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# Fifty thousand years of Arctic vegetation and megafaunal diet

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**Although it is generally agreed that the Arctic flora is among the youngest and least diverse on Earth, the processes that shaped it are poorly understood. Here we present 50 thousand years (kyr) of Arctic vegetation history, derived from the first large-scale ancient DNA metabarcoding study of circumpolar plant diversity. For this interval we also explore nematode diversity as a proxy for modelling vegetation cover and soil quality, and diets of herbivorous megafaunal mammals, many of which became extinct around 10 kyr BP (before present). For much of the period investigated, Arctic vegetation consisted of dry steppe-tundra dominated by forbs (non-graminoid herbaceous vascular plants). During the Last Glacial Maximum (25–15 kyr BP), diversity declined markedly, although forbs remained dominant. Much changed after 10 kyr BP, with the appearance of moist tundra dominated by woody plants and graminoids. Our analyses indicate that both graminoids and forbs would have featured in megafaunal diets. As such, our findings question the predominance of a Late Quaternary graminoid-dominated Arctic mammoth steppe.**

It can be argued that Arctic vegetation during the proximal Quaternary (the last circa 50 kyr) is less well understood than the ecology and population dynamics of the mammals that consumed it, despite the overall uniformity and low floristic diversity of Arctic vegetation[1,2]. Analyses of vegetation changes during this interval have been based mainly on fossil pollen. Although highly informative, records tend to be biased towards high pollen producers such as many graminoids (grasses, sedges and rushes) and *Artemisia*, which can obscure the abundance of other forms such as many insect-pollinated forbs[1]. Arctic pollen records are rarely comprehensively identified to species level, which underestimates actual diversity[3]. These problems are to some extent ameliorated by plant macrofossil studies (for example, ref. 4), which may provide detailed records of local vegetation. However, macrofossil studies are far less common, have their own taxonomic constraints, and usually cannot provide quantitative estimates of abundance.

In recent years, a complementary approach has emerged that uses plant and animal ancient DNA preserved in permafrost sediments[5]. Such environmental DNA[6] does not derive primarily from pollen, bones or teeth, but likely from above- and below-ground plant biomass, faeces, discarded cells and urine preserved in sediments[7–9]. Like macrofossils, environmental DNA appears to be local in origin[6,10–12] and, in principle, the survival of a few fragmented DNA molecules is sufficient for retrieval and taxonomic identification[13].

Environmental DNA can supply the fraction of the plant community not readily identifiable by pollen analysis and, to some extent, macrofossils, particularly in vegetation dominated by non-woody growth forms[7].

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For most plant groups, DNA permits identification at lower taxonomic levels than pollen[14]. In addition, environmental DNA records have proven to reflect not only the qualitative but also the quantitative diversity of above-ground plant[12] and animal taxa[9], as determined from modern subsurface soils.

Leaching of DNA through successive stratigraphic zones may be an issue in temperate conditions[9,11] but not in permafrost[6] or in sediments that have only recently thawed[15]. Re-deposition of sediments and organics can confound results, which is also the case for pollen and macrofossils[7,16], but can be avoided and accounted for by careful site selection and by excluding rare DNA sequence reads[16]. For Quaternary permafrost settings, at least, taphonomic bias due to differences in DNA survival across plant groups does not appear to be of concern (see Methods section 4.0 on taphonomy), as has been shown by a comparative permafrost ancient DNA study of plants and their associated fungi[8].

## Reconstruction of Arctic vegetation from permafrost

We collected 242 sediment samples from 21 sites across the Arctic (Fig. 1 and Extended Data Table 1). Ages were determined by accelerator mass spectrometry radiocarbon ($^{14}$C) dating, and are reported here in thousands of calibrated (calendar) years BP (Extended Data Fig. 1 and Supplementary Data 1). We sequenced the short P6 loop sequence of the *trn*L plastid (gene encoding chloroplast transfer RNA for leucine) region and a part of the ITS1 spacer region through metabarcoding (Methods section 3.0), generating a total of 14,601,839 *trn*L plant DNA sequence reads and 1,652,857 internal transcribed spacer (ITS) reads. Reads were identified by comparison with (1) the Arctic *trn*L taxonomic reference library[14], which we extended with ITS sequences for three families; (2) a new north boreal *trn*L taxonomic reference library constructed by sequencing 1,332 modern plant samples representing 835 species; and (3) GenBank, using the program ecoTag (Supplementary Data 2 and Methods section 3.0). Basic statistics, *in silico* analyses, and additional experiments were carried out to check data reliability (Extended Data Fig. 2 and Extended Data Table 2). We grouped the identified molecular operational taxonomic units (MOTUs) into three distinct intervals (Fig. 2a): (1) pre-Last Glacial Maximum (LGM) (50–25 kyr BP), a period of fluctuating climate; (2) LGM (25–15 kyr BP), a period of constantly cold and dry conditions; and (3) post-LGM (15–0 kyr BP), which includes the current interglacial, characterized by relatively higher temperatures[17].


**Figure 1 | Sample localities.** A total of 242 permafrost samples were collected from 21 sites, shown by green dots (1–21). Eight ancient megafauna gut and coprolite samples (A–H) are shown by grey hollow circles, and seven modern nematode localities are shown by grey hollow triangles (a–g). (1) Anadyr, (2) Baskura Peninsula, (3) Bol'shaya Balakhnaya, (4) Buor Khaya, (5) Cape Sabler, (6) Colesdalen, (7) Duvanny Yar, (8) Endalen, (9) Federov Island, (10) Goldbottom, (11) Khatanga, (12) Maine River, (13) Ovrazhny Peninsula, (14) Purgatory, (15) Quartz Creek, (16) Ross Mine, (17) Stevens Village, (18) Stuphallet, (19) Taimyr Lake, (20) Upper Taymyr River, (21) Zagoskin Lake, (A) Drevniy Creek Mammoth, (B) Bison, (C) Lyuba Mammoth, (D) Kolyma Rhino, (E) Last Chance Creek Horse, (F) Churapcha Rhino, (G) Mongochen Mammoth, (H) Finish Creek Valley Mammoth, (a) Blackstone River, (b) Ogilvie Mountains, (c) Eagle Plains South, (d) Eagle Plains North, (e) Little Atlin Lake, (f) Kluane Lake, (g) Carmacks.

## Shifts in plant community composition

To address compositional changes in vegetation across space and time we used a generalized linear model and permutational multivariate analysis of variance (PERMANOVA) (Supplementary Data 3 and Methods section 6.0). We find that (1) the composition of plant MOTU assemblages differed significantly across the three intervals (pseudo-$F = 6.77$, $P < 0.001$, Extended Data Fig. 3a–e), with pre-LGM and post-LGM plant assemblages differing the most (Extended Data Fig. 3f); (2) the greater the spatial distance separating a pair of samples within each time period, the less similar their composition ($P < 0.001$); and (3) LGM assemblages were the most homogeneous across space and post-LGM assemblages were the most heterogeneous (Fig. 2).

LGM pollen spectra show high floristic richness compared to other intervals (for example, ref. 1). This is due to the limited occurrence of woody taxa with high pollen production, which in turn proportionately emphasizes less-productive taxa. By contrast, our DNA data reveal that plant diversity was lowest during LGM relative to other intervals (Fig. 2a). Plant assemblages became more similar to each other and the estimated number of MOTUs decreased from pre-LGM to LGM (Fig. 2a), with many taxa absent that had previously been well represented

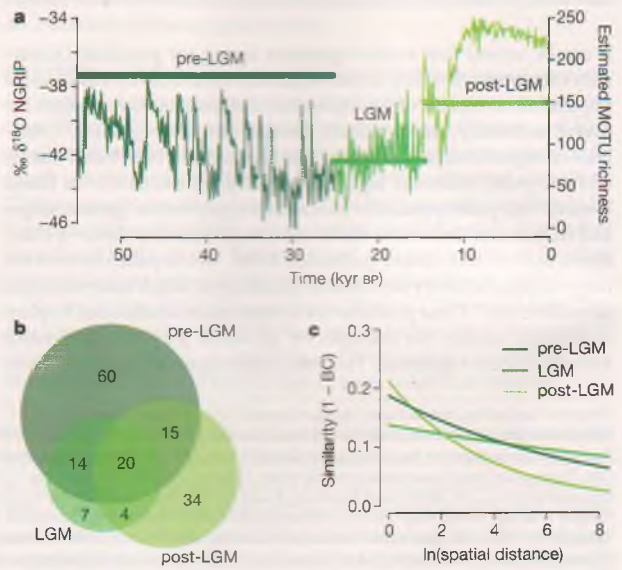


**Figure 2 | Taxonomic diversity of Arctic plant assemblages during the last 50 kyr.** Taxon composition was estimated by high-throughput sequencing of DNA from 242 permafrost samples. A total of 154 MOTUs were detected. **a**, Index of ambient temperature (continuous line; oxygen isotope concentration, North Greenland Ice Core Project, NGRIP[50]) and estimated MOTU number (horizontal bars; second-order jackknife) are shown for three palaeoclimatic periods: pre-LGM (>25 kyr BP, $n = 149$), LGM (25–15 kyr BP, $n = 32$) and post-LGM (<15 kyr BP, $n = 61$). **b**, MOTU counts recorded uniquely in each palaeoclimatic period and shared among periods. **c**, Modelled decline in similarity (1 − Bray–Curtis (BC) dissimilarity) between pairs of plant assemblages from the same palaeoclimatic period in relation to the spatial distance separating them.

(Fig. 2b). In addition, although the LGM flora was largely a subset of the pre-LGM flora, the post-LGM flora was different (Fig. 2b), with pronounced geographic differentiation (Fig. 2c).

## Steppe–tundra

Owing to the low taxonomic resolution of previously published vegetation reconstructions, it remains undetermined whether Arctic vegetation during the last part of the Quaternary was a form of tundra or more like steppe (for example, refs 18, 19). Small-scale contemporary analogues range from low-productivity fellfields and cryoxeric steppe communities to more productive dry Arctic steppe-to-tundra gradients. Our sediment DNA plant sequence data from ~50–12 kyr BP encompass taxa that typify both tundra and Arctic steppe environments. These include taxa that are today typical of dry and/or disturbed sites (for example, *Bromus pumpellianus*, *Artemisia frigida*, *Plantago canescens*, *Anemone patens*), saline soils (*Puccinellia*, *Armeria*), moist habitats (*Caltha*) and rocky or fellfield habitats (*Dryas*, *Draba*), plus a woody component dominated by *Salix* (Supplementary Data 4 and 5). A spatial and/or temporal mosaic of plant communities is indicated (Methods section 6.0), as is seen in floristically rich macrofossil records[4]. The most common MOTU in the pre-LGM and LGM samples is Anthemideae group 1 (*Artemisia*, *Achillea*, *Chrysanthemum*, *Tanacetum*), which underscores the importance in regional pollen assemblages of Asteraceae in general and *Artemisia* in particular[1]. *Equisetum* and *Eriophorum* are important only in postglacial assemblages, reflecting moister soil conditions. Increases in aquatic taxa (Supplementary Data 4 and 5) also indicate a predominance of moister substrates in the later part of the post-LGM period. These findings indicate a shift from dry steppe-tundra to moist tundra in the early part of the post-LGM period—a change widely reported in other proxy studies.

Nematode assemblage composition is known to change with vegetation cover[20], moisture[21] and organic resource inputs[22]. Therefore, to obtain a complementary proxy for vegetation cover and soil quality, we characterized the soil nematode fauna of contemporary mesic shrub tundra and subarctic steppe on well-drained loess soils in Yukon Territory, Canada (Fig. 1 and Extended Data Table 3). The relative proportion of the nematode families Teratocephalidae and Cephalobidae varied among vegetation types ($P < 0.001$, nested ANOVA), and indicator species analysis[23] confirmed that Teratocephalidae (indicator value = 0.98, $P = 0.001$) and Cephalobidae (indicator value = 0.98, $P = 0.001$) are very good indicators of tundra and steppe vegetation, respectively (Fig. 3).

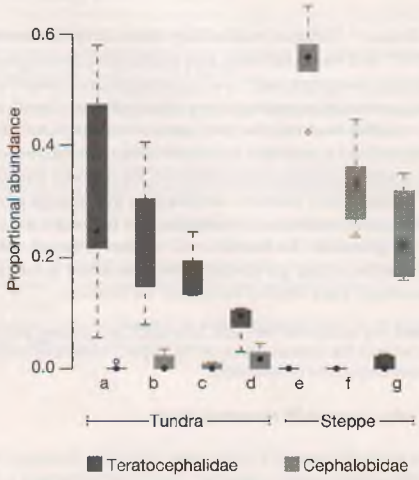


**Figure 3 | Proportional abundance of two families—Teratocephalidae and Cephalobidae—among the total soil nematode community at contemporary tundra and steppe sites in Yukon, Canada.** Teratocephalidae, dark; Cephalobidae, light. Letters a–g correspond to sample localities (Fig. 1). Median (central dot), quartile (box), maximum and minimum (whiskers) and outlying values (points) are shown.

These findings are in agreement with previous studies restricted to subarctic Sweden[24,25] and alpine and subalpine habitats[26,27]. We amplified short DNA sequences from these two taxa from 17 sediment samples analysed for plant DNA from Yukon and northeastern Siberia. We detected Cephalobidae DNA in almost all samples, whereas Teratocephalidae was detected at a higher frequency in samples younger than 10 kyr BP than in the pre-LGM and LGM samples (Extended Data Table 4). These results support our inferences from plant sequence data and indicate a transition from relatively dry tundra and steppe towards more moist tundra during the post-LGM interval.

## Forb dominance and megafaunal diets

To assess structural and functional shifts in the plant assemblages, we investigated temporal changes in the relative abundance of different growth forms. Our DNA results show that pre-LGM vegetation was dominated by forbs, the relative share of which increased during the LGM, whereas graminoids constituted less than 20% of the total read count (Fig. 4a). These results persisted when we corrected for observed modern representational bias[12] (Methods sections 4.0 and 5.3).

Continued forb dominance during the LGM implies that similar proportions of forbs and graminoids were maintained through this period, despite the significant decline in floristic diversity (Fig. 2a, b). Our findings contrast with pollen-based reconstructions, which have emphasized dominance of graminoids in the unglaciated Arctic and adjacent regions, particularly during the LGM, and are exemplified by the widely used term mammoth steppe[19]. Rather, our results show that vegetation was forb-dominated in both overall abundance of MOTUs and in floristic richness (Fig. 4a, b and Extended Data Fig. 3g, h), in agreement with macrofossil data that show a diversity of forbs of mixed ecological preference (for example ref. 4).

We explored whether forbs were prominent in habitats favoured by megafauna by analysing 25 dated (47–20 kyr BP) sediment samples from Main River, Siberia, using *trn*L plastid plant and 16S mitochondrial DNA mammal primers. We found that the mean proportion of forbs was higher in samples from which herbivorous megafaunal DNA had been retrieved ($n = 18$; for example, woolly mammoth, woolly rhinoceros, horse, reindeer and elk) than in samples lacking such DNA ($n = 7$; Fig. 4c and Extended Data Table 5). Although suggestive of co-occurrence of megafauna in forb-dominated settings, these results should be regarded as tentative, and further studies are needed to verify if this is indeed a general trend.

We also investigated whether megafaunal diets revealed the level of forb dominance observed in permafrost sediment samples. Using standardized methods, we genetically characterized intestinal/stomach contents and coprolites recovered from eight specimens of woolly mammoth, woolly rhinoceros, bison and horse from Siberia and Alaska, dated >55–21 kyr BP (Extended Data Table 6 and Methods sections 3.0 and 7.3). Although ingested plant remains are often difficult to identify morphologically, they can be accurately identified[28,29] and roughly quantified[30] using DNA. The majority of these samples are dominated by forbs, which comprise $0.63 \pm 0.12$ of the sequences, compared to $0.27 \pm 0.16$ expressing graminoid sequences (Fig. 4d and Supplementary Data 6). These results suggest that megafaunal species supplemented their diets with high-protein forbs rather than specializing more or less exclusively on grasses.

To confirm the reliability of our *trn*L approach for estimating herbivore diet, we analysed 50 rumen samples of sheep-feed diets with varying proportions of forbs (white clover (*Trifolium repens*)) and graminoids (ryegrass (*Lolium perenne*)) (Methods section 5.4). As seen in Fig. 4e, the Pearson correlation coefficient between the actual fraction of forbs in these diets and the proportion of forbs estimated with the DNA-based approach was highly significant ($r^2 = 0.75$, $P < 10^{-15}$).

## Discussion

Our observations of high forb abundance in the Terminal Pleistocene may merely reflect vegetation response to glacial climates, but there are

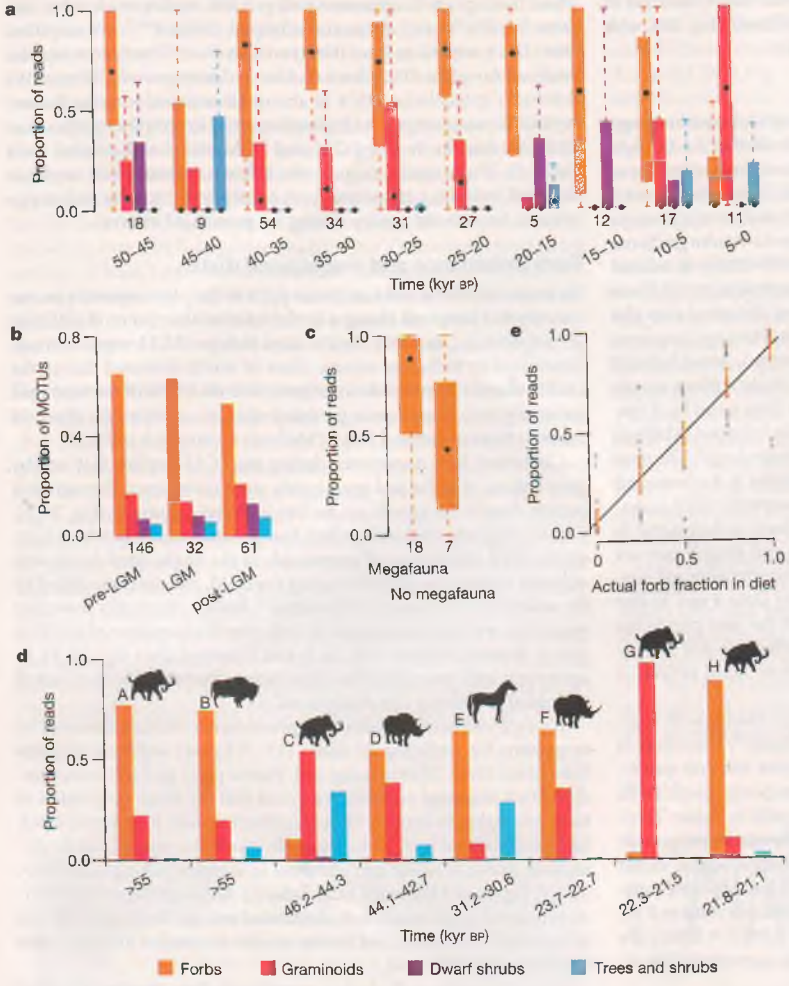


**Figure 4 | Plant growth form composition over time and across sample types, estimated by high-throughput sequencing of DNA from 242 permafrost samples.** **a**, Proportions of DNA reads corresponding to taxa exhibiting different growth forms, binned over 5 kyr time intervals. The analysis included all sediment samples except 21 Svalbard samples and three further samples for which no growth form information was available. **b**, Number of MOTUs exhibiting different growth forms as a proportion of total MOTU richness in all informative samples for each palaeoclimatic period. **c**, The proportional abundance of forbs in samples from Main River, Siberia (dated 47,100–19,850 yr BP) where megafauna were or were not detected. **d**, Proportions of DNA reads corresponding to different growth forms in megafauna diet, determined from analysis of eight gut and coprolite samples from late Quaternary megafauna species (woolly mammoth, woolly rhinoceros, bison and horse). Letters A–H correspond to the individual samples (Fig. 1). The 95.4% calibrated age range of each sample is shown; '> 55' indicates that the sample was too old to provide a finite radiocarbon age. **e**, Reliability of the *trn*L approach for estimating forb and graminoid abundance in diet analyses. Sheep were fed with known amounts of forbs (*Trifolium repens*) and graminoids (*Lolium perenne*), and the rumen content analysed using the same DNA-based approach as implemented above. Grey dots are raw data points, orange dots and lines represent the means and ± standard errors for diets containing different fractions of forbs. The grey line is a linear model fit. Numbers immediately below the columns in **a**, **b** and **c** indicate sample sizes. Median (central dot), quartile (box), maximum and minimum (whiskers) values are shown in **a** and **c**.

other possibilities[1]. An abundant megafauna would have caused significant trampling[31], enhancing gap-based recruitment[32], which could favour forbs[33]. Coupled with nitrogen input from wide-ranging herbivores[34], forbs may out-compete grasses[35]. Furthermore, a diet rich in forbs may help to explain how numerous large animals were sustained; forbs may be more nutrient-rich (for example, ref. 35) and more easily digested[36] than grasses. However, a feedback loop that maintained nutritious and productive forage and supported large mammalian populations in glacial climate regimes may have been impossible to maintain after deglaciation, as C:N ratios increased with global warming[37], and the potential breakdown of the megafauna–forb interaction would have been exacerbated by declining mammalian populations. In contemporary tundra and steppe (the latter often called grasslands), graminoids are generally perceived to be the dominant growth form in large herbivore habitats (for example, refs 38, 39). Our data, which unearth 50 kyr of Arctic vegetation history, call this perception into question.

## METHODS SUMMARY

Plant fragments or soil matrix organics were $^{14}$C-dated using accelerator mass spectrometry and measured ages were converted into calendar years[40]. Permafrost sampling, DNA extraction, PCR amplification and taxon identification (for example, ref. 41) followed established procedures. Most vascular taxa are covered by ref. 42, and nomenclature is provided accordingly; for the remaining taxa nomenclature follows ref. 43. Dissimilarity between plant assemblages was quantified using pairwise Bray–Curtis distance[44]. Variation in assemblage dissimilarity was decomposed using PERMANOVA[45] and visualized using non-metric multidimensional scaling[46,47]. We used a distance decay approach[48] and a generalized linear model to model variation in plant community assemblages over space and time. Growth form composition of communities was compiled from species trait databases[49]. Differences in the trait composition of assemblages in adjacent climatic periods were compared to a null model assuming random assortment from the previous interval. Nematode faunas of 35 contemporary sediment samples were morphologically determined. Presence of two indicator families (Teratocephalidae for tundra and Cephalobidae for steppe) was genetically determined in 17 ancient sediment samples. Megafaunal DNA and faeces and gut content were determined genetically following established methods. For a detailed discussion, see Methods.

**Online Content** Any additional Methods, Extended Data display items and Source Data are available in the online version of the paper; references unique to these sections appear only in the online paper.

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1. Anderson, P. M., Edwards, M. E. & Brubaker, L. B. in *The Quaternary Period in the United States. Developments in Quaternary Science* (eds Gillespie, A. E., Porter, S. C. & Atwater, B. F.) 427–440 (Elsevier, 2003).
2. Murray, D. F. in *Arctic and Alpine Biodiversity: Patterns, Causes and Ecosystem Consequences* (eds Chapin, F. S. & Körner, C.) 21–32 (Springer, 1995).
3. Lamb, H. F. & Edwards, M. E. in *Vegetation History. Handbook of Vegetation Science 7* (eds Huntley, B. & Webb, T. III) 519–555 (Kluwer Academic, 1988).

4. Kienast, F., Schirrmeister, L., Siegert, C. & Tarasov, P. E. Palaeobotanical evidence for warm summers in the East Siberian Arctic during the last cold stage. *Quat. Res.* **63,** 283–300 (2005).
5. Willerslev, E. *et al.* Diverse plant and animal genetic records from Holocene and Pleistocene sediments. *Science* **300,** 791–795 (2003).
6. Haile, J. *et al.* Ancient DNA reveals late survival of mammoth and horse in interior Alaska. *Proc. Natl Acad. Sci. USA* **106,** 22352–22357 (2009).
7. Jørgensen, T. *et al.* A comparative study of ancient sedimentary DNA, pollen and macrofossils from permafrost sediments of northern Siberia reveals long-term vegetational stability. *Mol. Ecol.* **21,** 1989–2003 (2012).
8. Lydolph, M. C. *et al.* Beringian paleoecology inferred from permafrost-preserved fungal DNA. *Appl. Environ. Microbiol.* **71,** 1012–1017 (2005).
9. Andersen, K. *et al.* Meta-barcoding of 'dirt' DNA from soil reflects vertebrate biodiversity. *Mol. Ecol.* **21,** 1966–1979 (2012).
10. Parducci, L. *et al.* Glacial survival of boreal trees in northern Scandinavia. *Science* **335,** 1083–1086 (2012).
11. Haile, J. *et al.* Ancient DNA chronology within sediment deposits: are paleobiological reconstructions possible and is DNA leaching a factor? *Mol. Biol. Evol.* **24,** 982–989 (2007).
12. Yoccoz, N. G. *et al.* DNA from soil mirrors plant taxonomic and growth form diversity. *Mol. Ecol.* **21,** 3647–3655 (2012).
13. Willerslev, E. & Cooper, A. Ancient DNA. *Proc. R. Soc. Lond. B* **272,** 3–16 (2005).
14. Sønstebø, J. H. *et al.* Using next-generation sequencing for molecular reconstruction of past Arctic vegetation and climate. *Mol. Ecol. Resour.* **10,** 1009–1018 (2010).
15. Hebsgaard, M. B. *et al.* The farm beneath the sand—an archaeological case study on ancient 'dirt' DNA. *Antiquity* **83,** 430–444 (2009).
16. Arnold, L. J. *et al.* Paper II - Dirt, dates and DNA: OSL and radiocarbon chronologies of perennially frozen sediments in Siberia, and their implications for sedimentary ancient DNA studies. *Boreas* **40,** 417–445 (2011).
17. Hopkins, D. M. in *Paleoecology of Beringia* (eds Hopkins, D. M., Matthews, J. V. Jr, Schweger, C. E. & Young, S. B.) 3–28 (Academic, 1982).
18. Ritchie, J. C. & Cwynar, L. C. in *Paleoecology of Beringia* (eds Hopkins, D. M., Matthews, J. V. Jr, Schweger, C. E., Young, S. B. & Stanley, V.) 113–126 (Academic, 1982).
19. Guthrie, R. D. *Frozen Fauna of the Mammoth Steppe* (Univ. Chicago Press, 1990).
20. Yeates, G. W. Diversity of nematode faunae under three vegetation types on a pallic soil in Otago, New Zealand. *NZ J. Zool.* **23,** 401–407 (1996).
21. Sohlenius, B. Influence of climatic conditions on nematode coexistence — a laboratory experiment with a coniferous forest soil. *Oikos* **44,** 430–438 (1985).
22. Yeates, G. W. Nematodes as soil indicators: functional and biodiversity aspects. *Biol. Fertil. Soils* **37,** 199–210 (2003).
23. Dufrêne, M. & Legendre, P. Species assemblages and indicator species: the need for a flexible asymmetrical approach. *Ecol. Monogr.* **67,** 345–366 (1997).
24. Ruess, L., Michelsen, A. & Jonasson, S. Simulated climate change in subarctic soils: responses in nematode species composition and dominance structure. *Nematology* **1,** 513–526 (1999).
25. Sørensen, L. I., Mikola, J., Kytoviita, M.-M. & Olofsson, J. Trampling and spatial heterogeneity explain decomposer abundances in a sub-Arctic grassland subjected to simulated reindeer grazing. *Ecosystems* **12,** 830–842 (2009).
26. Popovici, I. & Ciobanu, M. Diversity and distribution of nematode communities in grasslands from Romania in relation to vegetation and soil characteristics. *Appl. Soil Ecol.* **14,** 27–36 (2000).
27. Hoschitz, M. & Kaufmann, R. Nematode community composition in five alpine habitats. *Nematology* **6,** 737–747 (2004).
28. Poinar, H. N. *et al.* Molecular coproscopy: dung and diet of the extinct ground sloth *Nothrotheriops shastensis*. *Science* **281,** 402–406 (1998).
29. Hofreiter, M. *et al.* A molecular analysis of ground sloth diet through the last glaciation. *Mol. Ecol.* **9,** 1975–1984 (2000).
30. Soininen, E. M. E. *et al.* Analysing diet of small herbivores: the efficiency of DNA barcoding coupled with high-throughput pyrosequencing for deciphering the composition of complex plant mixtures. *Front. Zool.* **6,** 16 (2009).
31. Zimov, S. A., Zimov, N. S., Tikhonov, A. N. & Chapin, F. S. I. Mammoth steppe: a high-productivity phenomenon. *Quat. Sci. Rev.* **57,** 26–45 (2012).
32. Owen-Smith, N. Pleistocene extinctions: the pivotal role of megaherbivores. *Paleobiology* **13,** 351–362 (1987).
33. Austrheim, G. & Eriksson, O. Recruitment and life-history traits of sparse plant species in subalpine grasslands. *Can. J. Bot.* **81,** 171–182 (2003).
34. Wardle, D. A. & Bardgett, R. D. Human-induced changes in large herbivorous mammal density: the consequences for decomposers. *Front. Ecol. Environ.* **2,** 145–153 (2004).
35. Güsewell, S. N P ratios in terrestrial plants: variation and functional significance. *New Phytol.* **164,** 243–266 (2004).
36. Cornelissen, J. *et al.* Leaf digestibility and litter decomposability are related in a wide range of subarctic plant species and types. *Funct. Ecol.* **18,** 779–786 (2004).
37. McLauchlan, K. K., Williams, J. J., Craine, J. M. & Jeffers, E. S. Changes in global nitrogen cycling during the Holocene epoch. *Nature* **495,** 352–355 (2013).
38. van der Wal, R. Do herbivores cause habitat degradation or vegetation state transition? Evidence from the tundra. *Oikos* **114,** 177–186 (2006).
39. Bråthen, K. A. *et al.* Induced shift in ecosystem productivity? Extensive scale effects of abundant large herbivores. *Ecosystems* **10,** 773–789 (2007).
40. Reimer, P. J. *et al.* IntCal09 and Marine09 radiocarbon age calibration curves, 0–50,000 years cal BP. *Radiocarbon* **51,** 1111–1150 (2009).
41. Taberlet, P. *et al.* Power and limitations of the chloroplast *trn*L (UAA) intron for plant DNA barcoding. *Nucleic Acids Res.* **35,** e14 (2007).
42. Elven, R., Murray, D. F., Razzhivin, V. Y. & Yurtsev, B. A. *Annotated Checklist of the Panarctic Flora (PAF)* http://nhm2.uio.no/paf/ (Natural History Museum, Univ. Oslo, 2011).
43. Sayers, E. W. *et al.* Database resources of the National Center for Biotechnology Information. *Nucleic Acids Res.* **37,** D5–D15 (2009).
44. Bray, J. R. & Curtis, J. T. An ordination of the upland forest communities of southern Wisconsin. *Ecol. Monogr.* **27,** 325–349 (1957).
45. Anderson, M. J. A new method for non-parametric multivariate analysis of variance. *Austral. Ecol.* **26,** 32–46 (2001).
46. Shepard, R. N. The analysis of proximities: multidimensional scaling with an unknown distance function. I. *Psychometrika* **27,** 125–140 (1962).
47. Shepard, R. N. The analysis of proximities: multidimensional scaling with an unknown distance function. II. *Psychometrika* **27,** 219–246 (1962).
48. Nekola, J. C. & White, P. S. The distance decay of similarity in biogeography and ecology. *J. Biogeogr.* **26,** 867–878 (1999).
49. Klotz, S., Kühn, I. & Durka, W. *BIOLFLOR* (Bundesamt für Naturschutz, 2002).
50. NGRIP dating group, 2008. IGBP PAGES/World Data Center for Paleoclimatology Data Contribution Series # 2008-034. NOAA/NCDC Paleoclimatology Program, Boulder CO, USA. (2008).


**Supplementary Information** is available in the online version of the paper.

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**Author Information** All the raw and filtered data concerning plants, nematodes, megafauna and sheep diet are available either from Extended Data and Supplementary Data, or from the Dryad Digital Repository: http://doi.org/10.5061/dryad.ph8s5. Reprints and permissions information is available at www.nature.com/reprints. The authors declare competing financial interests: details are available in the online version of the paper. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to E.W. (ewillerslev@snm.ku.dk).