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Giant deer (*Megaloceros giganteus*) diet from Mid-Weichselian deposits under the present North Sea inferred from molar-embedded botanical remains

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ABSTRACT: The molar of a giant deer, *Megaloceros giganteus* (Blumenbach, 1799), was discovered in sandy deposits of the North Sea, about 10 km west of the present shoreline of the Netherlands. Compacted masticated plant remains were preserved in the molar's deep folds. A palaeoecological analysis of these plant remains shows the complete dominance of pollen from *Artemisia* (sage) and other Asteraceae Tubuliflorae (Compositae), indicating that the animal foraged in a steppe environment, and may have preferred to eat *Artemisia*, which contain a high level of nutrients such as calcium and phosphorous components, which are important for antler building. Radiocarbon dating indicates that the plant remains are of mid-Weichselian age [38 570 (+300, –290) ¹⁴C a BP, Greenland Interstadial-11]. This is the first study of the food choice of *M. giganteus* based on palaeoecological evidence. We hypothesize about links between the extirpation of *M. giganteus* at the onset of the Holocene in North-West Europe and the transition from a landscape with highly dynamic geomorphological processes and consequently prevalence of immature and nutrient-rich soils, to a less dynamic landscape with stable, leached and acidifying soils, and the decline of *Artemisia* and other calciphilous plants.

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KEYWORDS: food residue; Greenland Interstadial-11; late Pleistocene; microfossils; soil development.

Introduction

To strengthen the shoreline of the Netherlands south of The Hague, an artificial peninsula was created in 2011, using Late Pleistocene and early Holocene sediments dredged from the relatively shallow North Sea bed, about 10 km north of the Eurogeul area. This new land, formed by 21 million cubic metres of sandy deposits, functions as a coastal reinforcement and is called the Zandmotor. Three sediment units were used to create the Zandmotor: sediments from marine Holocene/recent sandbars, from early Holocene deposits and from underlying Late Pleistocene sandy sediments, originally deposited in ancient Rhine and Meuse deltas when, worldwide, sea levels were much lower than today (Laban and Rijdsdijk, 2002; van der Valk *et al.*, 2011; Langeveld, 2013).

When the Zandmotor was opened to the public, the area became a well-known locality for collecting fossils, including mammal bones and molluscs from Pleistocene and early Holocene sediments (van der Valk *et al.*, 2011). The well-preserved mammal bones, both from marine and from terrestrial species, are from the Last Glacial period (Weichselian) and the early Holocene. Bones of representatives of the Late Pleistocene mammoth fauna are common, and are well known from the southern bight of the North Sea between the British Isles and the European continent (Mol *et al.*, 2006, 2008). The fauna was dominated by large herbivores, such as woolly mammoth [*Mammuthus primigenius* (Blumenbach,

1799)], woolly rhinoceros [*Coelodonta antiquitatis* (Blumenbach, 1799)], steppe bison [*Bison priscus* Bojanus, 1827] and wild horse [*Equus caballus* Linnaeus, 1758]. Radiocarbon dated specimens show that representatives of this fauna lived between ca. 50 000 and 25 000 ¹⁴C a BP when North-West Europe was part of the mammoth steppe ecosystem (Mol *et al.*, 2006, 2008).

In the present paper we report an upper molar of a giant deer, *Megaloceros giganteus* (Blumenbach, 1799), that was found by one of us (CJMvdK) on 13 July 2015 along the beach of the Zandmotor. In its deep folds, the molar contained compacted, masticated plant remains that we studied using palynological and ancient DNA analyses.

Materials and methods

Molar

The molar (Fig. 1) is a complete right upper M3 (M3 sup. dext.) consisting of the crown and four roots; the two at the lingual side are almost entirely fused. At the base the crown is wider anteriorly (30 mm) than posteriorly (25 mm). The almost square shape of the molar indicates that it is an upper molar and the morphology shows that it is a last molar: an upper M3. The paracone is worn at the occlusal surface but the lower protocone is not entirely worn. The metacone and the hypocone show wear only at their posterior parts. The stages of wear indicate that the molar once belonged to an adult, but not a very old individual; the last molar was not yet in full use.

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Figure 1. The upper right molar (M3 sup. dext.) of the Zandmotor *Megaloceros giganteus*, after extraction of the plant remains from the infundibulum. Scale bar = 2 cm (A) Lingual view; (B) occlusal view; (C) buccal view.

The cingulum at the base of the hypocone is weakly developed, whereas the cingulum at the base of the protocone is pronounced. The surface of the enamel on the lingual side of the protocone and hypocone is of a fine, folded texture, which is a characteristic of deer molars. The space between the paracone and the protocone, as well as that between the metacone and the hypocone, is deep.

The space between the paracone and the protocone was filled with masticated plant remains, which were extracted for further study. Crown-height is low (maximum 27.5 mm at the buccal side and 26 mm at the lingual side), which is typical for large deer. Measurements of the molar at the occlusal surface are 30 mm antero-posterior and at their maximum 14 mm interior–exterior. The molar is deposited in the collection of the Natural History Museum Rotterdam and catalogued under number NMR999100013942. The studied microscope slides are catalogued under NMR999900012546.

Species identification

We compared the molar with upper molars of moose, *Alces alces* (Linnaeus, 1758), and *M. giganteus*; the fusion of the paracone and metacone at the buccal side and the surface texture of the paracone and metacone excluded moose. The overall size and morphology excluded other deer species, such as red deer (*Cervus elaphus* Linnaeus, 1758), fallow deer [*Dama dama* (Linnaeus, 1758)], reindeer [*Rangifer tarandus* (Linnaeus, 1758)] and roe deer [*Capreolus capreolus* (Linnaeus, 1758)]. The pronounced ridge on the posterior side of the crown and the horizontal basal ridges in buccal view indicated *Megaloceros* (Lister *et al.*, 2005). Comparison with a cranium with dental elements of a male *M. giganteus* present in the collection of the Natural History Museum Rotterdam (NMR 999100008426), from Late Pleistocene sediments of the province of Overijssel, the Netherlands, showed a superb match.

Confirmation of the species identity of the molar comes from ancient DNA analysis. Approximately 100 mg of bone powder was drilled from the tooth. DNA was extracted using a modified version of the protocol from Dabney *et al.* (2013): overnight incubation was performed at 42 °C instead of 37 °C; the Zymo-Spin columns were replaced by High Pure Large Volume Assembly 50 mL (Roche) columns; and elution was performed twice in 50 µL of Tris-EDTA-Triton X-100 Buffer (TET) with 10-min incubation time at 37 °C before each

elution. The DNA extract was transformed into an Illumina sequencing library in 25-µL reactions following Meyer and Kircher (2010) with some modifications: the initial DNA fragmentation was not performed and a MinElute kit (Qiagen) was used for the purification steps. The DNA library was indexed using KAPA HiFi uracil+ premix (KAPA Biosystems) polymerase with 13 cycles of amplification.

The indexed library was pooled with other sequencing libraries and sequenced on an Illumina MiSeq, yielding 981 545 reads. The reads were mapped to the Hohle Fels *M. giganteus* mitochondrial genome (Immel *et al.*, 2015) using Geneious 10.2.3 (Kearse *et al.*, 2012), resulting in three non-overlapping sequences [96 BP (tRNA-Asn), 60 BP (APTase6), 37 BP (COI)] which all matched 100% to the *M. giganteus* mitochondrial genome. The sequences were subject to BLAST searches in GenBank, and the closest match for APTase6 and COI differed from the query sequence by 2 and 1 BP, respectively (there are no *M. giganteus* sequences in GenBank for these mitochondrial gene regions). The tRNA-Asn sequence generated is identical across many Cervidae. While these results would be strengthened by additional sequencing depth and/or targeted sequence of a highly variable region of the mitochondrial genome, the 100% sequence match to the Hohle Fels genome, and difference in two of the genes from any other species, suggests that the tooth is from *M. giganteus*.

Radiocarbon dating

A sample of the plant remains was taken from the *M. giganteus* molar by sampling from the deepest part of the contents of the fold (see below). This plant sample was dated at the accelerator mass spectrometry (AMS) facility of the Centre for Isotope Research of Groningen University. The sample was chemically pre-treated using a 4% HCl acid bath (A-only) protocol (Mook and Streurman, 1983). After pre-treatment, the sample was combusted and turned into CO₂ by an elemental analyser (EA), coupled online with a stable isotope mass spectrometer (MS). The EA was also used for purifying of CO₂. In addition, the EA/MS system enables precise measurements of the δ¹³C value. CO₂ was reduced to graphite by reacting under an excess of hydrogen gas (Aerts-Bijma *et al.*, 2001). This graphite was pressed into a target holder that was placed in the ion source of the AMS. The Groningen AMS facility is based on a 2.5-MV accelerator,

Table 1. Results of isotopic measurements of the *M. giganteus* plant remains from inside the infundibulum of the molar.

Sample	Dated material	Radiocarbon ID	Age (^{14}C a BP)	Sigma	%C	%N	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	C/N	cal a BP 1 σ	cal a BP 2 σ
Plants	Compacted plant remains	GrA-68256	38 570	+300, -290	43.7	—	-27.39	—	—	42 800–42 370	43 030–42 160

and measures the ^{14}C concentration in the graphite (van der Plicht *et al.*, 2000).

Microfossil analysis of plant remains

The compacted masticated plant remains in the infundibulum between the paracone and the protocone were sampled using a preparation needle. Sampling occurred in five subsequent thin layers to determine if any recent contamination had occurred. If so, the contamination would be most pronounced in the outer layer that had been exposed to post-deposition disturbance: dredging, sea water, exposure to the air on the beach and exposure between the moment of discovery on the beach and sampling of plant remains in the laboratory. Subsample 1 was the outer, visible layer and subsamples 2–5 were subsequently obtained from deeper parts of the fold. The estimated total amount of available botanical material was ca. 0.5 cm^3 (after removal from the molar fold, and therefore no longer compacted). The preparation of microfossils was according to Faegri and Iversen (1989). The total amount of botanical material was small and therefore no separate macrofossil sample was available. As an alternative, the material that remained on the sieves during the initial phase of the microfossil preparation was studied for epidermis fragments and other botanical remains.

Ancient DNA analysis of plant remains

To further identify plant taxa, DNA analysis of the botanical remains was carried out as follows. A volume of $100\text{ }\mu\text{L}$ of ultrapure water was repeatedly flushed through the infundibulum. The resulting fluid and a control were then extracted using Lysis E and Sergey Bulat buffer (Haile, 2012). The extract was amplified using the short P6 loop sequence (usually 10–100 BP) of the *trnL* plastid region using universal primers (Taberlet *et al.*, 2007; Willerslev *et al.*, 2014). The arctic *trnL* taxonomic reference library (Sønstebo *et al.*, 2010) has been extended with sequences of the first internal transcribed spacer (ITS1) region of nuclear ribosomal DNA for Poaceae, Asteraceae and Cyperaceae (Willerslev *et al.*, 2014). Hence to increase the taxonomic resolution of these families, we also amplified the ITS1 region (45–90 BP) using four sets of 6-BP tagged primers. Amplicons and controls were visualized on a 2% agarose gel. There were no bands in the controls, and therefore only the amplicons were pooled and built into a library (Meyer and Kircher, 2010). The library was then pooled with others and sequenced on an Illumina MiSeq yielding a total of 2 108 196 reads.

Adaptor sequences and primers were removed and the sequences were separated based on their tag and primer combinations using OBITOOLS (Boyer *et al.*, 2016). Identical sequences were collapsed (Boyer *et al.*, 2016), and the sequences were used for BLAST searches in GenBank (Benson *et al.*, 2007), with the *trnL* sequences additionally BLASTed against an arctic plant reference database (Sønstebo *et al.*, 2010). Resulting BLAST hits were then visualized with MEGAN (Huson *et al.*, 2007) using default Lowest Common Ancestor algorithm (LCA) and analysis parameters.

Results

Radiocarbon dating

The ^{14}C dating result (Table 1) is reported in conventional radiocarbon years (^{14}C a BP), which includes correction for isotopic fractionation and use of the conventional half-life (Mook and van der Plicht, 1999). The background is determined by dating anthracite or wood of infinite age (on the ^{14}C timescale) and is in the range 50–45 000 a BP. The result of radiocarbon dating of the plant remains is 38 570 (+300, -290) ^{14}C a BP (GrA-68256). Calibration using the calibration curve IntCal13 (Reimer *et al.*, 2013) yielded a calendar age between 42 800 and 42 370 cal a BP (1 σ) and 43 030–42 160 cal a BP (2 σ). All numbers are rounded to the nearest 10. The unit 'cal a BP' is defined as calendar years relative to 1950 AD.

We also attempted to date the molar. Unfortunately, the extracted collagen from the molar material was not pure and contained low C and N yields, 6.2 and 2.2%, respectively. This does not satisfy the quality criteria as established by DeNiro (1985). The C and N percentages of the collagen were too low. A very small sample was dated but the results had to be rejected. We consider the date of the botanical material as representing an accurate age of the *M. giganteus* molar.

The age of the botanical remains in our molar fits well with radiocarbon dates produced by the Groningen and Oxford dating laboratories (Table 2) of skeletal elements of *M. giganteus* from the Southern Bight of the North Sea, including the area where the sediments for the Zandmotor were extracted. Mol *et al.* (2006) published a series of ^{14}C results from species of the Late Pleistocene megafauna, trawled from the bottom of the North Sea between the British Isles and the Netherlands. Radiocarbon dates of the megafauna suggest that species, adapted to a cold and dry steppe environment, lived in the area from at least 45 000 to 28 000 ^{14}C a BP. The obtained age of our *M. giganteus* from the Zandmotor fits well in this time span.

Microfossils and macroremains

Subsamples 1, 2 and 3 showed various small plant fragments, but were poor in pollen. There were no indications of contamination with modern material. The results of the

Table 2. Radiocarbon dates of *M. giganteus* skeletal elements from the North Sea area (after Mol *et al.*, 2006 and unpublished data).

Skeletal element	Laboratory number	Age (^{14}C a BP)
Cranium with antlers	GrA-38353	>45 000
Antler	GrA-32601	45 770 (+740, -680)
	GrA-32685	44 650 (+850, -760)
		45 200* (+700, -600)
Antler	GrA-32599	40 750 (+440, -380)
Metacarpal	OxA-6308	36 300 (+/-1100)

*Average of the two radiocarbon dates.

Table 3. Microfossil data from the two innermost subsamples of plant material embedded in the *Megaloceros giganteus* molar, indicating the number of pollen identified from each taxon. In both samples lumps of unripe *Artemisia* pollen grains were observed.

Taxon	Sample number		Total	Percentage
	5	4		
<i>Artemisia</i> (sage)	219	177	396	65.1
Asteraceae Tubuliflorae (Compositae)	94	73	167	27.5
Asteraceae Liguliflorae (Compositae)	—	1	1	0.2
Brassicaceae (mustard family)	4	5	9	1.5
Cyperaceae (sedge family)	1	2	3	0.5
<i>Helianthemum</i> (sun rose)	2	+	2	0.3
<i>Plantago</i> (plantain)	5	1	6	1.0
Plumbaginaceae (leadwort family)	—	+	+	+
Poaceae (grasses family)	8	+	8	1.3
<i>Alnus</i> (alder)	3	—	3	0.5
<i>Betula</i> (birch)	3	—	3	0.5
<i>Corylus</i> (hazel)	1	—	1	0.2
<i>Pinus</i> (pine)	1	—	1	0.2
<i>Salix</i> (willow)	5	3	8	1.3
Pollen sum			608	
<i>Sordaria</i> type (coprophilous fungi)	2	1	3	0.5
<i>Sporormiella</i> (coprophilous fungi)	5	1	6	1.0
HdV-1400 ascospores	2	—	2	0.3
HdV-128A	—	1	1	0.2

analysis of microfossils in subsamples 4 and 5 are shown in Table 3.

Subsamples 4 and 5 are dominated by pollen grains of *Artemisia* (sage) and other Asteraceae Tubuliflorae (Compositae) while a limited number of other herbaceous taxa are recorded, including the steppe-taxa *Plantago* (plantain), *Helianthemum* (sun rose) and Plumbaginaceae (leadwort family). Pollen grains of wind-pollinated trees (*Alnus*, *Betula*, *Corylus* and *Pinus* produce large quantities of pollen) show very low values, reflecting long-distance transport. Insect-pollinated *Salix* species (willows) release relatively low quantities of pollen, but *Salix* shows relatively high amounts (between 1 and 2%), and we therefore hypothesize that dwarf willow species were present in the landscape of our *M. giganteus*. Spores of coprophilous fungi (*Sporormiella* and *Sordaria* type) are low in frequency. Figure 2 shows some epidermises and other botanical macrofossils from the molar. These plant remains were well preserved but in small pieces and therefore were too fragmentary to be identified with certainty.

Ancient DNA

The results of the study of ancient plant DNA were inconclusive, as there were no species in common between those obtained using *trnL* primers and the ITS1 spacer region primers. Additionally, most results were either implausible or identified only at very high taxonomic level, and the few plausible plant species were present at lower sequence frequency than obviously impossible species (i.e. non-European plants). Although when using these primers it is not unusual to obtain strange results due to low-level and ever-present background levels of contamination (see e.g. Valentini *et al.*, 2009), these problems become greatly exaggerated when, as in this case, there is little or no endogenous plant DNA remaining. This result is perhaps not surprising given the poor DNA preservation in the tooth itself, which unlike the plant remains entrapped in the tooth fissure

also benefited from the environmental protection afforded by the tooth matrix.

Discussion

Food residues from molar folds

The epidermises and other botanical macrofossils from the molar fold were well preserved but highly fragmented. For comparison, we observed skulls in modern collections: four out of four moose skulls, two of two reindeer skulls and a fallow deer skull studied all have infundibula filled with small plant remains of the same compacted structure as our fossil specimen. Therefore, we suppose that our *M. giganteus* masticated the studied plant remains and that these were not forced into the molar fold through sedimentary pressure *post-mortem*.

Studies of the intestinal contents of frozen herbivores are quite common (e.g. Ukraintseva, 1979, 1993; van Geel *et al.*, 2008; Willerslev *et al.*, 2014), but studies of food residues from molar folds are scarce. Garutt *et al.* (1982) provided an overview of research on the food of the woolly rhinoceros and analysed the pollen and spore content of compacted food residues from the molars of an individual discovered in 1877 along the Khabui River in Siberia. Garutt *et al.* identified Poaceae (dominant), *Artemisia* (subdominant), *Alnus*, *Betula*, and small numbers of spores and pollen grains of herbaceous taxa of several families, pointing to an open landscape. Lazarev (2005) compared the pollen and spore taxa recorded by Garutt *et al.* (1982) with the pollen and spore contents of the gastrointestinal tract of the Churapchinsky rhino from Churapcha, central Yakutia (Lazarev and Tirkaya, 1975) and found much correspondence. Boeskorov *et al.* (2011) compared the pollen and spore analysis of the stomach contents of a woolly rhino from the Kolyma River, north-east Siberia, with the records obtained by Garutt *et al.* (1982) and by Lazarev and Tirkaya (1975) and found a close correspondence.

Guthrie (1990) extracted plant fragments from woolly rhino molars from Eurasia in the American Museum of Natural History in New York and found 96% grass fragments. In food residues from molars of Alaskan steppe bison, 80% grass was found. Guthrie (2001) presented the food residues from molars of several species, including steppe bison, helmeted muskoxen [*Bootherium bombifrons* (Harlan, 1825)], moose and reindeer, and from incisor pits of equids, all from the permafrost of Alaska and north-east Asia. He differentiated between feeding on grasses, sedges, cryptogams (lichens), forbs and woody species, and also between grazers, mixed feeders and browsers.

In the 1980s R. D. Guthrie examined the molars of the rich *M. giganteus* collection of the National Museum of Ireland (Dublin), hoping to find some food residues. As far as known (Nigel T. Monaghan, written comm., 2017) Guthrie recovered nothing but museum dirt.

Pollen and spore record from our molar

The dominant pollen grains in the analysis of samples from our molar are *Artemisia*. If the criteria given by Webb and Moore (1982) are applied, at least some of the *Artemisia* pollen grains (those with distinct echinae) are reminiscent of type A (i.e. *Artemisia cf. norvegica*). Today, this is one of the most widespread sub-arctic species (Taylor, 2006), and it is foraged by black-tailed deer [*Odocoileus hemionus* (Rafinesque, 1817)] in Alaska (Klein, 1965). Thus, *A. norvegica* (synonym *A. arctica*) may well have been the species consumed. Spores of the coprophilous fungi *Sporormiella* and

Figure 2. Molar of *Megaloceros giganteus* and plant remains from inside the infundibulum. (A) *M. giganteus* molar (occlusal view) from Zandmotor, showing compacted light-brown plant remains in infundibulum; (B) leaf tip of *cf. Artemisia*; (C) leaf tip of modern *Artemisia norvegica*; (D–F) epidermis fragments (*Artemisia*?), cells partly tanniniferous; (G) epidermis *cf. Salix*; (H) tissue showing secondary xylem; (I, J) transport tissue with pits – some with strongly reduced borders (half-bordered vessel-parenchyma pits; *Salix*?); (K) sclerenchyma fibres (testa cell layers?); (L) epidermis with irregularly arranged cells with undulate walls; (M) epidermis cells with undulate walls in a linear pattern; (N, O) cluster of hairs, O showing some curved hair bases.

Sordaria type in our samples support the existence of a population of herbivores.

Age, diet and environment

The Weichselian fossil record in the Netherlands has yielded numerous specimens of *M. giganteus* in association with other members of the Late Pleistocene megafauna. These specimens indicate that *M. giganteus* was a common species of the mammoth steppe environment. Sites include many dredging operations along the major rivers Rhine, Meuse and Waal and several sites in the North Sea, such as off the coast of the province of Zeeland (De Man, 1875, 1878; Mol *et al.*, 2008). The plant material from our molar was dated to Greenland Interstadial-11 at 1 σ level (Fig. 3); it extends into GS-11 at 2 σ level. In the terminology of geological and palynological studies in the Netherlands the molar is of Mid-Weichselian age. The vegetation composition in the Netherlands during Mid-Weichselian interstadials is reflected in various pollen records, which show that upland vegetation was dominated by Poaceae, Cyperaceae, *Betula nana*, dwarf willows (*Salix* spp.), *Artemisia* and *Thalictrum* (Brinkkemper *et al.*, 1987; Ran, 1990).

When comparing the pollen spectra of our molar with these data, we should bear in mind that the palynological studies of Mid-Weichselian material are based on cores from lake sediments and peat deposits. Individual samples from such sediments encompass a number of years, often more than 10 years per sample, thus including many complete flowering seasons. Sediments furthermore include pollen grains from various vegetation types across the landscape, from wet to dry habitats. In diagrams the local and extra-local taxa (*sensu* Janssen, 1973) of wet habitats will be over-represented, compared with upland taxa that come from further away.

Brinkkemper *et al.* (1987) and Ran (1990) showed that *Artemisia* formed 5–15% of the pollen sum during the Mid-Weichselian in the Netherlands. The complete dominance of pollen grains of *Artemisia* and other Asteraceae in our molar pollen spectra has no parallel in the pollen diagrams. We expect that our pollen spectra differ from pollen records of

sediments because the spectra composition from a molar content depends on (i) the availability and selection of edible plant species by the animal, (ii) the season, and especially what part of the flowering season is represented, and (iii) the possibility that flowers with yet-unreleased pollen formed part of the consumed plants and therefore some taxa may be strongly over-represented; indeed, some lumps of unripe *Artemisia* pollen in our molar samples indicate that unripe, not yet released pollen grains, still enclosed in the anthers of flowers, were consumed.

The need for calcium

We do not know if the choice for *Artemisia* was a preference of *M. giganteus* in general, or if it was just by chance that our specimen ate *Artemisia*. Primary data on the diet of *M. giganteus*, such as food residues from molars or from the gastrointestinal tract, are not yet available. Reconstructions based on secondary data (stable isotopes and microwear on teeth) have yielded varying results. Chritz *et al.* (2009) based conclusions on the analysis of stable isotopes in teeth, suggesting a grass- and forb-based diet, supplemented with browsing. According to Rivals and Lister (2016), *M. giganteus* in the late Middle to Late Pleistocene has tooth-wear patterns indicating a wide range of dietary behaviour including grazing, leaf browsing and mixed feeding, but generally tending towards mixed feeding and grazing.

Based on a theoretical approach, Geist (1998) calculated the nutritional requirements of *Megaloceros*. To satisfy the demands (energy, calcium, phosphate, protein, etc.; see also French *et al.*, 1956) during the growth of the massive antlers, males had to select plant species with high nutrient levels. Geist calculated that for growth of 45 kg of antlers each year a stag required forage containing at least 3.45% protein, 0.44% CaO (0.32% Ca) and 0.37% P₂O₅ (0.15% P). Forage meeting these requirements was considered to be rare, except for the buds and leaves of willows (*Salix* spp.), which would amply meet the demands. According to Geist (1998), who referred to nutritional needs as well as to tooth structure, males would have to feed on foliage of dwarf birches (*Betula* spp.) to grow their antlers, while females would feed more on

Figure 3. Selected part of the Greenland ice core record showing 20-year average oxygen isotope values from the three ice cores GRIP (red), GISP2 (green) and NGRIP (blue), adapted from Rasmussen *et al.* (2014). Interstadials are illustrated by grey shading. The 1 σ range of the calibrated radiocarbon date of the plant remains from the *Megaloceros* molar is plotted on the same time scale. Note that the Greenland ice core chronology is plotted in thousands of calendar ages relative to 2000; the 50-year difference with the calibrated ¹⁴C age is taken into account.

grasses, forbs and sedges. Antler growth was proposed to peak in early June and to be complete by mid-July. A model based on metabolic needs of moose predicted that in *Megaloceros* about 6% of the calcium and 10% of the phosphorus were resorbed from the skeleton in the summer and replenished by dietary intake over the following winter (Moen *et al.*, 1999). Female deer require forage containing relatively high amounts of calcium, phosphorus and protein especially during peak lactation, as demonstrated for moose by Chalyshev and Badlo (2002).

Deer build their antlers and lactate when forage is most abundant and of the highest nutritional quality. Klein (1965) investigated the percentages of protein, calcium and phosphorus in a number of forage plant species of black-tailed deer, including *A. arctica*. Most of the species exceeded the requirements as calculated by Geist (1998) for *Megaloceros*. *A. arctica* from limestone-derived soil contained 0.87–0.98% calcium, which is relatively high compared with the quantities in a number ($n=17$) of pharmaceutically important *Artemisia* species analysed by Ashraf *et al.* (2010): 0.06–1.07% (average 0.46%).

Being selective feeders, deer usually choose plants and parts of plants that are most suited to their current nutritional needs (Halls, 1984). If indeed a considerable quantity of *Artemisia* pollen from our molar originated from *A. norvegica*, then we hypothesize this *Artemisia* species may have been consumed by our *M. giganteus* because of its relatively high calcium content.

Hypotheses regarding diet and extinction of *M. giganteus*

The abrupt climate and vegetation changes of the Last Glacial/Interglacial Transition strongly affected the ranges of mammalian species (Moen *et al.*, 1999; Gonzalez *et al.*, 2000; Stuart *et al.*, 2004; Lister and Stuart, 2008; van der Plicht *et al.*, 2015). Ancient DNA studies show that contraction of species ranges and abundances caused loss of genetic diversity and presumably loss of adaptive flexibility (Lister and Stuart, 2008). After the Late Pleistocene/Holocene transition the steppe vegetation in northern Eurasia was replaced by tundra and forest, which was a change to adverse conditions for many of the large herbivore species that had lived on the mammoth steppe. Several hypotheses, including overkill by human hunters, were developed to explain the extirpation and extinction of large herbivores (Gonzalez *et al.*, 2000; Stuart *et al.*, 2004; Lister and Stuart, 2008). Here we focus on the extinction of *M. giganteus*.

Antler-extinction hypothesis

A clear understanding of the dietary preferences of *M. giganteus* is crucial to our understanding of how and why the species went extinct, and this is a central topic in the general megafaunal extinction debate (Chrutz *et al.*, 2009).

During the Bølling–Allerød interstadial (ca. 14.7–12.9 cal ka BP), *M. giganteus* populations were found in the increasingly forested habitats of North-West Europe. As climates shifted to colder conditions at the start of the Younger Dryas (ca. 12.9–11.7 cal ka BP), open vegetation returned and the growing season became shorter. The environmental conditions of the Younger Dryas led to the loss of the European population of *M. giganteus* (Stuart *et al.*, 2004). Various authors (e.g. Barnosky, 1986; Moen *et al.*, 1999) have linked antler growth and extinction. Barnosky (1986) claimed a decrease in the size of bones and antlers of *Megaloceros* that died during the Younger Dryas and suggested that the last surviving animals were nutritionally stressed (Barnosky,

1986). A productive environment was necessary to sustain the annual antler growth in males. According to Moen *et al.* (1999) sexual selection pressure for large antlers and large body size were countered by selection pressure for smaller antlers and smaller body size imposed by habitat degradation.

Female reproductive energetics hypothesis

O'Driscoll Worman and Kimbrell (2008) did not agree with the antler-extinction hypothesis and focused on the reproductive energetics of females as a possible contributor to extinction. Their model shows that the reproductive output of females may have been reduced by 50% due to a decline in the growing season at the start of the Younger Dryas. The authors conclude that nutritional stress on reproductive females probably contributed more to the extinction than nutritional stress on large-antlered males.

Soil and vegetation development and the decline of Ca-rich forage

According to Guthrie (1990), mammoth steppe vegetation provided more mineral nutrients for growth than is available today. A relatively high rate of evapotranspiration usually gives plants in arid regions a higher concentration of water-soluble elements than their counterparts in moist conditions. Guthrie (1990) also mentioned evidence supporting this idea. Hiking in the Alaskan bush, he often found shed antlers from contemporary moose and caribou showing gnaw marks made by the ungulates that once grew them. He even mentions entire antlers that have been reduced to their main beam, with tines and palms completely eaten. Herbivores chew on antler and bone because they are after the mineral nutrients.

Guthrie studied tens of thousands of Pleistocene bones and – in contrast to his observations of contemporary populations – he found that chew marks were infrequent. According to Guthrie, the comparatively low incidence of bone and antler chewing by herbivores during the Pleistocene indicates sufficient plant quality and lack of seasonal mineral nutrient deficiencies among herbivores. The higher mineral nutrient content of mammoth steppe plants, and probably soils as well, meant that Pleistocene grazers seldom lacked essential mineral nutrients (Guthrie, 1990).

Climate–soil–vegetation hypothesis

Based on our palynological results we would like to make a speculative comment regarding the extinction of *M. giganteus*. *Megaloceros* species are known from the late Middle Pleistocene to the early Holocene. As a mixed feeder, the species managed to survive interglacials and interstadials. With the loss of the mammoth steppe, we hypothesize *M. giganteus* would have had problems adapting to the new environment and vegetation composition. The onset of the Holocene, with rising temperatures and increased precipitation, caused the disappearance of the mammoth steppe, which had negative consequences for many large herbivores, including *M. giganteus* (Martin and Wright, 1967; Martin and Klein, 1984; Guthrie, 2001; Willerslev *et al.*, 2014). Pollen diagrams from northern Eurasia show a rapid decline of *Artemisia* at the onset of the Holocene (e.g. Hoek, 1997; Andreev and Tarasov, 2013). Treeless steppe areas became tundras, where *Artemisia* lost the competition with typical tundra taxa such as Ericales, Cyperaceae, mosses and lichens. Trees dispersed to the north, and erosion of soils by wind and water diminished because of increasingly dense herbaceous and arboreal vegetation. More stable soil formation occurred

under the temperate humid conditions of the Holocene. In this situation, two phenomena may well have played a crucial role.

Changes in vegetation that result in a decreasing availability of palatable plants with high-Ca biomass and an increase of trees with unpalatable biomass, such as *Pinus sylvestris*, would result in a decline of suitable forage. During the later part of the Allerød period and the early Holocene, *Pinus sylvestris* became a dominant tree species.

Second, during the late Weichselian, large parts of North-West Europe and the North Sea basin were covered by aeolian sands, particularly in its southern stretches (see e.g. Busschers *et al.*, 2007). These sands can be described as quartzitic, which probably held some calcium carbonate, but have an intrinsically low acid-neutralizing capacity after being decalcified (van Breemen *et al.*, 1984; Verstraten *et al.*, 1990; Kooijman *et al.*, 1998). During the later part of the Bølling–Allerød and the early Holocene, soil formation started with concurrent soil acidification, while calcium carbonate was rapidly leached.

Early soil successions on sands with a low acid-neutralizing capacity, such as Dutch inland drift sands and coastal dunes (both lime-rich and lime-poor), have been studied extensively (Sevink, 1991; Emmer, 1995; Kooijman *et al.*, 1998). Under the current temperate climatic conditions, acidification proceeds rapidly, *i.e.* within a few centuries pH values are such that podzolization starts. Ca contents of the above-ground biomass of dicots are known to depend strongly on pH and Ca availability in the soil (Thompson *et al.*, 1997) and rapid soil acidification is thus accompanied with a decline in Ca content, particularly of the shallow-rooting herbaceous biomass, with an additional change from calciphilous to acidophilous plant species.

Whether resulting from a vegetation change because of rising temperatures or a succession-driven change in soil conditions, or a combination of both, it is likely that the availability of Ca-rich forage declined during the Lateglacial and the early Holocene. In large parts of its range *M. giganteus* then became extinct, with some remnant populations remaining during the Younger Dryas in secluded environments. *M. giganteus* had the largest antlers of any known cervid. The extreme size and weight of these antlers made the animals vulnerable to a decline in the mineral content of their food. Due to the allometric relationship between antler size and body size in cervids (Gould, 1974), *M. giganteus* would have suffered the most from the deterioration in food quality (Moen *et al.*, 1999), compared to other (smaller) deer. The rapid decline of *Artemisia* and other calciphilous plant species may have contributed to the Lateglacial *M. giganteus* extirpation. Although speculative, the possible link between soil formation, the decline of *Artemisia* and other calciphilous steppe plants, and the extinction of *M. giganteus* (compare with Barnosky, 1986, regarding the *Betula nana* decline) should be kept in mind in future studies.

Continued survival of *M. giganteus* in western Siberia

M. giganteus had disappeared from most of Eurasia by the onset of the Holocene, yet survived in the forest steppe of a relatively small area in the Urals/western Siberia until ca. 7700 cal a BP (Stuart *et al.*, 2004; van der Plicht *et al.*, 2015). Compared to the abrupt and intense Lateglacial changes of the Pleistocene/Holocene transition there were no major climatic changes during this period.

The areas for which these late *M. giganteus* were reported are in the northern border zone of the Western Siberian loess

belt. Soils in this northern extension of the loess belt, although still classified as Chernozems, today exhibit decalcified and more acid upper soil horizons, and distinct clay translocation. Further north, even more pronounced soil acidification is described in the form of predominantly Podzoluvisols that are polygenetic in nature, with an earlier stage of development under less severe leaching conditions.

For southern Siberia, Chytrý *et al.* (2007) describe a clear pattern in the current plant composition that is directly linked to the extent of soil acidification and leaching, with steppe vegetation occurring on dry calcium-rich soils, while tundra and forest are marked by distinctly more leached and acid soils in areas with higher precipitation and lower evapotranspiration. Chytrý *et al.* (2007) additionally mention that the dry cold steppe was the habitat of large Pleistocene herbivores.

Evidently, the temporal trends in soil development in the loess belt as described above were associated with a concurrent change in vegetation composition, notably the increase of calcifuge plants and the disappearance of calciphilous dry steppe elements. For the southern part of the West Siberian Lowland, Blyakharchuk (2003) dated the change from steppe to dense forest and associated changes in soil development to ca. 7000 BP. For the Russian Altai, Schlütz and Lehmkuhl (2006) reported drier climatic conditions for the Late Pleistocene and early Holocene, with steppe vegetation and lime-rich soils, followed by soil formation reflecting wetter climatic conditions evidenced by palaeosols from the mid-Holocene (ca. 7000 BP).

Based on these studies, we hypothesize that the continued availability of lime-rich soils and calciphilous plant species in Western Siberia may have played a role in the long survival of *M. giganteus* in the region. Furthermore, we propose that mid-Holocene soil degradation, related vegetation changes, and decreased food quality may well have induced the loss of *M. giganteus* from western Siberia around 7000 BP and contributed to the final extinction of the species. Our hypothesis does not exclude other factors in the extinction dynamics of *M. giganteus*, such as loss of genetic diversity and adaptive flexibility (Lister and Stuart, 2008), 'overkill' by human hunters and the negative effects of the Younger Dryas cold phase (Stuart *et al.*, 2004).

Conclusions

We present the first palaeoecological study of a food residue from a *M. giganteus* molar. *Artemisia* appeared to be the most frequent pollen type and this might well reflect a nutritional-based selection of food (high nutrient level; calcium, phosphorous in *Artemisia*) by this individual *M. giganteus*. We speculate about a possible link between the regional extirpation and global extinction of *M. giganteus* and a decline of the calcium content of soils and calciphilous plant species during the Lateglacial period and during the early Holocene.

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Abbreviations. AMS, accelerator mass spectrometer; EA, elemental analyser; MS, mass spectrometer.

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