not elephantids. Therefore, a physiological adaptation that is elephantid-specific, such as a possible (metabolic) adaptation to compensate for low digestion efficiency (Kuitems *et al.*, 2015c), remains a credible explanation for the high δ^{15}N values observed in woolly mammoths. Data from this thesis are even in favour of such an explanation. As shown in Chapter 4 and 6, samples from straight-tusked elephant (*Palaeoloxodon antiquus*) reveal that these older elephantids do yield higher δ^{15}N values than those of coeval living large herbivores; a similar pattern as observed for woolly mammoths.

In addition to the data presented in Chapter 6, stable isotopes of three omnivores and two carnivores from Schöningen have been measured (Table 7.1). These new data are interesting to compare with the herbivore data. The δ^{15}N values for straight-tusked elephants and/or woolly mammoths are shown in Fig. 7.1, relative to the δ^{15}N values of other terrestrial herbivores, omnivores and carnivores from the same region (specifically, North Sea and Schöningen). The figure shows that in both regions the δ^{15}N values of straight-tusked elephants are higher than those of most other, non-proboscidean herbivores, and more similar to those of carnivores. The range of δ^{15}N values of woolly

Table 7.1 Isotope results of omnivorous and carnivorous mammals from Schöningen.

species (Latin)	species (English)	layer	material	%C	%N	C:N	δ^{13}C (‰)	δ^{15}N (‰)
Canis lupus	wolf	Schö 13 II-4	bone	37.1	13.1	3.3	-20.1	8.7
Vulpus vulpus	fox	Schö 13 II-4	bone/dentine	34.7	12.3	3.3	-20.3	9.3
Ursus spelaeus	cave bear	Schö 12B	bone	41.4	14.8	3.3	-22.9	4.7
Sus scrofa	wild boar	Schö 12B	bone/dentine	38.8	13.7	3.3	-22.0	6.0
Sus scrofa	wild boar	Schö 12B	bone/dentine	40.9	14.5	3.3	-22.4	4.3

mammoths from the North Sea partly overlaps with the lowest values of the straight-tusked elephants and the highest values of the non-proboscidean herbivores.

In contrast to the woolly mammoth, the straight-tusked elephant is assumed to be a browser preferring wooded environments (Stuart, 2005). Recent tooth wear investigations on straight-tusked elephants from other Middle Pleistocene localities correlated with MIS 11 in Germany (Steinheim; Rivals and Ziegler, 2018) and in England (Clacton, Hoxne, and Swanscombe; Rivals and Lister, 2016) support that the straight-tusked elephant's diet consisted partly or predominantly -depending on the location- of browse (Rivals and Ziegler, 2018). In general, browsers tend to have lower δ^{15}N values than grazers (Ambrose, 1991, Drucker *et al.*, 2010). This, and the fact that straight-tusked elephants are believed to have inhabited quite humid and temperate environments, makes the high δ^{15}N values of straight-tusked elephant tissues even more intriguing

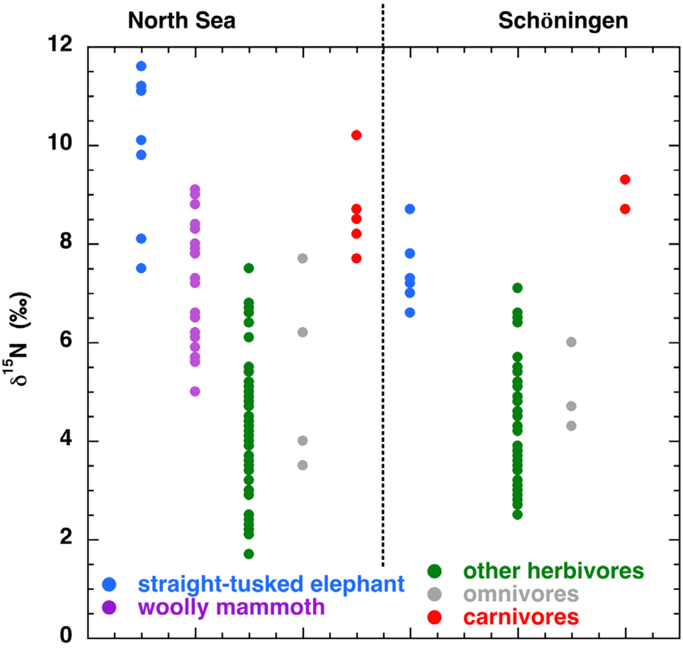


Figure 7.1 δ^{15}N values of straight-tusked elephants and woolly mammoths compared to δ^{15}N values of other terrestrial herbivores, omnivores and carnivores. Blue dots indicate δ^{15}N values of mammals from Schöningen; open circles indicate δ^{15}N values of mammals from the North Sea area.

than those of the woolly mammoth. These data make a family-wide physiological cause for the observed high $\delta^{15}N$ values more plausible and form a promising basis for further investigation and debate.

7.2 Looking for a model of the past

Ambrose and DeNiro (1986) showed that the $\delta^{15}N$ value in collagen of a free-ranging wild modern African elephant living in East Africa is higher than stable $\delta^{15}N$ values of other ungulates and almost as high as the $\delta^{15}N$ values of the carnivorous species in the same study. This result resembles the pattern observed in Pleistocene herbivores inhabiting the mammoth steppe environment, and was the incentive to use extant relatives of the woolly mammoth, the African elephant (*Loxodonta africana*) and in particular Asian elephant (*Elephas maximus*) in order to get information about the $\delta^{15}N$ values in modern elephantids living under controlled conditions in Kuitems *et al.* (2015c).

In addition to considering results in the context of what is known from other proxies, $\delta^{13}C$ and $\delta^{15}N$ values are practically always discussed relative to those from the same species in different times or areas, or to other species that live in the same area and period or share certain characteristics. Also, in order to gain insights into potential factors underlying specific isotopic compositions of extinct species, one needs to use modern 'model' species. The use of (modern) models is not confined to stable isotope studies, but is inherent to any study that attempts to make assumptions about what might have happened in the past. Appropriate model species share relevant characteristics with the 'target' species (that is, here, the species one attempts to decipher by information obtained from the model species).

However, choosing an appropriate model is not always straightforward and may sometimes even be problematic, as becomes also clear from various examples throughout the current thesis. The species chosen will usually meet a determined taxonomic equivalency, but as examples in the present thesis show, the best fitting model is not per se genetically the closest related. Also, a target species may have various model species, each of them being most suitable for a particular situation or purpose. The appropriateness certainly depends on the research question, but also on availability. Finally, comparison with model species can be informative, but care must be taken when generalizing from one species to another. For instance, a species in the past may have had characteristics that differ from what is generally assumed. In that case, the fundamental idea or 'baseline' which is used for comparison with the target species is already biased.

The choice of appropriate baselines becomes dramatically clear when using models in order to convert stable isotope values into estimates of the proportion of different food sources in a diet. The use of isotope mixing models for these purposes has taken off during the last decade. Although recently developed models allow more and more for a certain amount of uncertainties, using inappropriate priors and/or interpreting the outcomes as hard evidence, lead to erroneous conclusions (Caut *et al.*, 2008; Phillips *et al.*, 2014).

Controlled feeding studies proved to be valuable for interpreting stable isotope results and for understanding specific fractionation processes (Ambrose, 2000; Sponheimer *et al.*, 2003a; Nardoto *et al.*, 2006; O'Connell *et al.*, 2012), and would have been excellent to get insights in the high mammoth δ^{15}N values. Performing such a study with extinct animals would be impossible for obvious reasons. Also in other studies, physical or ecological aspects of modern elephants are compared to those of extinct proboscideans. Sometimes the authors make explicit why the comparison between extinct elephants and modern elephants is appropriate and relevant (see for example, Olivier, 1982; Valente, 1983; Marchant and Shoshani, 2007; Smith *et al.*, 2017), but other do not (e.g., Cammidge *et al.*, 2019). Recently, Meyer *et al.* (2017) discovered that the straight-tusked elephant's genome was closely to the genome of the extant African elephant than previously thought. Would this mean the African elephant would be, in a comparison study, an appropriate model for the straight-tusked elephant?

An example from this dissertation where the choice of a model was not obvious, can be found in Chapter 5, in which the isotope composition of Siberian unicorn (*Elasmotherium sibiricum*) samples have been discussed. This extinct kind of rhinoceros had enormous, continuously-growing cheek-teeth. This extreme form of hypselodonty is an adaptation to dietary components which lead to major dental abrasion. Its dental morphology and the angle of its neck and head indicate adaptation to feeding close to the ground or even consumption of underground plant parts such as plant bulbs and roots. Until recently, very little was known about this peculiar animal. Not only its morphology, but also its stable isotope values diverge from other Pleistocene rhinoceroses and many other large herbivores, including those from woolly rhinoceros and woolly mammoth from the same spatio-temporal range. A modern inhabitant of this region, which today is characterized by dry steppe environments and (semi) deserts, is the saiga antelope (*Saiga tatarica*). Also, Late Pleistocene fossil remains of the saiga antelope have been found in this region. Surprisingly, their δ^{13}C and δ^{15}N values overlap considerably with those of Siberian unicorn. This overlap could be interpreted as an indication of sharing (parts of) a dietary niche, but in contrast to the Siberian unicorn, saiga antelope is medium-sized, has different (digestive) physiology and is not specialised in eating underground plant parts. The relation between δ^{13}C values and the consumption of underground plant parts would be an interesting topic for further investigation.

An animal that does include underground plant parts in its diet is the wild boar (*Sus scrofa*). Wild boars have brachyodont molars and the species is generally a forest dweller, and did not inhabit the mammoth steppe. As discussed in Chapter 6, one of the reasons not to assume that the landscape in Schöningen was completely open and glacial conditions prevailed during depositional level 4, is the presence of fossils of wild boar (for example, Schö 13 II-4). Recently, stable isotopes were measured in two wild boar samples (see Table 7.1). The samples originate from depositional level 1 (Schö 12 B), the assumed interglacial optimum. Their $\delta^{13}C$ values (-22.0‰ and -22.4‰) agree with the $\delta^{13}C$ values of other animals from the interglacial optimum (n = 17, average -22.2‰).

However, according to the threshold presented in Chapter 6, these $\delta^{13}C$ values do not reflect the canopy effect. Of course, a number of two samples is very limited and there is a significant risk of over-interpretation. But among the possible explanations for the fact that the wild boar samples do not reflect the canopy effect, is that the value below which $\delta^{13}C$ values are expected to reflect the canopy effect (-22.5‰) must be somewhat adjusted towards a less negative number in the case of the Schöningen fossils. This $\delta^{13}C$ value of -22.5‰ is based on the results of previous studies on fossil remains and modern samples from different large herbivores, ages and localities by Drucker and colleagues, such as Late-Glacial and Early Holocene red deer samples from the French Jura (Drucker *et al.*, 2003a; Drucker and Bocherens, 2009). The circumstances in these localities may have not been a suitable equivalent for those in Middle Pleistocene Schöningen. As for $\delta^{13}C$ values from fossils, the canopy effect-$\delta^{13}C$ threshold may be expected to vary through time and space. Hence, this canopy effect-threshold was indicated with a dotted line and must be considered as an estimation for the canopy effect-threshold for the Schöningen fossils, instead of as a strict boundary.

Another possible explanation is that the consumption of underground plant parts, which seem to have higher $\delta^{13}C$ values than photosynthetic plant parts (Badeck *et al.*, 2005) make the visibility of the canopy-effect on the $\delta^{13}C$ values less clear. Other possible explanations are that the Schöningen wild boars inhabited forested parts of the landscapes which were not densely closed (N.B. more open forest types would not cause the canopy effect), or these Middle Pleistocene wild boars were less restricted to forest than their modern counterparts. In the latter case, the characteristics of the species have changed over time.

The fact that characteristics such as habitat or diet of a modern species does not have to match those in previous times, becomes clear from the isotope signatures of horses from Schöningen (see Chapter 6). Horses are generally considered to be typical obligate grazers. However, the $\delta^{15}N$ values of the horses from Schöningen, which are lower than all other investigated species from the same locality, provide supporting evidence for a browsing diet. Morphologically, the horse molars diverge from those found in other periods and sites, with unfamiliar pointed cusps and a dental wear pattern indicative that browsing formed a substantial part of the diet (Rivals *et al.*, 2014). Besides, the study of Rivals *et al.* (2014) shows that the variety of dental wear of horses across time and space is extremely wide. Richards *et al.* (2017) found that $\delta^{13}C$ and $\delta^{15}N$ values of collagen of horses from an archaeological site in France changed considerably between ~ 150,000 and 50,000 years ago following environmental and/or climatic changes. The wide variety in isotope values they found seems to support flexibility in diet. But as the authors state, it is unclear whether the range of $\delta^{13}C$ and $\delta^{15}N$ values from collagen reflects either changing isotope compositions of diachronic similar dietary components, or reflects dietary change (Richards *et al.*, 2017). The horse stable isotope data show a flexibility that is most probably not restricted to horses. One can assume that also other mammalian species have a degree of flexibility in their diet and their environmental constraints. A flexibility that is important, in particular for herbivores, to survive the Pleistocene changes in climatic conditions and vegetation. A lack of flexibility might lead to extinction.

7.3 The fatal blow for woolly mammoths

Many of the data discussed in this thesis come from fossils of now extinct animal species. Most of these species vanished as part of the 'Late Quaternary megafaunal extinctions' (Stuart, 2005; Stuart and Lister, 2012; Kosintsev *et al.*, 2019; Lister and Stuart, 2019). During the Weichselian and the Early Holocene, about 65% of the terrestrial large mammals (weighing more than 45 kg) became extinct across various continents (Mann *et al.*, 2015, 2018; Di Febbraro *et al.*, 2017; Rabanus-Wallace *et al.*, 2017). There is a long and still ongoing debate about what caused the megafauna extinctions that started towards the end of the Pleistocene (Koch and Barnosky, 2006; Haynes, 2009; Stuart, 2015). The Late Quaternary is a period characterized by major climate fluctuations. In addition, humans developed more advanced hunting techniques and they expanded their geographical range into three new continents and farther more to the north (Sykes, 2018; Hufthammer *et al.*, 2019; Ineshin and Teten'kin, 2019; Pitulko *et al.*, 2019; Vachula *et al.*, 2019). These factors have undoubtedly been critical for the extinction of certain species. But despite the number of species that became extinct within the Late Quaternary, the reasons for extinction may need to be studied per species and per region rather than a common explanation to be sought for the entire complex of extinctions (Broughton and Weitzel, 2018). Inter- and intraspecies spatiotemporal differences are observed for their extinction dates, and hence, it is obvious that they did not suffer a synchronous collapse (Lorenzen *et al.*, 2011; Stuart, 2015; Puzachenko *et al.*, 2017; Puzachenko and Markova, 2019). The disappearance of certain species may itself have triggered the extinction of other species, occasionally dramatically altering the functioning of entire ecosystems (Rule *et al.*, 2012; Gill, 2014).

Also, the mammoth steppe ecosystem, including many of its inhabitants came to an end in this period. Among the prevailing hypotheses explaining the extinction of the woolly mammoth, are 1) the hypothesis that humans are mainly responsible (over-hunting and introducing diseases; Martin, 1984; Braje and Erlandson, 2013; Boivin *et al.*, 2016), 2) the idea that climate change played major part (Cooper *et al.*, 2015; Rabanus-Wallace *et al.*, 2017), and 3) the assumption that both humans and climate played a crucial role (Haynes, 2007; Nikolskiy *et al.*, 2011; Wan and Zhang, 2017). Moreover, exotic causes have been suggested, such as impacts from the cosmos (Firestone *et al.*, 2007; Pinter *et al.*, 2011). Frequently-mentioned problems include the numerous preceding glacial-interglacial cycles that the species did survive, the long-term coexistence of woolly mammoths and hominins in some of their geographical regions dismissing prey naivety as contributing factor over there, the surviving of numerous coeval living species, and the lack of enough diachronic high-quality data (Stuart, 2015; Di Febbraro *et al.*, 2017; Rabanus-Wallace *et al.*, 2017; Vachula *et al.*, 2019).

At the margins of a biotope, significant information can be found about determining factors for the maximum spread of that specific biotope (Chase and Leibold, 2003), albeit potentially a reflection of predominantly suboptimal conditions (Braunisch *et al.*, 2008). Several biotic and abiotic factors, rather than behaviour and habitat selection, limit the distribution a species. These include vegetation type, sea level rise, widespread peat formation and the configuration of impassable rivers, mountain chains and ice sheets (Lister and Stuart, 2008; Kahlke, 2015).

Changes challenge the tolerance of a species, their niche breadth, rather than on the preference of the species; tolerance draws the line between survival and extinction. The disappearance of a species might be triggered by several factors. The ability to (episodically) expand or shift the actual range of a species into other areas within its potential range (that is, where it can survive and reproduce) often plays an essential role for survival. Habitat loss and fragmentation are inextricably linked to climate change, but geographically interconnected areas of suitable habitat are critical for maintaining the survival of a species (Aitken and Whitlock, 2013; Di Febbraro *et al.*, 2017).

The woolly mammoths survived numerous climatic changes caused by alternating glacials/stadials and interglacials/interstadials throughout their existence in the Pleistocene. Though their range was contracted into refugia during interglacials, woolly mammoths inhabited a gigantic geographic region with local variations due to natural features such as mountain ranges and rivers, which shows that woolly mammoths had a high level of flexibility and the ability to adapt to a range of environmental conditions.

Although situated on the northern peripheries of the mammoth steppe biotope, North-eastern Siberia was more or less continuously occupied by the woolly mammoth (Puzachenko *et al.*, 2017). Apparently here, life conditions remained suitable enough throughout the various Late Pleistocene stadials and interstadials. Favourable circumstances were served by, for instance, the lowlands of many parts of North-eastern Siberia remaining ice-free due to aridity during the LGM, whereas many other regions along the Northern Hemisphere were then covered by ice sheets (Gualtieri *et al.*, 2005; Hoffecker and Elias, 2007; Stauch and Gualtieri, 2008; Möller *et al.*, 2015).

It was also North-eastern Siberia where the woolly mammoth survived the longest before getting extinct. This also applies to various other mammoth steppe dwellers (Boeskorov, 2006; Di Febbraro *et al.*, 2017), such as woolly rhino (Stuart and Lister, 2012), steppe bison (Stuart, 2015) and wild horse (Boeskorov *et al.*, 2018). Indeed, whereas mammoths started to disappear from different parts of their range between around 20,000 and 11,000 years ago, fossils from much younger mammoth populations are recovered from islands in the Bering Sea, such as St Lawrence, St Matthew, the Pribilof Islands, and in particular Wrangel. The fact that the mammoth survived so long on Wrangel Island, and the fact that there is no clear evidence that humans occupied the island before the mammoth's disappearance (Gerasimov *et al.*, 2006; Nikolskiy *et al.*, 2011), makes Wrangel Island an interesting area to study the conditions under which the very last woolly mammoths became extinct.

As presented and discussed in Chapter 3, the $\delta^{13}C$ and $\delta^{15}N$ values of woolly mammoth samples from Wrangel remain similar through time. Recently, these conclusions were confirmed by Arppe *et al.* (2019). The stable isotope data do not give direct information about the extinction of the woolly mammoth on Wrangel Island. But the stable isotope composition of samples from woolly mammoth show no changes that point to any changes in for instance the moisture or nutrient availability on the island, as was previously suggested for St. Paul Island (Graham *et al.*, 2016). These results are in line with conclusions from Fox *et al.* (2007): climate signals recorded in oxygen isotope values in serially sampled tusks from North-eastern Eurasia, including Holocene tusks from Wrangel Island do not point to climate change that could be regarded as the direct cause of the woolly mammoth extinction (Fox *et al.*, 2007). Also our mammoth stable isotope data from Wrangel do not indicate any climate change just before its ultimate extinction.

Although the current dataset and results of study of various other proxies point to stability through time in this region, changes in habitat and/or diet at some point cannot be absolutely ruled out based on diachronic similarity of stable isotope data. For instance, a transition to another diet would go unnoticed in the stable isotope values of the tissues of the consumer, if the isotope values of the new food have more or less the same isotope values as the other diet. A stable isotope value of fossil tissue of a consumer can be generated by many potential means, instigated by for instance soil processes, dietary isotope composition, physiological aspects. However, also the opposite is true. Variation in $\delta^{13}C$ and $\delta^{15}N$ values in an herbivore tissue following environmental and/or climatic changes, could reflect either variation in isotope values of the consumed plants in response to the climate fluctuations, or a switch to another diet (Rabanus-Wallace *et al.*, 2017; Richards *et al.*, 2017). The latter would indicate adaptability and flexibility of the animal, whereas the previous would not per se.

Nevertheless, climate change is not likely to have been the direct cause of the woolly mammoth extinction on Wrangel Island. Nutrition and the absence of predators are not the only factors critical for a species' survival. The small population size in combination with the dispersal barriers of Wrangel- inextricably linked to the isolated nature of an island- has probably been the bottleneck for the Wrangel mammoths. Recent investigation on the genome of one of the last surviving Wrangel mammoths (dated to about 4,300 yr BP) demonstrates reduced genetic variation. It also shows that the last mammoths on Wrangel Island likely suffered from genetic diseases that reduced fitness (Palkopoulou *et al.*, 2015; Pečnerová *et al.*, 2016; Rogers and Slatkin, 2017; Fry *et al.*, 2018). The actual population size might have been large considering the small size of the island and might even have increased dramatically around the onset of the Holocene (Zimov *et al.*, 2012). But it was not large in terms of the measure of genetic exchange, which hampers maintaining a healthy and reproductive population. Already 12,000 years ago, at about the time when Wrangel became separated from the mainland (Brigham-Grette and Gualtieri, 2004; Keigwin *et al.*, 2006), the woolly mammoth genome indicates a major reduction in effective population size (Palkopoulou *et al.*, 2015). Along the way, the reduced genetic variation was probably harmful for the reproductive success of the Holocene Wrangel mammoths. That must have had a disastrous impact on their viability and might have contributed to their extinction (Palkopoulou *et al.*, 2015).

The youngest dated mammoth from Wrangel is dated to 3685 ± 60 ^{14}C yr BP (Ua-13366; Vartanyan *et al.*, 2008), which calibrates to 2276-1905 BCE (at 95.4% probability by using IntCal13, Reimer *et al.*, 2013). This youngest find does not represent the last animal per se, since in theory, younger remains may be found at a later stage, in particular considering the incompleteness of the fossil record or so-called 'Signor-Lipps effect' (Signor and Lipps, 1982). But an estimation for the extinction date of the woolly mammoth produced by Bayesian age modelling, indicates that this youngest find belonged to one of the very last representatives of this species. So far, 153 ^{14}C dates of woolly mammoth fossils from Wrangel Island have been published, including the new dates as presented in Chapter 3. The majority (n = 132) are younger than 12,000 years ago, and therefore date to the time after Wrangel became an island isolated from the mainland (Brigham-Grette and Gualtieri, 2004; Keigwin *et al.*, 2006). The 132 Holocene ^{14}C dates of woolly mammoths from Wrangel Island can be incorporated within a Single Phase model in OxCal version 4.3.2 (Bronk Ramsey, 2009). In the model, the end boundary provides an estimate of the last appearance of the woolly mammoth on the basis of the 132 ^{14}C data. The callibration curve IntCal13 was used for calibrating the ^{14}C dates (Reimer *et al.*, 2013). The model (see SI Chapter 7 for the code) generates an end boundary of 4218-3886 cal BP (at 95.4% probability). This means that the last appearance for the woolly mammoth was around 2269-1937 BCE and means the very end of this intriguing species.