Collapse of the mammoth-steppe in central Yukon as revealed by ancient environmental DNA

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The temporal and spatial coarseness of megafaunal fossil records complicates attempts to disentangle the relative impacts of climate change, ecosystem restructuring, and human activities associated with the Late Quaternary extinctions. Advances in the extraction and identification of ancient DNA that was shed into the environment and preserved for millennia in sediment now provides a way to augment discontinuous palaeontological assemblages. Here, we present a 30,000-year sedimentary ancient DNA (sedaDNA) record derived from loessal permafrost silts in the Klondike region of Yukon, Canada. We observe a substantial turnover in ecosystem composition between 13,500 and 10,000 calendar years ago with the rise of woody shrubs and the disappearance of the mammoth-steppe (steppe-tundra) ecosystem. We also identify a lingering signal of Equus sp. (North American horse) and Mammutthus primigenius (woolly mammoth) at multiple sites persisting thousands of years after their supposed extinction from the fossil record.
Humans evolved and dispersed throughout the continents in an epoch dominated by giant terrestrial mammals. Megafauna (body mass ≥ 44 kg) only exist in comparable densities today within small refugia (mainly Africa) where most of their populations are in states of decline, and many of these species are threatened or endangered. The ecological reverberations associated with the Late Pleistocene (130,000–11,700 years before present [BP]) loss of approximately 101 of 150 genera of Earth’s largest terrestrial animals is thought to have restructured the terrestrial biosphere, impacting vegetation composition and diversity, biogeochemistry, and climate feedback systems. This rearrangement of terrestrial ecosystems, including massive biogeographic range shifts, local extirpations, and widespread extinctions, is argued by some to be the direct result of rapid climate change and attendant environmental feedbacks during the late Pleistocene. Others contend that factors unique to the last glacial period are to blame, such as the coincident dispersal of a new predator—Homo sapiens. It is likely that no single factor can account for the staggered magnitudes of such losses globally, but rather that each ecosystem experienced a variable set of locally complicating pressures. Taphonomic processes challenge attempts to tease apart the palaeoecological nuances of the late Quaternary extinctions (LQE), necessitating relatively precise estimates for megafaunal population declines and last appearance dates, for timings of ecological shifts (e.g. changes in plant community structure), as well as for robust archaeological evidence of anthropogenic impacts.

In the case of eastern Beringia (unglaciated regions of Yukon, Canada and Alaska, U.S.A.), Guthrie and Mann et al. argue that the expansion of woody shrubs and peatlands following an increased moisture regime during the late Pleistocene was the leading contributor to the loss of megafaunal grazers, including mammoth, horse, and bison. By contrast, Zimov et al. contend that megafaunal extirpations preceded a rise in woody shrubs, with the loss of keystone megaherbivores having led to the disappearance of the graminoid and forb dominated, mammoth-steppe biomes. Disentangling the relative timings of ecological restructuring versus megafaunal population declines often exceeds the resolution of Quaternary records.

Here, we present hybridization capture enriched sedimentary ancient DNA (sedaDNA) data derived from loessal silts preserved in permafrost and recovered from four sites in the Klondike goldfields—an unglaciated region of west-central Yukon Territory, Canada—dating to ca. 30,000–4000 calibrated (calendar) years before present (cal BP). This work builds on the methodological results reported in Murchie et al. in which North American horse (Equus sp. and Mammuthus primigenius) DNA was unexpectedly identified in a permafrost sample dating to ~9700 cal BP. This post-dates the last macrofossil evidence (such as bones, teeth, and soft-tissues) of these animals in Alaska by some 3300 years. Such a late date is supported by some 3300 years. Such a late date is supported by macrofossil evidence (such as bones, teeth, and soft-tissues) of the last Glacial Maximum (LGM, 26,500–19,000 cal BP) which in eastern Beringia included the loss of Homotherium latidens (scimitar-toothed cat) and Arctodus simus (short-faced bear). The second wave occurred during the late Pleistocene was the leading contributor to the loss of megafaunal grazers, including mammoth, horse, and bison. By contrast, Zimov et al. contend that megafaunal extirpations preceded a rise in woody shrubs, with the loss of keystone megaherbivores having led to the disappearance of the graminoid and forb dominated, mammoth-steppe biomes. Disentangling the relative timings of ecological restructuring versus megafaunal population declines often exceeds the resolution of Quaternary records.

Background

Late Quaternary losses on the eastern mammoth-steppe. Megafaunal extinctions and extirpations after 40,000 cal BP in the Holarctic (i.e., northern Eurasia, Beringia, and North America) arguably followed a two-stage pattern suggested by dated macrofossils (Supplementary Table 1). The first wave seems to have occurred prior to or during the Last Glacial Maximum (LGM, 26,500–19,000 cal BP) which in eastern Beringia included the loss of Homotherium latidens (scimitar-toothed cat) and Arctodus simus (short-faced bear). The second wave occurred during the late Pleistocene was the leading contributor to the loss of megafaunal grazers, including mammoth, horse, and bison. By contrast, Zimov et al. contend that megafaunal extirpations preceded a rise in woody shrubs, with the loss of keystone megaherbivores having led to the disappearance of the graminoid and forb dominated, mammoth-steppe biomes. Disentangling the relative timings of ecological restructuring versus megafaunal population declines often exceeds the resolution of Quaternary records.

Beringia’s environment during the late Pleistocene has been characterized as a graminoid and forb-dominated steppe-tundra mosaic generally referred to as the mammoth-steppe. It is thought to have been the most extensive terrestrial biome on Earth during the late Pleistocene, stretching from the Siberian Peninsula eastward across Eurasia and into Canada, although the extent and character of this ecosystem remains controversial for some. This paradoxically productive high-latitude mosaic biome supported a diverse abundance of large bodied fauna, facilitating higher biotic productivity (energy and nutrient turnover) than many habitats existing at high latitudes today.

Owen-Smith proposed the keynote herbivore hypothesis based on the ecology of extant African mega fauna to explain the role of megaherbivores in transforming vegetation structure and composition. Nutrients locked in leaves and stems are liberated when used by fauna, accelerating biogeochemical cycling. In this top-down model, megafauna are critical for maintaining and promoting biodiversity in open-mosaic environments, and for controlling the abundance of woody vegetation that can limit biodiversity. A severe reduction of these mega fauna engines is proposed to have resulted in the conversion of mosaic, steppe grasslands and wood pastures to more uniform forests and prairies, shrinking mosaic ecotones—high productivity transition areas between biological communities—thereby reducing the carrying capacity of terminal Pleistocene environments. This in turn could have led to a positive-feedback response wherein diminishing populations of megafauna were increasingly unable to control woody shrub...
expansion, further reducing the biotic productivity of the mammoth-steppe.

Alternatively, an increasing moisture regime during the Bølling–Allerød interstadial (ca. 14,690–12,890 cal BP) is argued to have caused the rise of mesic-adapted woody shrubs that were highly defended against herbivory, replacing the diet of Pleistocene grazers (woolly mammoth, steppe bison, and horse) along with the paludification of Beringia (the spread of peatlands). In this line of bottom-up arguments, climate change and attendant environmental feedbacks led to the disappearance of the mammoth-steppe in eastern Beringia, along with the megafauna it supported.

Power and limits of sedimentary ancient DNA. Much of the LQE debate has been limited by the inability of dated macrofossils (primarily from detrital contexts) to convey the spatio-temporal resolution necessary to untangle the causative versus correlative ecological transformations associated with the Pleistocene-Holocene transition. Molecular (micro) methods are increasingly able to augment discontinuous macrofossil records. This can aid in identifying cryptic populations (or ghost ranges), independently assess population declines, and estimate the timings of functional extinctions/extirpations—the point at which undercrowding and inbreeding depression lead to a loss of fitness through Allee effects to the degree that a species no longer significantly contributes to ecosystem functioning, becoming a trace presence in records before completely disappearing.

Ancient environmental DNA (eDNA) is a powerful method for directly assessing the local presence of animals, plants, fungi, and microbota through time. Most eDNA is quickly metabolized by bacteria or otherwise degraded through a variety of chemical and physical processes. SedaDNA (referring to a subset of ancient eDNA sample types) can survive these degradative processes, even in the absence of visible fossils, because cellular material can bind to sedimentary minerals, protecting these molecular fragments for millennia, especially when perennially frozen. Sediment samples as small as 100 mg can contain tens of billions of DNA fragments from all forms of life in a local ecosystem. However, there are several SedaDNA challenges to be aware of: (1) determining whether the recovered SedaDNA is stratigraphically accurate; (2) whether the wet-lab recovery and targeting strategy, genetic reference databases, and taxon assignment approach (bioinformatic parameters) can accurately assess the breadth of eDNA or is prone to false-positive/negative assignments; (3) assessing whether eDNA abundance retains a correlation with a population’s living biomass; and (4) determining the degree to which sedimentary inhibitors or differential degradation may bias the SedaDNA signal. Of particular importance for the kinds of permafrost SedaDNA analyzed in this report are the factors of reworked SedaDNA and leaching.

Perennially frozen SedaDNA has the potential to undergo erosion and redeposition while remaining chemically intact. Arnold et al. found evidence of reworked periglacial sediments in high-energy fluvial contexts within large catchments and local thermokarst deposits. They caution against sampling from settings where DNA can be readily reworked and redeposited within younger materials. In this study, we targeted loessal silts to mitigate the potential for fluvial reworking, although aeolian processes are certainly capable of reworking nanoscale SedaDNA complexes. Further, there is evidence throughout the Klondike of an early Holocene thaw unconformity that Mahony identified at Upper Goldbottom and Upper Quartz (among other...
sites) that presents the possibility for the localized reworking of sedaDNA during our early Holocene core samples. The Lucky Lady II section by contrast is continuous from >16,500–8500 cal BP, sits toward the middle of a broad valley, and shows no evidence of erosion or redeposition by slope wash or thermokarst-induced slumping—suggesting that reworked early Holocene sedaDNA is of less concern at the Lucky Lady II site.

The vertical movement of free DNA has been found to be negligible in perennial frozen settings91. Studies have found synchronous palaeoecological shifts when comparing palynological and macrofossil evidence with sedaDNA reconstructions101,102, in addition to age-dependent DNA damage patterns103–105. Leaching is not considered to be a problem in perennally frozen sediment because there is minimal movement of liquid water33,37,102,106–108.

### Results

#### Age-modelling and palynology

Age-depth models were first reported for cores used in this study by Sadoway109 and Mahony100. To refine the chronologies of these records we developed Bayesian age-depth models for each site using Oxcal v.4.4.2110 and the IntCal20 calibration curve111 (Supplementary Figs. 5–7 for Bayesian age-models. Dates reported as calendar/calibrated years before present (cal BP).

| Site               | Corea | IDb | Calibrated age-modelc (2σ) | Inputd | PalaeoChip mapped & MEGAn assignede |
|--------------------|-------|-----|-----------------------------|--------|-----------------------------------|
| **Bear Creek**     |       |     |                             |        |                                   |
|                   |       |     | Median From To               |        |                                   |
| Bear Creek         | BC 4-2B PHP-1 | ≥30,000 | 1.05 | 5,650,809 | 241,143 (4.27%)                  |
| Lucky Lady II      |       |     |                             |        |                                   |
|                   |       |     | Median From To               |        |                                   |
| Lucky Lady II      | LL2S-189-E PHP-2 | 8707 | 8919 | 8637 | 0.9 | 8,667,496 | 9,908 (0.11%)                  |
| Upper Goldbottom   |       |     |                             |        |                                   |
|                   |       |     | Median From To               |        |                                   |
| Upper Goldbottom   | MM12-118B PHP-11 | 9,246 | 8698 | 8637 | 2.35 | 7,983,035 | 541,521 (6.78%)                  |
| Upper Quartz       |       |     |                             |        |                                   |
|                   |       |     | Median From To               |        |                                   |
| Upper Quartz       | MM12-QC-10 PHP-19 | 3849 | 3999 | 3727 | 1.5 | 9,244,928 | 190,555 (2.06%)                  |

**Table 1 SedaDNA permafrost samples.**

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|--------------------|-------|-----|-----------------------------|--------|-----------------------------------|
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#### SedaDNA palaeoecology

Through the targeted capture36 of organelle eDNA preserved in loessal permafrost silts (Supplementary Fig. 4), *Bison priscus* (steppe bison), *Mammuthus primigenius* (woolly mammoth), *Equus* sp. (specifically limited to caballine horse), and *Lagopus lagopus* (willow ptarmigan) constitute most of the identifiable DNA reads of direct interest within Animalia (Fig. 2). This is in addition to less abundant organisms such as *Rangifer tarandus* (caribou/reindeer), and *Ovis sp.* (likely Dall sheep). Many reads expectedly lack taxonomic specificity at species and genus ranks (as many regions of the mitochondrial genome are variably conserved), and as such a large portion of reads could only be confidently assigned to higher ranks such as Caprinae, Pecora, Perissodactyla, and Elephantidae. In some cases, such as with hits to order Perissodactyla and superorder Atherotheria, we can be confident that they represent *Equus* (or its familial relative, *Haringtonhippus* [stilt-legged horse]), and *Mammuthus*, respectively, as unique members of their clades in the late Quaternary record of this region. There is also a low biomolecular signal from predators in this dataset including *Canis lupus* (grey wolf) and *Martes sp.* (marten). A variety of rodents were identified, including *Urocitellus* sp. (likely arctic ground squirrel), *Microtus xanthognathus* (taiga vole), and *Dicrostonyx groenlandicus* (northern collared lemming). Human DNA was identified despite not being targeted with the PalaeoChip baits. However, human DNA was also observed in the
negative controls. We do not consider this human signal to be reliable without further investigation.

Overall, this taxonomically rich sedaDNA dataset reflects a gradual decline in the megafauna signal through time (Fig. 3). Elephantidae is one of the first to decrease in sedaDNA abundance after ~20,000 cal BP. This is followed by declining signals for Bovidae and Equidae (Fig. 2), until a punctuated decrease occurs near the Pleistocene-Holocene transition as their sedaDNA signals almost disappear while those for *Aelos alces* (moose) and *Cervus sp.* (likely *Cervus canadensis* [elk/wapiti]) enter the dataset. Our admittedly temporarily coarse set of permafrost samples suggests a delay in the disappearance of megafaunal grazing species in the Klondike to between ca. 13,000–10,000 cal BP, but we also observe a lag in the final appearance of *Equus* and *Mammothus* sedaDNA as late as ca. 6000 cal BP. Cores from Lucky Lady II, Upper Goldbottom, and Upper Quartz retain 100+ DNA sequences assignable to those taxa well beyond their last dated macrofossils (Fig. 2).

Plant sedaDNA clearly reflects a major environmental turnover between 13,500–10,000 cal BP (Figs. 3–4). Pleistocene graminoids (grass-like, herbaceous [non-woody] plants) such as Poaceae (grasses), Cyperaceae (sedges), along with a variety of forbs (herbaceous flowering plants) such as *Artemisia* (sagebrush), *Lupinus* (lupine), *Saxifraga* (rockfoil), *Papaver* (poppy), and *Ranunculus* (buttercup) were identified in relative sedaDNA abundance from 30,000–13,500 cal BP, Woody taxa such as *Salix* (willow), *Populus* (poplar), *Betula* (birch), *Rhododendron*, *Arctous* (bearberry), and *Picea* (spruce) were identified with increasing relative sedaDNA abundances after ~13,500 cal BP, along with *Equisetum* (horsetail), *Gymnocarpium* (oak ferns), and *Sphagnum* (peat moss). Our data suggests that forbs and graminoids (in this case Poaceae and Asteraceae) were dominant from ~30,000–13,500 cal BP while woody shrubs were comparatively rare (Fig. 3), indicating that the conventional idea of the mammoth-steppe holds until at least this late.

**Assessing aDNA authenticity.** All 13 negative controls had negligible library adapted molecules prior to indexing (Supplementary Fig. 8) and had minuscule molarities after targeted

| Permafrost Sites | Lower LAD | Yukon LAD | Alaska LAD |
|------------------|-----------|-----------|------------|
| Permafrost Sites | 12,989 (+) | 14,211 (+) | 15,523 (+) |
| Lower LAD | 12,781 (+) | 14,595 (+) | 15,393 (+) |
| Yukon LAD | 12,558 (+) | 14,720 (+) | 15,642 (+) |
| Alaska LAD | 12,969 (+) | 14,487 (+) | 15,211 (+) |

Fig. 2 Metagenomic comparison of animal reads assigned using BLASTn to MEGAN. Values indicate unique reads assigned to that taxon node. Source data are provided as a Source Data file.
enrichment (Supplementary Fig. 9). Despite using the entire post-enrichment eluate for each of the controls during equimolar pooling, these blanks received minimal sequenced reads (102,440 and even fewer reads that could be taxonomically binned (81,08%). Shotgun sequencing blanks processed with a subset of these libraries in Murchie et al.36 were almost entirely (>95%) adaptamers (adapter chimeric DNA), and likewise contained no signal persisting into the Holocene. This signal can be used to infer the presence of ancient DNA characteristic damage patterns (Supplementary Figs. 29–33), suggesting that reworking and leaching have contributed minimally (if at all) to the ecological reconstructions.

**Discussion**

We observe four main trends within this dataset. (1) There is a surprising taxonomic richness and spatio-temporal consistency in the metagenomic signal across all sampling locations, suggesting that these reconstructions are representative of palaeoecological trends in the Klondike region. (2) Megafaunal sedaDNA declines gradually after the LGM with the *Mammuthus primigenius* signal being the first to drop out, followed by *Bison priscus* and *Equus*. (3) Signal dominance for forbs and graminoids is coeval with grazing megafauna, whereas the local transition towards woody shrubs is associated with a diminished faunal signal. Megafaunal sedaDNA reduces substantially after the Younger Dryas, by which time grazing megafauna had become functionally extirpated in the Klondike. (4) Despite this turnover, a low megafaunal sedaDNA signal persists into the Holocene. This signal—identified as a ghost range here—is suggestive of a late persistence of megaflora in a high latitude refugium, apparently outliving the
functional extinction and complete loss of other continental populations.

Ecological turnover and collapse of the mammoth-steppe. *Mammuthus, Equus, and Bison* presence are closely associated in our dataset with forbs and graminoids characteristic of the mammoth-steppe biome. During the relative sedaDNA rise in Salicaceae (likely willow shrubs) ca. 13,500 and 10,000 cal BP, Asteraceae and Poaceae correspondingly decline in relative sedaDNA abundance while grazing megafaunal DNA largely disappears from our dataset. This suggests that, at least in the Klondike, *Mammuthus primigenius* may have been the first
Fig. 5 Example mapDamage plots. Minimum size 24 bp, minimum map quality 30. See Supplementary Figs. 13-28 for a full breakdown of fragment misincorporation plots and a discussion of on/off target mapping. Source data are provided as a Source Data file.

Fig. 6 Metagenomic Principal Coordinates Analysis (PCoA) produced in MEGAN using a chi-square ecological index. LAD last appearance date based on dated macrofossils (Fig. 2). Source data are provided as a Source Data file.

megafaunal species to undergo a local population reduction after ~20,000 cal BP.

It is difficult to say whether sedaDNA signal decay reflects an actual reduction in the regional abundance of animals or is reflective of other stochastic and unknown factors unique to this proxy such as variable eDNA release and turnover, biochemical changes to eDNA stabilization processes such as organo-mineral binding, or shifting microbial and other taphonomic pressures. As Equus sedaDNA remains relatively consistent until the rise of mesic-hydric wood shubs during the Allerød warming (~13,500 cal BP) (Fig. 3B), this is suggestive of longer-term local declines of Mammuthus (and perhaps Bison) eDNA input rather than a shifting of biomolecular taphonomy because we would generally expect animal eDNA to breakdown or become mineral-bound at similar local rates. Further, Lagopus DNA spikes during major declines in megafaunal DNA. Again, implying local ecological rather than just taphonomic or methodological factors driving shifts in relative animal sedaDNA recovery. As this study used a capture enrichment approach targeting organelle genomes (with replicates [Supplementary Table 14]) rather than PCR metabarcoding amplicons, relative changes in metagenomic signal abundance are arguably somewhat correlated with shifting eDNA inputs during periods of otherwise stable climate. As such, we suggest that major shifts in relative DNA abundance, such as Mammuthus sedaDNA nearly dropping out of the dataset after 20,000 cal BP, are ecologically informative.

The decline in local mammoth abundance that we infer from this sedaDNA record is consistent with a low frequency of 14C-dated mammoth macrofossils from the Klondike. *M. primigenius* macrofossils are comparatively rare in central Yukon relative to more northerly sites around Old Crow (Supplementary Fig. 1), but still relatively few of those northern macrofossils have been successfully radiocarbon dated because most are beyond...
radiocarbon range\textsuperscript{117,118}. Much of the <30,000-year fossil record in Beringia is represented by a small number of well-preserved, and logistically accessible sites (Supplementary Fig. 1). Our faunal sedaDNA dataset somewhat conflicts with these macrofossil abundances in that the highest frequencies of DNA reads identified as \textit{Mammuthus}, \textit{Equus}, or \textit{Bison} are distributed between 30,000–15,000 cal BP. Conversely, dated faunal remains of \textit{Mammuthus} and \textit{Bison} in eastern Beringia have a median concentration around 15,000 cal BP, with only \textit{Equus} bones being predominant nearer to 25,000 cal BP (Supplementary Fig. 1).

Relative \textit{Bison} and \textit{Equus} sedaDNA signals decrease in read counts after ~15,000 cal BP. However, there is a subsequent increase around the onset of the Younger Dryas (ca. 12,900 cal BP) that may be associated with previously described \textit{Bison} dispersals\textsuperscript{40,119} or other local factors (e.g. shifting mosaic vegetation patches\textsuperscript{25}, herbivore land use, or taphonomy). It is worth noting that this inference is limited by there only being a single Younger Dryas core sample in this dataset. After the Younger Dryas (during the early Holocene) grazer sedaDNA nearly disappears (Fig. 3), which is correlated with the ecological turnover favoring forbs and graminoids to woody shrubs—predominantly \textit{Salix} sp.—and a rise in avian fauna, rodents, and cervid browsers. There is a pronounced transition in the ecological signal after ~13,500 cal BP from forbs and graminoids to woody shrubs in the Lucky Lady II cores, with rises in \textit{Salix} reads tightly associated with a sharp increase of \textit{Lagopus lagopus} (willow ptarmigan) sedaDNA—a grous whose habitat and subsistence patterns are based on woody shrubs\textsuperscript{120} (Fig. 3).

Keesing and Young\textsuperscript{121} observed on the African savanna that when large grazing mammals were removed from an area, the rodent populations doubled, which increased the populations of predators that target small-bodied animals. Our data mirrors this observation with an increase in rodent sedaDNA after ~10,000 cal BP (Fig. 2), along with the appearance of the small forest dwelling carnivore \textit{Martes} sp. (martens) who may also now be present because of trees on the landscape.

After \textit{Mammuthus primigenius} and \textit{Equus} sp. were functionally extirpated from the Klondike, the local ecosystem began transitioning towards boreal taxa with an associated rise in \textit{Picea} (spruce) and mosses (Fig. 4). Despite significant declines in grazing megafaunal DNA, reads extend beyond their last dated macro-remains—perhaps even as late as the mid-Holocene— which has already been observed for \textit{Bison priscus}\textsuperscript{39,40}.

Our plant dataset is consistent with Willerslev et al\textsuperscript{33} in which forbs were found to proportionally dominate their metacoded sedaDNA signal during and after the LGM. Nichols et al\textsuperscript{112} argue that the forb dominance observed in Willerslev et al\textsuperscript{33} was partly caused by polymerase and GC biases of their PCR metabarcoding approach favoring forbs over graminoids with the Platinum HiFi Taq polymerase targeting the short \textit{trnL} (P6 loop) locus\textsuperscript{122}. We have used a capture enrichment approach (indexd and reamplified with the KAPA SYBR FAST qPCR Master Mix) targeting much larger regions of the chloroplast genome (\textit{trnl [~500 bp]}, \textit{rbcL [~600 bp]}, and \textit{matK [~800 bp]})\textsuperscript{36} where overall GC content is generally equivalent between the three major target families identified in Fig. 3 (see supplementary Fig. 56). We suspect that beyond the PCR biases argued to have influenced Willerslev et al\textsuperscript{33} (i.e. the greater relative abundance of forb sedaDNA compared to graminoids and woody plants) that this is likely the result of eDNA release and preservation characteristics of forbs with higher rates of biomass turnover. This more rapid turnover thus potentially leads to an eDNA over-representation of forbs compared with typical palynological findings. It has been argued that interpreting relative floral abundances with eDNA requires calibration. Yoccoz et al\textsuperscript{95}, for example, observed that their above-ground vegetation surveys were accurately mirrored in modern environmental soil DNA, but that functional groups (woody plants, graminoids, and forbs) varied in their proportional eDNA representation. Woody plants were most affected by this trend, being proportionally under-represented in eDNA compared to above ground biomass by 1:5\textsuperscript{25}, while graminoids were under-represented by 1:1.5. Conversely, forbs were over-represented by 2:5:1. GC and polymerase bias coupled with eDNA release variation, beyond simple growth form categories, complicates this further\textsuperscript{112}. Nevertheless, the substantial abundance of forb DNA, even if cut by half, likely reflects an abundance of flowering herbs on the Pleistocene mammoth-steppe. This may also be under-represented palynologically due to varied pollen production between entomophilous (insect-pollinated) forbs and anemophilous (wind-pollinated) graminoids\textsuperscript{123}.

The rise in Pinaceae (notably spruce, see Fig. 4) around ~10,000 cal BP, and its growing dominance through our mid-Holocene samples, is consistent with other records from Yukon in regard to the initial development of the taiga/boreal forest\textsuperscript{124,129}, and is consistent with pollen grains identified in samples younger than ca. 9200 cal BP (Supplementary Table 4). We observe similar relative sedaDNA increases in boreal flora (Fig. 4), albeit with a comparatively less abundant signal for \textit{Betula}. Palynological studies frequently report an abundance of \textit{Betula} with a comparatively small initial influx of \textit{Salix} in the Alaskan-Yukon interior during the terminal Pleistocene shrub expansion\textsuperscript{125}. While we observe a distinct rise in relative \textit{Betula} sedaDNA during the Bolling–Allerød and post-Younger Dryas chronozones that persists into the Holocene, the number of \textit{Betula} sedaDNA molecules are comparatively dwarfed by the immense abundances of Salicaceae (\textit{Salix} [willow]) (Figs. 3–4) DNA. \textit{Betula} is known to be over-represented by pollen, whereas \textit{Salix} is often under-represented\textsuperscript{130–133}. Our sedaDNA data suggests that \textit{Salix} was more important in Beringian shrub expansion than palynological records have yet indicated.

The relative over-abundance of \textit{Salix} sedaDNA compared to \textit{Betula} is relevant to testing the shrub-expansion extinction model of Guthrie\textsuperscript{16}, who contended that an increasing moisture regime and rise of mesic-hydric vegetation, with chemical defenses against herbivory (notably \textit{Betula nana exilis} [resinous dwarf birch], but also including \textit{Salix})\textsuperscript{134–136}, drove regional extirpations of grazing megafauna in eastern Beringia. If \textit{Salix} was substantially more abundant in the Beringian shrub expansion than \textit{Betula} as our sedaDNA dataset suggests, this questions whether the rise of defensive vegetation was a major driver in the extirpations as \textit{Salix} is the most preferred and palatable shrub among extant subarctic browser\textsuperscript{135,137}. While \textit{Bison} and \textit{Equus} are considered closer towards the obligate grazer end of dietary guilds\textsuperscript{30,138}, both have been observed to exhibit variable grazing and even mixed feeding\textsuperscript{139,140}. \textit{Mammuthus}, \textit{Equus}, and \textit{Bison} coprolites suggest that these taxa had a diet variably rich in forbs and graminoids, with a smaller but notable proportion of woody shrubs/trees (including alder [\textit{Alnus}], birch [\textit{Betula}], larch [\textit{Larix}], spruce [\textit{Picea}]), and willow [\textit{Salix}]\textsuperscript{33,141,142}. If mammalian sedaDNA abundances are rough indicators of palaeo-biomass (a correlation in need of further research), it is unclear why \textit{Mammuthus primigenius} and \textit{Bison priscus} (Fig. 2) relative sedaDNA signals decline prior to an expansion of woody shrubs during the Bolling-Allerød warming (Fig. 3). The abrupt increases in Cyperaceae (\textit{Carex} [sedges]), Ericaceae (\textit{Arctous} [bearberry], \textit{Rhododendron}), Betulaceae, and Salicaceae during the Allerød are suggestive of a transition toward a moist dwarf-shrub ecosystem by ca. 13,500 cal BP. The presence of these plants suggests more continuous ground cover, better insulation, shallower permafrost, and likely boggy, wet conditions. The early Allerød rise of Fabaceae sedaDNA (particularly \textit{Astragalus} and
Oxytropis, Fig. 4) may also be indicative of a rise in flora with anti-herbivory defenses as these locoweeds are toxic to grazing fauna. Notably, despite otherwise rapid faunal and floral turnover, our single Younger Dryas core sample may suggest that the mammoth-steppe locally persisted through the Bolling-Allerod chronozone in the Klondike.

Mann et al.28,29 and Rabanus-Wallace et al.30 contend that a shifting moisture gradient from xeric to mesic-hydrlic, with the paludification of eastern Beringia, best accounts for the loss of dryland-specialists (Equus, Mammuthus), whereas mesic and mixed feeding seasonal fauna (Rangifer, Cervus, Ovibos) retained suitable habitats and hydric specialists (Alces, Homo sapiens) were able to invade new Beringian niches. While warming, an increasing moisture regime, the arrival of cervid browsers, and the rise of woody shrubs may explain much of the terminal signal decay observed for grazing specialists, this does not explain the relative declines of Mammuthus and Bison sedaDNA prior to the Bolling–Allerod chronozone (assuming that sedaDNA abundance retains a correlation with palaeo-biomass). Guthrie143 found that horses had undergone body-size declines after the LGM until their extirpation in Beringia, which likewise suggests longer-term pressures predating shrub expansion. The rise of woody plants in this dataset thus may be partially explained by both the gradual reduction in local megaherbivore ecosystem engineering over millennia and by a warming climate and shifting moisture regimes.

Unknowns in sedaDNA release, preservation, and recovery restrict what we can confidently infer from differences in relative signal abundance, but there are indications of both top-down and bottom-up contributions to the collapse of the mammoth-steppe in central Yukon. While the decline of faunal sedaDNA is likely influenced to some degree by shifting taphonomic processes (such as warming and increasing moisture causing more DNA degradation), substantial declines in megafaunal sedaDNA predate the Bolling–Allerod interstidal (14,690–12,900 cal BP)68. This could be seen as partially supporting Zimov and colleagues’5,23 keystone megaherbivore decline model that these animals were increasingly unable to maintain suitable steppe habitats due to declining populations. However, there is a substantial time lag in our dataset between a declining megafaunal sedaDNA signal after 20,000 cal BP and the relative rise of woody shrub sedaDNA ca. 13,500 cal BP. By contrast, the rise of mesic-hydrlic woody shrub DNA during the Allerod oscillation (13,900–12,900 cal BP) and early Holocene are clearly associated with an abrupt decline in megafaunal sedaDNA, strongly supporting the climate-induced shrub and peatland expansion model of Guthrie16 and Mann et al.28–30. There is potential support in this dataset for both bottom-up and top-down pressures influencing megafaunal extirpations in the Klondike. Further research is needed to determine whether indications of longer-term top-down pressures are real or an artifact of sedaDNA methodology, and to what degree each Beringian megafaunal species was differentially impacted and/or responsive to shifting ecological pressures.

The degree to which humans may have been involved in any of these transformations is hard to gauge from available evidence (see Supplementary Notes 1.2). Early (>14,000 cal BP) human presence in eastern Beringia is controversial but has been suggested based on possible anthropogenic cutmarks at Bluefish Caves144–147 and the identification of allegedly human fecal biomarkers and a coinciding rise of fire activity on the Alaskan North Slope148–151. However, these records lack unambiguous artifacts, features, or other clear indications of middle Upper Palaeolithic lifeways as seen in eastern Siberia152–156. At this time, there is no clear evidence for an ecologically significant human presence in eastern Beringia prior to ca. 14,000 cal BP (Supplementary Figs. 2–3). Thereafter, low fecundity megafauna72,157, who had already undergone millennia of oscillating climatological and ecological pressures, may have been vulnerable to novel anthropogenic forces158–162 that lack archaeological visibility due to the emergence of post-LGM, high mobility lifeways156,163. Currently, evidence of anthropogenic contributions to the ecological turnover in eastern Beringia remain functionally absent, being at most but one enigmatic component in a synergistic set of compounding pressures. SedaDNA analyses of Pleistocene permafrost targeting human DNA may prove key to addressing lingering unknowns in the peopling of Beringia.

Evidence of a cryptic refugium. The persistence of Equus and Mammuthus until ~9200 cal BP and perhaps as late as ~5700 cal BP (Fig. 2), as suggested by our sedaDNA records, lies well beyond the last dated macrofossils for these taxa (Fig. 7). However, interpreting cryptic populations with sedaDNA necessitates caution. As noted previously, Arnold et al.31 found that although permafrost contains a wealth of well-preserved eDNA, the favourable characteristics of perennally frozen ground increases the likelihood for allochthonous organisms to survive transport and be redeposited within younger strata. They argue that while reworking is of lesser concern when assessing first appearance dates and “abundant” sedaDNA signals, reworking of older sediments can be an inherent problem when assessing last appearance dates in high-energy fluvial contexts or in areas of thermokarst where older sediments thaw and mobilize followed by potential re-aggradation of permafrost. Arnold and colleagues highlight the careful analysis of loess sediments from the Stevens Village site in central Alaska where Haile et al.37 utilized 14C, OSL, extensive eDNA sampling on and off site, and careful sedimentological analyses to plausibly infer the late survival of Mammuthus and Equus to as late as ~10,000 cal BP. While the sediments targeted here are also loessial silts100, these materials were not recovered in the field with ancient DNA in mind, but were instead later reselected to follow-up on results presented by Murchie et al.36. Although we acknowledge that the signals for late megafaunal persistence should be interpreted with careful skepticism, and require additional supporting evidence for verification (particularly given early Holocene thaw unconformities147–149 in the Klondike as identified at Upper Goldbottom and Upper Quartz100), these signals are reasonable and worthy of further study for the following reasons.

First, the ghost range signals observed for Mammuthus and Equus is observed at three different sites in 9 (Mammuthus) to 12 (Equus) separate permafrost cores and are correlated with substantial and consistent changes in vegetation. In samples younger than ~13,500 cal BP there is a complete restructuring of vegetation. To our knowledge, no plant taxa completely disappeared during the transition, which limits our ability to use the plant data to chronologically test for allochthonous sedaDNA. However, the megafaunal signal observed in the alleged ghost range cores are comparable to those observed during periods of known presence. It is reasonable to ask how many reads are sufficient to say an organism was truly present, but there cannot be any simple answer to that question. At the same time, >50 unique sedaDNA molecules identified as Elephantidae at ~9500 cal BP is significant relative to older cores considering the otherwise substantial ecological turnover.

Certainly in the case of cores with sediments younger than 13,000 cal BP, the presence of Mammuthus and Equus extending to ~9500 cal BP is highly consistent with other investigations in both northern Asia and northwestern North America37,41,164,165. Furthermore, reads for these megafaunal taxa are observed at two
sites across all subsampled replicates and are associated with a completely different plant ecological signature. Here the point is that we would expect both floral and faunal sedaDNA would be reworked at roughly similar rates, which is not what is observed. Graminoids and forbs do persist, as would be expected, but there is no obviously mixed ecological signal as woody species dominate the plant metagenomics data (Fig. 4); this is despite otherwise having been observed in modern experiments to be proportionally under-represented genetically compared to their biomass by a factor of ~1.595.

The youngest signatures for Equus and Mammuthus (ca. 5700 cal BP) are of great interest because they imply local survival long after the Pleistocene-Holocene transition. Aside from very late insular occurrences of mammoths from the Bering and Chukchi Seas41, there are no accepted radiocarbon dates on mammoth or horse fossils in mainland Beringia that fall anywhere close to the mid-Holocene (Figs. 2, 7, Supplementary Fig. 1). Although the authenticity of the identifications is not in question (Supplementary Fig. 57), the wide temporal gap between these sedaDNA molecules and dated bones is concerning. This difference needs to be evaluated in context. Palaeontological and archaeological records across much of the Arctic and Subarctic are notably sparse. Small refugial populations might have survived in remote pockets at sizes too small to be readily detected by macrofossil collections derived largely from a small set of resource extraction and development sites. The case of Mammuthus survival on St. Paul and Wrangel islands, until 5500 and 4000 cal years BP, respectively41,42, is interesting because until recent decades neither population was known to have persisted into the mid-Holocene. Bison priscus was likewise discovered to have survived throughout the Holocene in southern Yukon39,40. Conroy et al.165 observed the presence of coprophilous fungi in the Alaskan interior at Windmill and Jan Lakes until ~9000 cal BP and 4500 cal BP. This abundance of spores postdates the megafaunal turnover and is perhaps most likely related to a replacement with browsing cervids. Alternatively, it is also possible that cryptic, refugial populations of Pleistocene grazers were contributing sources. The way forward is through further testing and confirmation, which can be achieved through multiproxy sampling from a broader suite of high latitude sites where reworking is negligible, and conditions favour the widest possible metagenomic spectra.
Summary. This taxonomically rich sedaDNA dataset tracks the ecological turnover of fauna and flora in central Yukon across the Pleistocene-Holocene transition. We identify the coeval eDNA turnover of megafaunal grazers with forbs and graminoids relative to the rise of woody shrubs and boreal flora at ca. 13,500–13,000 cal BP and after 10,000 cal BP, along with the gradual decline of faunal sedaDNA after 30,000 cal BP. There is also a consistent, multi-site signal of late persistence for Equus and Mammuthus, perhaps surviving some 7000 years longer than their last dated macrofossils in eastern Beringia would indicate. Top-down versus bottom-up perspectives on the collapse of the mammoth-steppe cannot be entirely resolved with this dataset, perhaps because both are relevant. The ancient eDNA data presented here have indications of both long term, top-down ecological pressures impacting megafaunal populations in the Klondike, arguably supporting the keystone megaherbivore model of the mammoth-steppe (albeit with unknowns regarding Klondike, arguably supporting the keystone megaherbivore model ecological pressures impacting megafaunal populations in the Klondike, last dated macrofossils in eastern Beringia would indicate. BP 16,284 – 24,000 years ago sediment inputs (0.34 m 4 – 8 ka) effectively tease apart the complexity of factors involved in the sparse in well-dated and geographically diverse macrofossils to engineering controlling woody expansion and the abruptness of the transition there- after is arguably indicative of both a potential lack of megafaunal the ecological turnover. The abruptness of the transition there- after is arguably indicative of both a potential lack of megafaunal engineering controlling woody expansion (4,23,31,121,166) and the persistence of mammoth-steppe taxa at our single Younger Dryas characterized of the Klondike by 10,000 cal BP. The late Pleistocene palaeoentological record of Beringian megafauna is extensive compared to many other areas of Eurasia and the Americas (4,56). However, even with this richness, it is too sparse in well-dated and geographically diverse macrofossils to effectively tease apart the complexity of factors involved in the Pleistocene-Holocene transition and the collapse of the mammoth-steppe. The data presented in this study highlights the power of environmental DNA for the recovery of highly complex signals of ecological change from exceptionally small sediment inputs (0.3–1.35 grams), even in the absence of macro biological tissues. Using targeted enrichment for sedaDNA is also far more cost effective for high-throughput sequencing applications compared to a shotgun approach. Murchie et al. (2012) used a shotgun approach for their much larger dataset, which is more detailed and comprehensive. However, the results presented in this study suggest that the mammoth-steppe locally persisted through the Bolling–Allerød warming, but that subsequent early Holocene transformations significantly shifted the ecological character of the Klondike by 10,000 cal BP.

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Summary. Twenty-one core samples of loessial permafrost silts recovered from the Klondike region of Yukon, Canada (Fig. 1, Table 1)—dating between 30,000–4,000 calibrated years before present (cal BP)—were processed for sedimentary ancient DNA (sedaDNA) to evaluate changing biomolecular signals of plants and animals during the late Pleistocene-Holocene transition in eastern Beringia. We used Yasukawa megabase modelling in conjunction with stratigraphic and cryostratigraphic observations to estimate core dates, and a subset of the core samples were processed in parallel for palynology. Despite the exceptional preservation and richness of the sedaDNA, very few pollen grains could be found in the samples (Supplementary Table 4).

For ancient DNA processing, we utilized a sedaDNA modified Dabney et al. extraction procedure with the long cold spin inhibitor removal technique as described in Murchie et al. (2012), and prepared double stranded, dual-indexed libraries (4,4,1,46,100) for targeted enrichment. We used the PalaeoChip Arctic-1.0 plant and animal baits (4,25) to capture enrich these libraries for chloroplast barcoding loci (trnL, matK, rbcL) of Arctic/Subarctic plants and for whole mitochondrial genomes (or singular loci where mitogenomes were unavailable at the time of bait-design in 2017) of extinct and extant northern animals (focused on megafauna). Libraries were sequenced on an Illumina HiSeq 1500 with 2 x 90 paired-end read chemistry, trimming, merging, and the sequencing reads were processed using the same software used to taxonomically identify the reads to the top 600 hits against a July 2019 local copy of the GenBank database (4,1,57), which was used as the input for MEGAN Community Edition (4,57,54, v6.19.7, https://github.com/husonlab/megan-ce) and PIA (4,53) (pipA (pipA (pipA)) (pipA). The outputs from MEGAN are plotted in the main text, while plots of individual extraction replicates from both MEGAN and PIA are included in the supplement (Supplementary Figs. 34–55). MapDamage (4,2,3, https://gimolhac.github.io/mapDamage/) was used to assess the aDNA damage signals of taxonomically identified taxa (Supplementary Figs. 13–28).

Field sampling. The cores used in this analysis were previously studied by D’Costa et al. (4,4,1,15), Mahony (4,4,1,100), and Sadoway (4,4,1,109) and have since been kept in cold storage at the University of Alberta. Permafrost cores were collected between June and August of 2010, 2012, and 2013 with research permits issued to DF from the Yukon Heritage Branch. These cores were sampled at placer gold mining exposures chosen for the quality of the exposure and expected age of the sediments. Prior to sample collection, the sampling area was cleared of eroded materials back to frozen sediments to create a fresh coring surface for a 10 cm diameter coring tube – 30 cm in length. Horizontal cores were drilled with a small portable gas-powered drill (Echo), recovered frozen, stored individually in plastic bags, immediately placed in a –20 °C chest freezer, and transported in the −20 °C freezer to the University of Alberta. Permafrost cores were collected from Bear Creek, Upper Quartz, and Upper Goldbottom. Vertical cores were taken from Lucky Lady II (Fig. 1, Supplementary Methods 2.1).

Radiocarbon dating and Bayesian age-depth modelling. Plant macrofossils were picked from thawed samples using a dissecting microscope, dried and preserved for AMS dating at the University of Alberta along with known-age wood standards (c.f. Mahony (4,4,1,108)). Pre-treatment of all samples followed standard acid-base-acid procedures. Solutions heated to 70 °C and placed in 1 M HCl for 30 min, followed by 60-min washes in 1 M NaOH until the solution became clear. Finally, samples were washed in 1 M HCl for 30 min and rinsed with ultrapure water until they became neutral. Measurements of CO2 production, graphitization and radiocarbon abundance in all samples were completed at the Keck-Carbon Cycle AMS facility (UCIAMS).

Age-depth models for Lucky Lady II, Upper Goldbottom and Upper Quartz were first presented by Sadoway (4,4,1,109) and Mahony (4,4,1,109). To refine the chronologies of these records we developed new Bayesian age-depth models for each study site using Oxcal v.4.4.210 and the IntCal20 calibration curve (4,4,1,101). In each case a P.Sequence depositional model was developed along sampling transects or vertical cores using a variable K parameter (increments per unit length) (4,4,1,109) and a general Outlier_Model, with a 5% prior probability of any 14C date being a statistical outlier (4,4,1,102). Boundaries were placed at the contacts between sediment units where changes in accumulation rates are likely to have taken place. The Upper Quartz age-depth model (Supplementary Fig. 5) is developed from two P.Sequence depositional models run either side sedimentological boundary at 3.8 m which represents an unconformity of several thousand years. The lower P.Sequence includes two 14C dates (UCIAMS-111733 and UCIAMS-114710) and provides chronology for SedanaDNA samples; PHP-22, PHP-23, PHP-24, and PHP-25.
25. The upper P_Sequence includes three 14C dates (UCIAMS-114899, UCIAMS-114733, and UCIAMS-114710) and provides chronology for SedaDNA samples; PHP-19, PHP-20, and PHP-21. Lucky Lady II includes three vertical cores which were sampled for SedaDNA (LIII-12, LL2C, and LL2S) (Supplementary Fig. 6). A prominent palaeo-soil that can be traced laterally for hundreds of metres around the exposure is present in the LIII-12 and LL2C cores, and 14C dates associated with this horizon were used to combine these cores with a single P_Sequence model (UCIAMS-56390, UCIAMS-114725, UCIAMS-143307, and UCIAMS-143308). Core LIII-12 includes three 14C dates (UCIAMS-240139, UCIAMS-122284, and UCIAMS-122273), as well as a palaeo-soil isochron, and provides chronology for SedaDNA samples PHP-9, PHP-8, and PHP-7. Core LL2C includes three 14C dates (UCIAMS-142212, UCIAMS-142197, and UCIAMS-142197), as well as the palaeo-soil isochron, and provides chronology for SedaDNA samples PHP-4, PHP-5, and PHP-7. A boundary was placed at 3.5 m (at the contact between units 1 and 2) where grey silts including granodiorite vegetation are replaced by organic-rich grey and black silts with in situ shrub vegetation. Core LL2-S includes three 14C dates (UCIAMS-143296, UCIAMS-142197, and UCIAMS-143306) and provides chronology for SedaDNA samples PHP-3 and PHP-2.

The Upper Goldbottom age-depth model (Supplementary Fig. 7) is developed from two P_Sequence depositional models run either side of the sedimentological boundary at 22.5 m which represents a maximum of several thousand years. The lower P_Sequence provides chronology for sedaDNA samples PHP-14, PHP-13, and PHP-15, and includes six 14C dates (UCIAMS-122282, UCIAMS-114712, UCIAMS-142208, UCIAMS-114716, and UCIAMS-122274) as well as the Dawson tephra which has been dated to 29,055 ± 29,470 cal BP179. The depths of three 14C dates obtained from Arctic ground squirrel middens (UCIAMS-114712 and UCIAMS-114714) were adjusted by 0.8 m to account for burial depth. This is likely to be a conservative estimate of active-layer depths during cold stages in the Yukon which were deeper than present. One 14C date caused a significant age-reversal and so was excluded from the age-depth model (UCIAMS-240141). Boundaries were placed at 12.6 m and 18.6 m (Fig. 7) to incorporate the core to core boundary in the LLII-12 and LL2C cores, and to separate the core into the overlying organic-rich sediments containing the palaeosol and overlying organic-rich grey and black silts. This model is used to combine these cores with a single P_Sequence model (UCIAMS-56390, UCIAMS-114725, UCIAMS-143307, and UCIAMS-143308).

Lysis and purification. We followed the lysis and sedaDNA extraction procedure described in Murchie et al.36. The first round of sediments (PHP) were lysed with an input of 0.3 g. Subsequent experiments determined that this resulted in a higher inhibitor load for certain samples leading to ~10–20% failed or suboptimal adapter ligation efficiencies during library preparation (Supplementary Figs. 8–9). For the second round of extractions (PHP), we reduced the input to 0.15 g, but used two PowerBead lysis tubes per sample that were pooled on the same Roche column following the long cold spin.

Subsamples were lysed with a digest solution (Supplementary Table 5) preloaded into Dneasy PowerBead tubes, then vortexed for 20 min using a TissueLyser II. Therefore, the tubes were briefly centrifuged to remove liquid from the lysis tube and proteinase K was pipetted into each tube individually. The tubes were then briefly finger vortexed to disturb the sediment- bead pellets that had formed at the bottom of the tubes and were loaded in an incubator to oscillate overnight at 35 °C. The next day, the PowerBead tubes were centrifuged at 10,000 × g for 5 min and the supernatant was transferred to a 1 mL MAXYMum Recovery tube and stored at −20 °C for later purifications.

For sedaDNA purification, the digestion supernatant (~1.25 mL) was thawed, buffered to pH 7.0 with Tris, and added to 2.5 mL volume of high-volatility guanidinium binding buffer (Supplementary Table 6) in a 50 mL falcon tube and mixed by repeated inversion. The 50 mL tubes were loaded into a refrigerated centrifuge for the Murchie et al.36 long cold spin, where they were centrifuged at 2500 × g at 4 °C for ~20 h. Thereafter, the falcon tubes were carefully removed from the centrifuge buckets, and the supernatant was decanted, taking care not to disturb the darkly coloured pellet that had formed during the cold spin. The binding buffer was passed through a high-volume silica-column (High Pure Extender Assembly, Roche Diagnostics) over multiple rounds of centrifugation and extraction proceeded as per Danby et al.187 with binding and wash centrifugation at 3300 × g, two rounds of PE wash, followed by two 30 s dry spins at 16,000 × g with the tubes rotated 180° between spins to minimize the chance of ethanol retention. Purified DNA was eluted off the silica columns with two volumes of 25 µL EBT (each while waiting 5 min after EBT loading to maximize elution, then centrifuging at 16,000 × g for 1 min). Prior to all subsequent experiments the eluted DNA were further centrifuged at 16,000 × g for 25 min to pellet any remaining co-eluted inhibitors. Care was taken when subsampling these extracts to avoid disturbing any pellet precipitates.

Library preparation, quantitative PCR, and indexing. Doubled stranded libraries were prepared for each extract as described in Meyer and Kircher189 with modifications from Kircher et al.169 and a modified end-repair reaction to account for the lack of uracil excision (Supplementary Table 7). Samples were purified after blunt-end repair with a QIAquick Nucleotide Removal Kit (QIAGEN) (to maximally retain small fragments) and after adapter ligation (Supplementary Tables 8–9) and indexing (Supplementary Table 10) with a MinElute PCR Purification Kit (QIAGEN). Pre-indexing total library-adapter DNA concentrations were estimated as a filtering step to determine whether a sample was successfully converted into libraries with the short amplification qPCR assay (Supplementary Table 11).

Targeted capture with PalaeoChip. In-solution enrichments were carried out using the previously designed PalaeoChip Arctic v1.0 bait set190. This bait set targets whole mitochondrial genomes from approximately 180 extinct and extant Holarctic fauna, and the chloroplast barcoding loci (rLm, rbcL, and matK) from approximately 2100 species of plants. See Murchie et al.36 for further details on the design of PalaeoChip Arctic-1.0.

Enrichments were performed using a modified version of the myBaits v4.1 protocol (Diacel Arbor Biosciences). In summary, hybridization and bait mixes were prepared to the concentrations in Supplementary Table 12. For each library, 7 µL of template was combined with 5 µL of the library block master mix (using xGens, Human Cot-1 DNA, and Salmon Sperm). Hybridization and bait mixes were combined and pre-warmed to 60 °C before being combined with the library-block mixture. The final reaction for batch 1 (PHP) was incubated for 48 at 53 °C overnight, and hybridized chimeric double-stranded DNA was enriched with a hybridization temperature of 60 °C over ~72 h to improve off-target exclusion.
After the hybridization, beads were dispensed (20 µL per reaction [rxn]), washed with 200 µL/rxn of binding buffer, then resuspended in 20 µL/rxn binding buffer and aliquoted into PCR strips. Beads were washed using 20 µL/rxn of the binding buffer, incubated at 55 °C for 2.5 min (60 °C for the second round), finger vortexed and spun down, then incubated for another 2.5 min. Beads were pelleted and the supernatant (the non-captured library fraction) was removed and stored at −20 °C as per Khank et al.185. The beads were resuspended in 180 µL of 60 °C Wash Buffer X, per tube and washed four times following the Mybaits v4.1 protocol. Beads were resuspended in 18.8 µL EBT, PCR reamplified for 12 cycles (Supplementary Table 13), then purified with MinElute columns following manufacturer’s protocols and eluted in 15 µL EBT.

Total quantification, pooling, size selection, and sequencing. Libraries were quantified using the long-amplification total library qPCR assay (Supplementary Table 13) and pooled to equimolar concentrations. Pools were size-selected using gel excision following electrophoresis for molecules ranging between 150–500 bp. Gel plugs were purified using the QIAquick Gel Extraction Kit (QIAGEN), according to manufacturer’s protocol, then sequenced on an Illumina HiSeq 1500 with a 2 × 90 bp paired-end protocol at the Farncombe Metagenomics Facility (McMaster University, ON).

Bioinformatics. Reads were demultiplexed with bcl2fastq (v1.8.4), converted to bam files with fastq2bam (https://github.com/grenaud/BCL2BAM2FASTQ), then trimmed and merged through eLoom184,186 using ancient DNA specific parameters (–ancientdna). Reads were mapped to a concatenation of the PalaeoChip Arctic-1.0 plant and animal probe references with network-aware-BWA185 (https://github.com/mpseva/network-aware-bwa) with a maximum edit distance of 0.01 (–n 0.01), allowing for a maximum two gap openings (+o 2), and with seeding effectively disabled (+s 16500). Mapped reads that were merged or unmerged but properly paired were extracted with libbam (https://github.com/grenaud/libbam), collapsed based on unique 5′ and 3′ positions with biohazard (https://bitbucket.org/ustenzel/biohazard) (for PCR deduplication), and converted to fasta files and restricted to a minimum length of 24 bp. Fasta files were additionally filtered to remove any reads with lying sequence similarity to the illumina adapter sequences (…/fasta2oneline.pl input.fasta | grep –v -1 AGATCGGAA | grep –v -1 TCCGATCT | tr ’/” “’ | tail –n +2 | output.fasta) and were string deduplicated using the NGSRemoveDuplicates module of NGSExplore (https://github.com/kmeteon/NGSExplore).

These filtered fastas were used as the input for BLASTn179, which were aligned against a July 2019 local copy of the GenBank NCBI (National Center for Biotechnology Information;171,175) nucleotide database set to return the top 600 alignments (unique accession hits) per read with e-values less than 1.0E–5 (flags: –num_alignments 600 -max_hsps 1 -evalue 0.00001). The BLASTn outputs were then passed to MEGAN (Community Edition, v.6.19.7173,174) where the BLASTn outputs were filtered to return the top 600 –values less than 1.0E–20 (default minimum complexity), then passed to MEGAN-LCA and PIA taxonomic binning approaches. All samples were mapped to these references using the same aforementioned procedures but with an additional map-quality filter set to ≥30 with samtools (https://github.com/samtools/samtools), then assessed for ancient DNA typical damage signals using mapDamage277 (v 2.0.3).

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability. The sEdna sequence data generated in this study that can be mapped to the PalaeoChip bait reference sequences have been deposited as bam files in the NCBI SRA database under BioProject: PRJNA722670, Accessions: SRR14265632–SRR14265692. Metagenomic data derived from these mapped reads are provided in the supplementary information and source data files. PalaeoChip reference and bait sequences are available at https://doi.org/10.5281/zenodo.5643845. Source data are provided with this paper.

Code availability. All software utilized in this analysis are available online and are referenced and linked to here in the supplementary information.

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Competing interests
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Additional information
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