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Latest Pleistocene shrub expansion and steppe-tundra persistence in easternmost Beringia

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ABSTRACT

The collapse of the mammoth steppe ecosystem at the end of the Pleistocene fundamentally restructured northern communities across Beringia, yet the spatial variability of this transition remains poorly understood. We present a multi-proxy analysis of the Mint Gulch site in the Klondike region, Yukon Territory, spanning ca. 16,000 to 13,000 calibrated years before present (cal yr BP), integrating sedimentary ancient DNA (sedaDNA), macrofossils, pore-ice isotopes ($\delta^{18}O$), and radiocarbon chronology. Pore-ice isotope values increase from −33.9‰ at ca. 16,000 cal yr BP to −21.4‰ by ca. 13,180 cal yr BP, reflecting regional warming and changing hydroclimate. SedaDNA records document grazing megafauna (woolly mammoth, horse, steppe-bison) and grasses and forbs until ca. 13,910 cal yr BP, when shrub-dependent taxa and browsing herbivores (moose, willow ptarmigan) first appear, or appear in greater abundance. However, macrofossil evidence from an Arctic ground squirrel midden (ca. 13,680 cal yr BP) reveals continued presence of steppe-tundra indicator taxa, including *Artemisia* sp., *Potentilla* sp., and the weevil *Connatichela artemisiae*. Our results demonstrate that despite regional hydroclimate change beginning ca. 14,000 cal yr BP, steppe-tundra vegetation persisted in locations where topography and aspect favoured xeric taxa. The Mint Gulch site provides rare documentation of the transitional period between steppe-tundra and shrub-tundra ecosystems, capturing co-occurrence of both communities around 13,910 cal yr BP. This study highlights the spatially heterogeneous nature of late Pleistocene ecosystem transitions in eastern Beringia and the importance of multi-proxy approaches for understanding local-scale persistence of steppe-adapted taxa.

1. Introduction

The Klondike region of central Yukon Territory was once part of Beringia, the unglaciated landmass spanning from eastern Asia to the Yukon Territory during cold stages of the Pleistocene (Hopkins, 1982; Guthrie, 2001; Froese et al., 2009). Beringia was characterised by the mammoth steppe, a loess-fed cold and arid grassland-forb ecosystem that supported diverse megafauna (Guthrie, 1990; Zazula et al., 2007). Although the mammoth steppe has no exact modern analogue, it is best described as a dry steppe-tundra ecosystem with relatively warm soils and deep active layers (Schweger, 1982; Guthrie, 1990; Sanborn et al., 2006; Monteath et al., 2025). The collapse of this ecosystem at the end of the Pleistocene resulted in dramatic restructuring of northern communities (Guthrie, 2006; Murchie et al., 2021a, 2023; Monteath et al., 2023; Clarke et al., 2024) and has been proposed to be due to either a bottom-up forcing by a change in hydroclimate regime and/or

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top-down pressure from reduced megafaunal herbivory caused by human overhunting (e.g., Zimov et al., 1995, 2012; Guthrie, 2001; Mann et al., 2015; Monteath et al., 2021; Williams, 2022). Human overhunting may have been a factor in the extinction of many megafauna species, however, current findings suggest that shrubs began appearing across eastern Beringia prior to these extinctions, supporting a climate-driven shift in environmental conditions (Monteath et al., 2021; Williams, 2022). The underlying permafrost, with characteristically deep active layers during cold stages of the Pleistocene, was fundamentally changed in response to increasing effective moisture, thicker organic soils, warmer temperatures, and shallower active layers that encouraged the expansion of shrubs (Guthrie, 2001; Anderson et al., 2003; Monteath et al., 2023).

In east Beringia, the loss of the mammoth steppe ecosystem is most clearly documented at sites in the Klondike goldfields of central Yukon Territory and from interior Alaska using multiproxy analyses of sedimentary ancient DNA (sedaDNA), pollen, insects, and pore-ice isotopes (Murchie et al., 2021a; Monteath et al., 2023; Clarke et al., 2024). Clarke et al. (2024) hypothesised that despite this collapse, steppe-tundra persisted at a local scale where topography and aspect favoured more xeric taxa. However, the location, extent, and timing of this persistence remain poorly resolved and most traditional proxy records preserved broad-scale landscape transitions. Similarly, Murchie et al. (2021a) suggested woolly mammoths and horses survived into the Holocene in Yukon, but these records only exist in sedaDNA data, and to date no bones have ever been recovered to support this hypothesis. In Yukon, it is plausible that the persistence of steppe-tundra would be preserved at the local scale, primarily through records of vegetation that may be similar in composition to the azonal steppe communities existing on south-facing slopes in present-day SW Yukon Territory (Laxton et al., 1996; Vetter, 2000; Zazula et al., 2007). From the Ural Mountains, the importance of topography for preserving habitat suitable for the survival of arctic-alpine plant communities is highlighted by sedaDNA data from Clarke et al. (2019). The authors report an expansion of woody taxa around 14,000 cal yr BP and the decline of arctic-alpine plant diversity, but not the complete loss of taxa. It is from these example that we must consider abiotic controls, like aspect and topography, when considering where and how climate-sensitive taxa can survive in an increasingly warming Arctic.

The application of sedaDNA has played an increasingly important role in improving our capacity to reconstruct entire Beringian ecosystems by extracting ancient DNA directly from sediments (e.g., Willerslev et al., 2014; Murchie et al., 2021a, 2023; Clarke et al., 2024; Edwards et al., 2026). Prior to sedaDNA, palaeoecological studies in the Arctic have applied traditional palaeoecological proxies such as fossils, insects, pollen, and plant macrofossils to reconstruct ecological changes over time (e.g., Cwynar, 1982; Elias, 2000; Walker et al., 2003; Zazula et al., 2003, 2007; Harington, 2011). In the loessal landscapes of Beringia, the success of traditional proxies can vary considerably due to factors like low presence (pollen, e.g., Murchie et al., 2021a), the requirement for large sediment sample sizes (insects, e.g., Kuzmina, 2017), and sporadic preservation, dispersal, and production (e.g., plant macrofossils, e.g., Birks, 2014). In contrast, we only require 0.25 g of permafrost-preserved sediments per sample to extract ancient DNA (Murchie et al., 2021b). However, sedaDNA does not function without biases (e.g., Arnold et al., 2011; Pedersen et al., 2015; Edwards, 2020; Seeber et al., 2024), but the capacity to reconstruct biotic activity in lieu of complementary proxies has proven to be an exceptional addition to the palaeoecologist's toolset (Edwards, 2020).

The comparison between traditional proxies and sedaDNA has been the subject of several studies from lake and peat deposits (Pedersen et al., 2013; Parducci et al., 2015), which generally conclude that sedaDNA is a valuable additional proxy to pollen and macrofossil analyses. For pollen and macrofossils, dispersal characteristics provide a well-established framework for interpreting source area. For example, bisaccate pollen grains, such as pine, can be transported long distances (e.g., Campbell et al., 1999) and are therefore weak indicators of local presence, whereas stomata are unlikely to have a wide dispersal and therefore more reliably reflect vegetation within the catchment (e.g., MacDonald, 2002; Pisaric et al., 2001). For sedaDNA, however, systematic studies of source area are relatively fewer (e.g., Alsos et al., 2018). One example from modern vegetation surveys on Svalbard was completed by Edwards et al. (2018). The authors concluded that soil-derived eDNA provides a highly localised snapshot of plant communities, mostly detecting species growing within 1 m of the sampling point. These findings suggest that sedaDNA source area is highly context dependent that can vary with factors such as topography, catchment size, and vegetation structure. Accordingly, to capture a more complete record of vegetation, especially in palaeoecological studies, multiple spatially distributed samples are necessary (e.g., Edwards et al., 2026).

This paper presents a high-resolution multi-proxy analysis of a latest Pleistocene site, Mint Gulch, spanning ca. 16,000 to 13,000 cal yr BP using sedaDNA, plant and invertebrate macrofossils, and pore-ice isotopes. Specifically, we address the following questions: (1) Do steppe-tundra taxa persist locally at Mint Gulch as regional shrub expansion initiates around 14,000 cal yr BP? (2) What is the timing and community composition of shrub (dependent) taxa arriving at Mint Gulch? (3) Do sedaDNA, macrofossils, and pore-ice isotopes data provide consistent or contrasting reconstructions of ecosystem change at Mint Gulch?

2. Methods and materials

2.1. Site description and sample collection

The Mint Gulch site (63.56, −139.54) is located 25 km southeast of Dawson City in the Klondike region of central Yukon and in the Traditional Territory of the Tr'ondëk Hwëch'in First Nation (Fig. 1). Active placer gold mining at the site had exposed a 7 m high exposure of ice rich loessal sediments. This exposure includes two primary stratigraphic units separated by a prominent palaeosol (Fig. 2A). We collected horizontal sediment cores, grab samples, and an Arctic ground squirrel (*Urocitellus parryii*) midden in 2018. Horizontal core samples were collected using a gasoline-powered drill with a 3-inch diameter core barrel. We removed thawed sediments to reach permafrost and drilled our horizontal cores in pairs (Fig. 2A). We collected a total of 25 cores (24 collected in pairs) from the lower stratigraphic unit and two grab samples (DF18-48 & −49) from upper unit. We also collected an Arctic ground squirrel midden (DF18-37) that was found less than 1 m below the prominent palaeosol (Fig. 2A). The midden was chipped out of the exposure using a chisel and hammer. All of our samples were placed in a cooler and transported frozen to Dawson City and then to the Permafrost Archives Science Laboratory at the University of Alberta where the remained frozen until analysis.

2.2. Radiocarbon chronology and age-depth modelling

We obtained radiocarbon dates from plant macrofossils found in five of the samples (Table 1). We pre-treated samples following standard ABA (Acid-Base-Acid) (Reyes et al., 2010) at the University of Alberta. CO_2 production, graphitisation and measurement of radiocarbon abundance was completed at the University of California Irvine Keck-Carbon Cycle AMS facility (UCIAMS). We calibrated our radiocarbon dates and constructed a Bayesian age-depth model using OxCal version 4.4 (Bronk Ramsey, 2009a) and the IntCal20 radiocarbon calibration curve (Reimer et al., 2020). The *P_Sequence* age-depth model includes four of the radiocarbon dates (Table 1) and was run with a variable *k* parameter (Ramsey, 2008; Bronk Ramsey and Lee, 2013) and a *General_Outlier* model (Bronk Ramsey, 2009b). Modelled and calibrated ages are reported at two-sigma uncertainty or as median ages, rounded to the nearest 5 years.

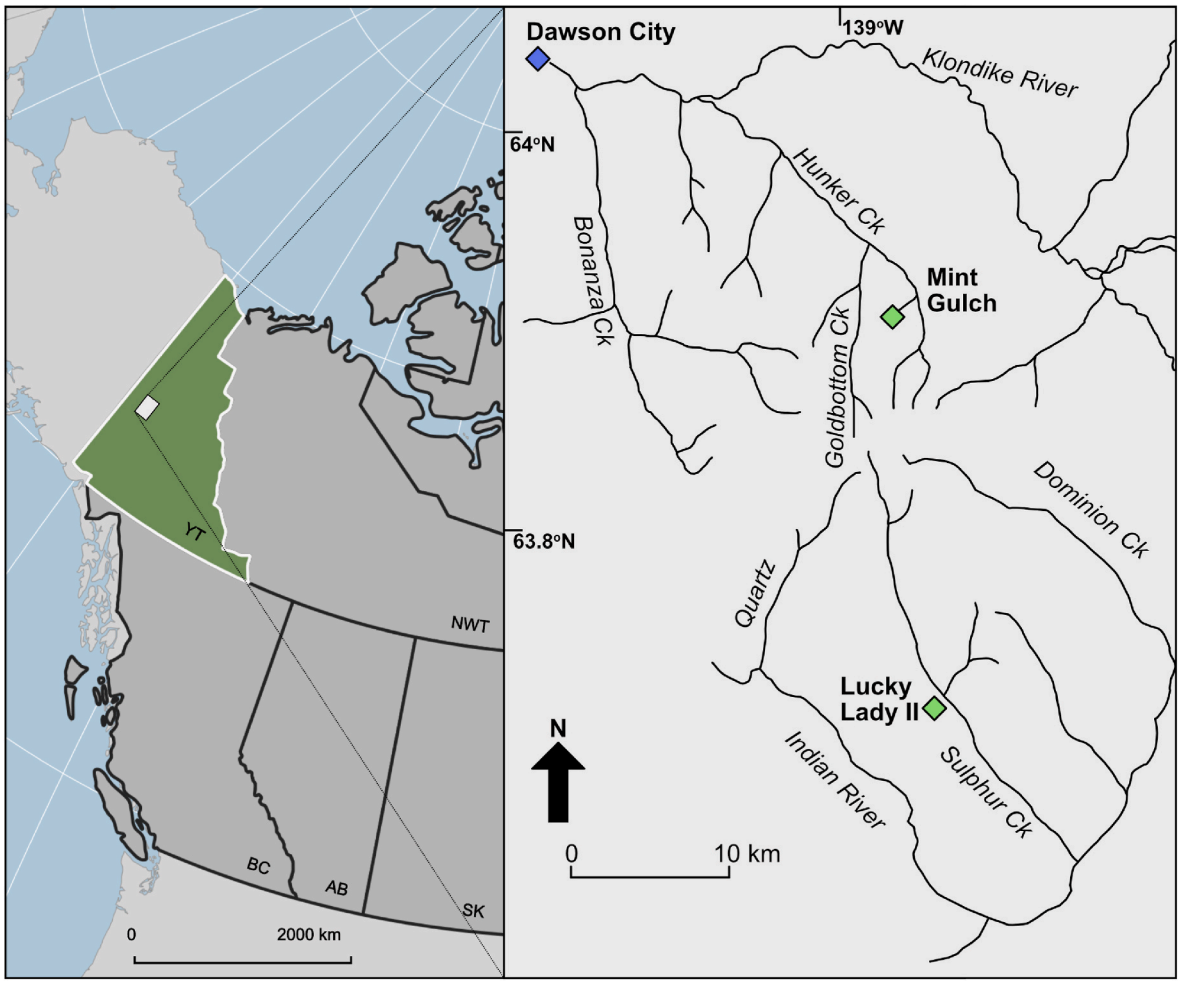

Fig. 1. Map of Klondike goldfields with the Mint Gulch and Lucky Lady II study sites.

2.3. Water isotopes

We measured pore-ice water isotopes ($\delta^{18}O$ and $\delta^{2}H$) using a Picarro L2130-I water isotope analyser by extracting waters from fifteen sediment cores in addition to waters from the Arctic ground squirrel midden by thawing the samples in vacuum-sealed bags at 4 °C and extracting the waters using a 0.2 μm filtered syringe into 2 mL autosampler vials. Raw $\delta^{18}O/\delta^{2}H$ values were normalized to the V-SMOW-SLAP scale using USGS-45 and USGS-46 or USGS-46a and USGS-48 isotope reference waters along with an internal reference water (QCDI) that was used to track run performance. Our analytical precision is estimated at less than 0.2‰ for $\delta^{18}O$ and 1.0‰ for $\delta^{2}H$ based on routine measurement of the internal reference water. After measurements were performed, we used the software ChemCorrect to calibrate the data. Calibrated $\delta^{18}O$ and $\delta^{2}H$ (‰) values are reported in Table 2.

2.4. Macrofossil preparation

We picked invertebrate and plant macrofossils from thawed sediment core sub-samples and our Arctic ground squirrel midden under a dissecting microscope. We first thawed and then wet sieved the samples through a 500 μm and 150 μm sieve and the resulting fractions were analysed individually. We present our invertebrate macrofossil data as minimum number of individuals (MNI) and we quantified our plant macrofossils using a Relative Abundance Index (RAI) (e.g., Spaulding et al., 1990; Latorre et al., 2002; Zazula et al., 2007). RAI categories reflect an estimated abundance and are bound by the following limits: 0 = absence, 1=<1%, 2 = 1–5%, 3 = 6–25%, 4 = 26–50%, 5 = 51–75% and 6=>75%.

We identified our invertebrate remains using subfossil reference collections curated by S. Kuzmina that are housed at the University of Alberta, as well as with reference to Zazula et al. (2007), Monteath et al. (2023) and modern reference specimens from the University of Alberta E.H. Strickland Entomological Museum. We identified plant macrofossils using reference collections at the Royal Alberta Museum (Quaternary Environments). Additional resources included previous publications by Zazula et al. (2005, 2007, 2011), Gaglioti et al. (2011), and Langeveld et al. (2018). We sourced the plant ecological information from Cody (2000), Zazula et al. (2007), Klinkenberg (2023), and Flora of North America Editorial Committee (1993+) and nomenclature for vascular plants is based on the Database of Vascular Plants of Canada online database (Brouillet et al., 2010). We obtained ecological information for the invertebrates from Anderson (1984), Zazula et al. (2007), and Kuzmina (2015) and taxonomic nomenclature follows various resources (see Table 3). If plant or invertebrate macrofossils could only be identified to genus level, we inferred the ecology from the most common representative in previous eastern Beringian macrofossil studies (e.g., Zazula et al., 2006, 2007) or from genera currently found in central Yukon (e.g., Anderson, 1984; Cody, 2000). Where materials could not be identified to genus level or a likely representative species could not be determined, we deemed the specimens as indeterminate (Indet.). Macrofossils that were not identified are listed as unknowns (Table 3).

2.5. Sedimentary ancient DNA

We conducted sedaDNA analysis on a total of nine sediment samples

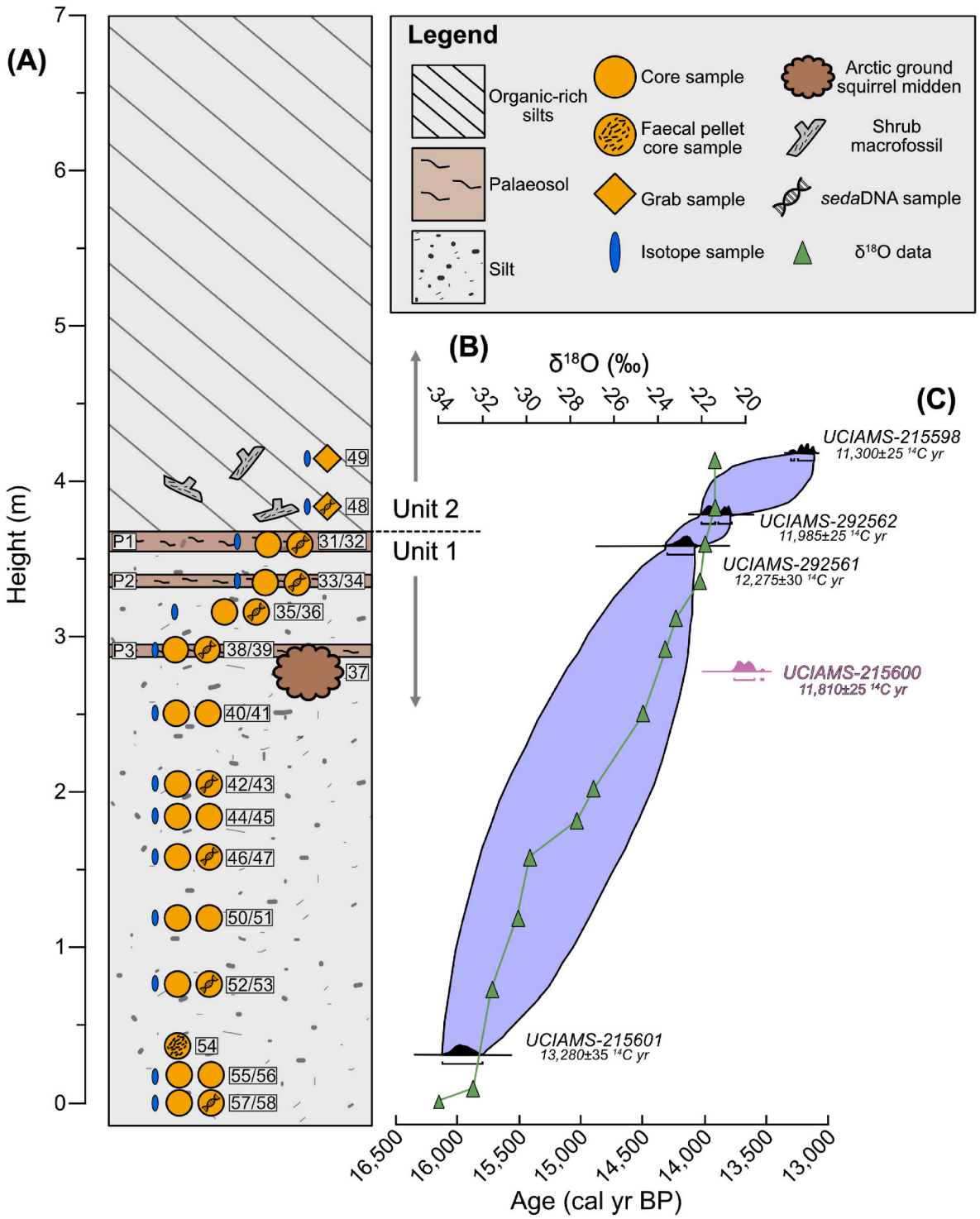

Fig. 2. Stratigraphic sections and chronology from the Mint Gulch site. (A) Stratigraphy as described in 2018. (B) Pore-ice isotopes ($\delta^{18}O$ (‰)). (C) *P_Sequence* age-depth model aligned with the stratigraphic log depth scale.

from the Mint Gulch site. We subsampled material from the interior of each core using sterilised sampling tools following methods outlined in Murchie et al. (2021a, 2021b). We took care to ensure that no interior surfaces were exposed to exogenous contamination. We first sterilised our metal sampling tools using bleach, followed by a wash in 70% molecular grade ethanol, and at least 30 min of UV irradiation. We repeated this sterilisation process for each sample. Once we collected at least 5 g of sample, we took triplicate subsamples of 250 mg, which were each added to a PowerBead tube (QIAGEN). We then extracted the triplicate subsamples independently to ensure quality and reproducibility of the results. Our extractions occurred in two batches, each with an extraction blank.

We extracted ancient DNA in a dedicated clean facility at the Permafrost Archives Science Laboratory at the University of Alberta following Murchie et al. (2021b), which uses an additional cold-spin step to optimise DNA recovery from permafrost sediments. We then prepared single-stranded DNA sequencing libraries for each extract following Kapp et al. (2021) in a dedicated ancient Paleogenomics Lab at

Table 1
Radiocarbon (^{14}C), calibrated and modelled data for the Mint Gulch site. Dates were calibrated using OxCal version 4.4 (Bronk Ramsey, 2009a,b) and the Intcal20 calibration curve (Reimer et al., 2020). All calibrated age ranges are reported at 2σ uncertainty.

Sample ID	Height in section (cm)	Lab ID (UCIAMS)	^{14}C Age (BP)	Age (cal. yr BP)	Median Age (cal. yr BP)	Modelled Age (cal. yr BP)	Median Modelled Age (cal. yr BP)	Sample type
DF18-49	420	215598	11,300 ± 25	13,290–13,115	13,185	13295–13115	13,200	Grab
DF18-48	380	292562	11,985 ± 25	14,020–13,790	13,910	14025–13790	13,935	Grab
DF18-31/32	360	292561	12,275 ± 30	14,770–14,080	14,185	14300–14075	14,155	Core
DF18-33/34	330	-	-	-	-	14715–14090	14,290	Core
DF18-35/36	310	-	-	-	-	14890–14110	14,400	Core
DF18-38/39	295	-	-	-	-	15010–14130	14,485	Core
DF18-37[a]	280	215600	11,810 ± 25	13,760–13,535	13,680	-	-	Midden
DF18-40/41	250	-	-	-	-	15335–14245	14,750	Core
DF18-42/43	205	-	-	-	-	15620–14440	15,015	Core
DF18-44/45	185	-	-	-	-	15720–14540	15,135	Core
DF18-46/47	160	-	-	-	-	15830–14690	15,280	Core
DF18-50/51	120	-	-	-	-	15965–14950	15,520	Core
DF18-52/53	75	-	-	-	-	16070–15305	15,775	Core
DF18-54	35	215601	13,280 ± 35	16,100–15,795	15,950	16115–15800	15,960	Faecal pellets/ Core
DF18-55/56	10	-	-	-	-	16300–15975	15,960	Core
DF18-57/58	0	-	-	-	-	16370–16045	16,140	Core

UCIAMS – University of California Irvine Accelerated Mass Spectrometer.
[a] Sample was not used to develop the age-depth model.

Table 2
Pore-ice isotope data from Mint gulch.

Sample ID	Height in section (cm)	$\delta^{18}O$ (‰)	$\delta^{2}H$ (‰)	Sampled for sedaDNA analysis?	Median Modelled Age (cal. yr BP)
DF18-49	420	−21.4	−165.90	No	13,200
DF18-48	380	−21.4	−166.44	Yes	13,935
DF18-31/32	360	−21.9	−171.18	Yes	14,155
DF18-33/34	330	−22.1	−172.92	Yes	14,290
DF18-35/36	310	−23.3	−181.13	Yes	14,400
DF18-38/39	295	−23.7	−186.32	Yes	14,485
DF18-37[a]	280	−22.5	−177.58	No	-
DF18-40/41	250	−24.8	−195.13	No	14,750
DF18-42/43	205	−27	−207.28	Yes	15,015
DF18-44/45	185	−27.7	−210.71	No	15,135
DF18-46/47	160	−29.8	−226.44	Yes	15,280
DF18-50/51	120	−30.4	−233.81	No	15,520
DF18-52/53	75	−31.6	−240.48	Yes	15,775
DF18-54	35	-	-	No	15,960
DF18-55/56	10	−32.5	−252.32	No	15,960
DF18-57/58	0	−33.9	−255.37	Yes	16,140

[a] DF18-37 is an Arctic ground squirrel midden and values were extracted from waters, not pore-ice.

the University of California, Santa Cruz (UCSC). Our in-solution enrichments followed Murchie et al. (2021b) using the PalaeoChip Arctic v1.0 bait set which targets whole mitochondrial genomes of ~180 extant and extinct fauna and chloroplast genes of ~2100 species of Holarctic plants. We then pooled and sequenced our libraries on an Illumina NextSeq 2000 instrument using a P2 sequencing kit with paired-end 2 x 61 cycle chemistry at the UCSC Paleogenomics sequencing facility.

For our bioinformatic pre-processing, we demultiplexed raw sequencing reads with bcl2fastq (RRID:SCR_015058). Then, using fastp (Chen et al., 2018), we removed adapters from raw sequencing reads, filtered them for minimum length of 30, a complexity of 70%, and sequence quality (phred) ≥ Q25, and merged paired-end reads. We removed duplicate sequences with PRINSEQ-lite (Schmieder and Edwards, 2011) and confirmed the quality of the resulting filtered FASTQ files with FastQC (Andrews, 2010).

For taxonomic profiling, we used a competitive mapping approach in which each read is mapped against many genomes from different species. This approach reduces mapping biases and ensures that the taxonomic identification is as accurate as possible (Feuerborn et al., 2020). We used Bowtie2 (Langmead and Salzberg, 2012) to map reads to the NCBI-nt, REFSeq (downloaded March 2023) (Sayers et al., 2022) and PhyloNorway (Alsos et al., 2020) databases with the following parameters: *bowtie2 -q -k 100 –no-unal*. We retained the top 100 hits to the databases to enable downstream lower common ancestor (LCA) analyses. After mapping, we used samtools (Li and Durbin, 2009) to merge and sort all BAM files associated with each sample. Next, we used ngsLCA (Wang et al., 2022), an LCA program that used the NCBI taxonomy for taxonomic assignments, with default parameters. We ran the ngsLCA output through bamdam (De Sanctis et al., 2025) to visualise and authenticate ngsLCA-assigned reads (see Supplementary material S1). We used a minimum cutoff of ten uniquely mapped reads to validate both our animal and plant taxa and excluded reads with a

Table 3
Macrofossil remains for Mint Gulch.

Sample ID		Taxon (elements)	MNI (Invertebrates and mammals)	RAI (Plants)	Invertebrate and mammal ecology	Habitat – Yukon (Cody 2000)	Habitat - North America (Flora of North America, Illustrated Flora of British Columbia)
DF18-48 (Grab sample)	Invertebrates	Coleoptera					
		Curculionidae					
		Connatichela artemisiae Anderson	1		Found along dry river banks and dry south-facing slopes (steppe) in association with *Artemisia*[c]		
		Gastropoda					
		Indet. Succineidae	1		Most occur in moist riparian habitats but also under rocks, sticks, logs, and leaf litter in forests.[k]		
	Plants	Indet. Amblystegiaceae		2			Habitats vary
		Unidentified fruit "UK1"		2			
		Woody materials, likely from angiosperm shrubs		3			
DF18-49 (Grab sample)	Invertebrates	Coleoptera					
		Curculionidae					
		Connatichela artemisiae Anderson	1		Found along dry river banks and dry south-facing slopes (steppe) in association with *Artemisia*[c]		
		Lepidophorus lineaticollis Kirby	1		Wet to dry tundra, southern steppe, and river shorelines[c]		
		Staphylinidae					
		Tachinus cf *brevipennis* Sahlberg	1		Mesic to moist plant litter and fluvial flood debris in tundra regions[i]		
		Indet. Carabidae	1				
		Indet. Gastropoda			Habitats vary		
	Plants	Indet. Amblystegiaceae		3			
		Unidentified fruit "UK1"		2			
		Woody materials, likely from angiosperm shrubs		3			
DF18-31/32 (Core)	Invertebrates	Coleoptera					
		Byrrhidae					
		Morychus sp.	1		Species found on azonal steppe slopes[b]		
		Curculionidae					
		Connatichela artemisiae Anderson	1		Found along dry river banks and dry south-facing slopes (steppe) in association with *Artemisia*[c]		
		Lepidophorus lineaticollis Kirby	2		Wet to dry tundra, southern steppe, and river shorelines[c]		
		Indet. Gastropoda	1		Habitats vary		
	Plants	Indet. Amblystegiaceae		1			
		Unidentified seed or achene		1			
DF18-37 (Midden)[a]	Invertebrates	Coleoptera					
		Curculionidae					
		Lepidophorus lineaticollis Kirby	1		Wet to dry tundra, southern steppe, and river shorelines[c]		
		Scarabaeidae					

(*continued on next page*)

Table 3 (*continued*)

Sample ID	Taxon (elements)	MNI (Invertebrates and mammals)	RAI (Plants)	Invertebrate and mammal ecology	Habitat – Yukon (Cody 2000)	Habitat - North America (Flora of North America, Illustrated Flora of British Columbia)
	Siphonaptera consentaneus Lectotype	3		Coprophilous dung beetle found in grasslands[d]		
	Ceratophyllidae *Oropsylla alaskensis* Baker	1		*Urocitellus parryii* hosts and burrows[e]		
	Thysanoptera Indet. Thripidae	1		Widely distributed		
	Astigmata Glycyphagidae *Fusacarus* sp. Michael	31		Found in mammal and bird nests in North America[f]		
	Mesostigmata Laelapidae: Haemogamasinae cf. *Haemogamasus* sp. Berlese	1		Rodent parasite[g]		
	cf. Macronyssidae	1		Parasitic mites, widely distributed[h]		
	Salicaceae *Salix* sp. Linnaeus		2			Arctic, boreal, temperate
	Salix cf. *arctica* Pallas		2		Mesic to dry meadows, slopes, ridges, heath, and thickets in subalpine and alpine zones	Distribution in arctic and alpine, in open wet to dry habitats including, meadows and wetlands, tundra, patterned permafrost polygons, snow beds, on slopes and cliffs, and substrates including moraines/till, and calcareous
	Indet. Poaceae		2			
	Cyperaceae *Carex* cf. *myosauroides* Villars (synonym *Kobresia myosauroides* (Villars) Fiori)		2		Dry locations, usually calcareous sandy heath and windswept ridges	Dry (to wet) areas including tundra, grassland, heathland, bare and rocky areas
	Carex sp. Linnaeus		2			Cosmopolitan in diverse and broad habitat types
	Brassicaceae cf. *Lepidium* sp. Linnaeus		3		Dry disturbed areas and waste places in steppe and montane zones	Mucilaginous seeds are suggested to contribute to long-distance transcontinental dispersal
	Asteraceae *Artemisia* sp. Linnaeus		4		Dry southerly slopes, sandy banks, grasslands, and open forest patches in montane to subalpine zones	Mainly occur in North America and Eurasia. Diverse habitats. Taxonomically challenging taxon
	Solidago sp. Linnaeus		3			Mostly in North America, with some occurrence in South America and Eurasia in broad habitats. Often in open canopy to forests including wetlands, meadows, and thickets
	Plantaginaceae *Penstemon* cf. *gormanii* Greene		3		Dry rocky slopes, sand dunes, sandy or gravelly river terraces.	Dry open area including rocky slopes, sand dunes, gravel stream terraces, forest clearings
	Rosaceae *Potentilla* cf. *glaucophylla* Lehmann		4		Mesic to dry meadows, tundra, rocky slopes, gravel bars, grasslands, and open forests in montane to alpine zones	*P. glaucophylla* var. *glaucophylla* occurs in mesic to dry areas including meadows, gravelly flats, slopes, grasslands, and tundra in the Yukon and other areas. *P. glaucophylla* var. *perdissecta* is restricted to the Northern Rockies.
	Fabaceae *Oxytropis-Astragalus* type		1			Habitats vary.
Mammal	Rodentia Cricetidae Indet. Arvicolinae (faecal pellets)	1				Indeterminate, likely colonisation of vacant midden
	Sciuridae					

(*continued on next page*)

Table 3 (*continued*)

Sample ID	Taxon (elements)	MNI (Invertebrates and mammals)	RAI (Plants)	Invertebrate and mammal ecology	Habitat – Yukon (Cody 2000)	Habitat - North America (Flora of North America, Illustrated Flora of British Columbia)
	Urocitellus parryii Richardson	1		From Boreal Forest meadows, tundra and alpine meadow habitats[i]		Cold, arid grasslands, alpine tundra, and dry open meadows (steppe-tundra)
DF18-54 (core)	Mammal Rodentia Sciuridae *Urocitellus parryii* Richardson (faecal pellets)	1		From Boreal Forest meadows, tundra and alpine meadow habitats[i]		

[a] Data from Cocker et al. (2025c)
[b] Kuzmina (2022);
[c] Anderson (1997);
[d] Krell (2024);
[e] Nadler and Hoffman (1977);
[f] Halliday and Walter (2006);
[g] Whitaker and Wilson (1984);
[h] Baulieu et al. (2019);
[i] Campbell (1988);
[j] McLean (2018);
[k] Forsyth (2005)

taxonomic assignment above the family level. Despite stringent measures to prevent contamination in the laboratory, false positive results from ecologically improbable taxa may still arise in sedaDNA studies due to sequence homology between related species and contamination in published genomes (Pach et al., 2024). To mitigate false positives, we removed taxa that were not represented in the Palaeochip-1.0 bait set before continuing with further analyses (Murchie et al., 2021b) with the exception of *Taraxacum* (dandelion), which is absent from the bait set but is known to have been present in the region (REFS). After filtering, we used *BLAST-n* (Camacho et al., 2009) to test suspected misidentified taxa that were closely related to ecologically probable species. We identified purported *Cervus* (wapiti) sequences in samples DF18-58 and DF18-48 that were homologous with other Cervidae species expected in the region, including *Rangifer* (caribou) and *Alces* (moose). We excluded these reads at the genus level but retained them at the family level under total Cervidae reads. Authentication data and sequencing results from our samples and blanks are available in Supplementary material S1.

3. Results

3.1. Site stratigraphy and chronology

The stratigraphy of the Mint Gulch site (Fig. 2) is divided into two primary units which are temporally constrained by the Bayesian age-depth model.

Unit 1 (0–360 cm) comprises organic-poor loessal silts and contains three palaeosols: a well-developed palaeosol at a height of 360 cm (P1, DF18-31/32; ca. 14,185 cal yr BP), and two less-developed palaeosols at 330 cm (P2, DF18-33) and 295 cm (P3, DF18-38). The unit also contains an Arctic ground squirrel midden (DF18-37) at 280 cm, with a direct median calibrated age of ca. 13,680 cal yr BP. However, due to stratigraphic non-conformity, caused by burrowing behavior, DF18-37 is excluded from the age-depth model (but is shown in Fig. 2B for reference). The base of Unit 1 is marked by faecal pellet sample DF18-54 (at 35 cm), which provides the oldest radiocarbon age at the site: ca. 15,950 cal yr BP.

Unit 2 (360–~700 cm) consists of organic-rich sediments, of which the uppermost ~2 m was largely inaccessible at the time of sampling. However, two radiocarbon-dated samples were obtained: DF18-48 at 380 cm (ca. 13,910 cal yr BP) and DF18-49 at 420 cm (ca. 13,180 cal yr BP), with DF18-49 representing the youngest dated sample in the stratigraphic sequence.

3.2. Water isotopes

Local scale co-isotope variability (δ^2H-δ^{18}O) is characterised using reference data of modern δ^2H and δ^{18}O measurements from the Global Network of Isotopes in Precipitation (GNIP) stations at Mayo (1986-1989) and Whitehorse (1961-1965 and 1985-1989) (IAEA/WMO). We compare co-isotope variability between the Local Meteoric Water Line (LMWL) with a slope of 6.4 and an intercept of −31.6‰ ($r^2 = 0.98$) and Mint Gulch with a slope of 7.2, and an intercept of −13.3‰ ($r^2 = 1$). Although slope and intercept values between the LMWL and Mint Gulch do not strongly agree, we attribute this to the low sample size of isotope values for Mint Gulch (n = 15) in comparison with the LMWL (n = 128) and the larger number of depleted δ^{18}O values at the Mint Gulch site. The LMWL exhibits a lowest value of −30.3‰ in comparison to the Mint Gulch site with a value of −33.9‰. We still contend that this co-isotope variability is consistent with a meteoric origin for the pore waters at the Mint Gulch site (Fig. 3). Our data follow similar trends to previous studies from the region (e.g., Porter et al., 2016; Monteath et al., 2023).

We analysed fifteen samples for pore-ice water isotopes (δ^{18}O and δ^2H) (Table 2), with an average sampling interval of 30 cm. Pore-ice oxygen isotopic (δ^{18}O) data are plotted by depth in Fig. 2. The lowest δ^{18}O value in Unit 1 of −33.9‰ occurs in the lowest sample, DF18-57 (ca. 16,140 cal yr BP), and gradually increases to palaeosol P1, DF18-

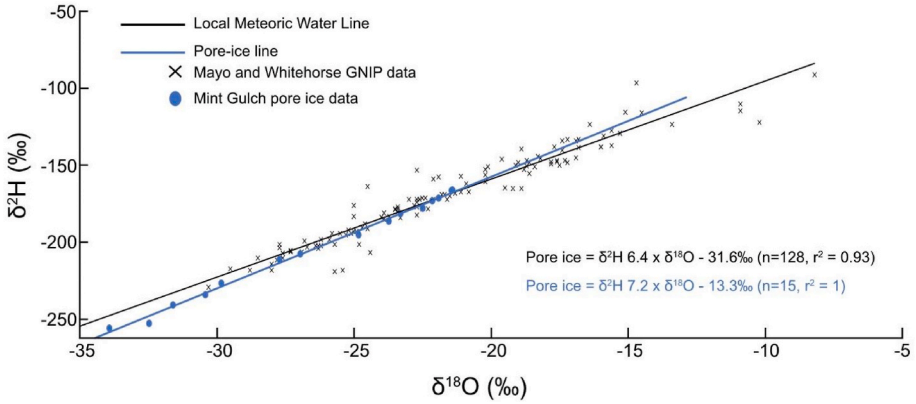

Fig. 3. A comparison between δ^2H and $\delta^{18}O$ measurements from Mayo and Whitehorse GNIP stations plotted on the Local Meteoric Water Line (LMWL) and relict pore-ice δ^2H and $\delta^{18}O$ values from the Mint Gulch site.

31/32 (ca. 14,185 cal yr BP), with a value of −21.9‰. Increased $\delta^{18}O$ values are also present in both Unit 2 samples, DF18-48 (ca. 13,910 cal yr BP) and DF18-49 (ca. 13,185 cal yr BP), both with values of −21.4‰. An enriched $\delta^{18}O$ isotope value of −22.5‰ is recorded for midden DF18-37 (ca. 13,680 cal yr BP).

3.3. Sedimentary ancient DNA

We obtained animal and plant sedaDNA from nine permafrost samples (Figs. 4 and 5) (data are available in Supplementary material S1). We identified at least 16 animal taxa of interest with the majority of reads mapping to subfamilies Bovinae (likely steppe-bison, *Bison* cf. *priscus*) and Caprinae (e.g., members of sheep genus, *Ovis*) and to the genera *Mammuthus* (likely woolly mammoth, *M. primigenius*) and *Equus* (horses). Arvicoline rodents (Arvicolinae) and caribou (*Rangifer tarandus*) were detected across almost all samples but with an overall lower number of mapped reads. Moose (*Alces alces*) and willow ptarmigan (*Lagopus lagopus*) either appear for the first time, or with a greater abundance in the youngest sedaDNA sample at the site (DF18-48), which has an age of ca. 13,910 cal yr BP. Sample DF18-36 had a far lower diversity with the only taxa being Phasianidae and Arvicolinae.

The plant sedaDNA taxonomic profile is dominated by forbs (herbaceous flowering plants) and grasses in terms of diversity and trees and shrubs in the family Salicaceae when considering total mapped read counts. A variety of forbs such as Asteraceae (daisies), Primulaceae (primroses), Boraginaceae (forget-me-nots), Orobanchaceae (broomrapes), Fabaceae (legumes), Rosaceae (roses) and Brassicaceae (mustards) are present across all samples at relatively consistent sedaDNA abundance. The youngest sample, DF18-48 (ca. 13,910 cal yr BP), records the arrival of shrub taxa like *Rhododendron*, *Arctostaphylos* (bear berries) and *Pyrola* (wintergreen), as well as an increase in the relative sedaDNA abundance of other forbs such as *Lupinus* (lupines).

Fig. 4. A heatmap of the most common animal taxa based on read counts.

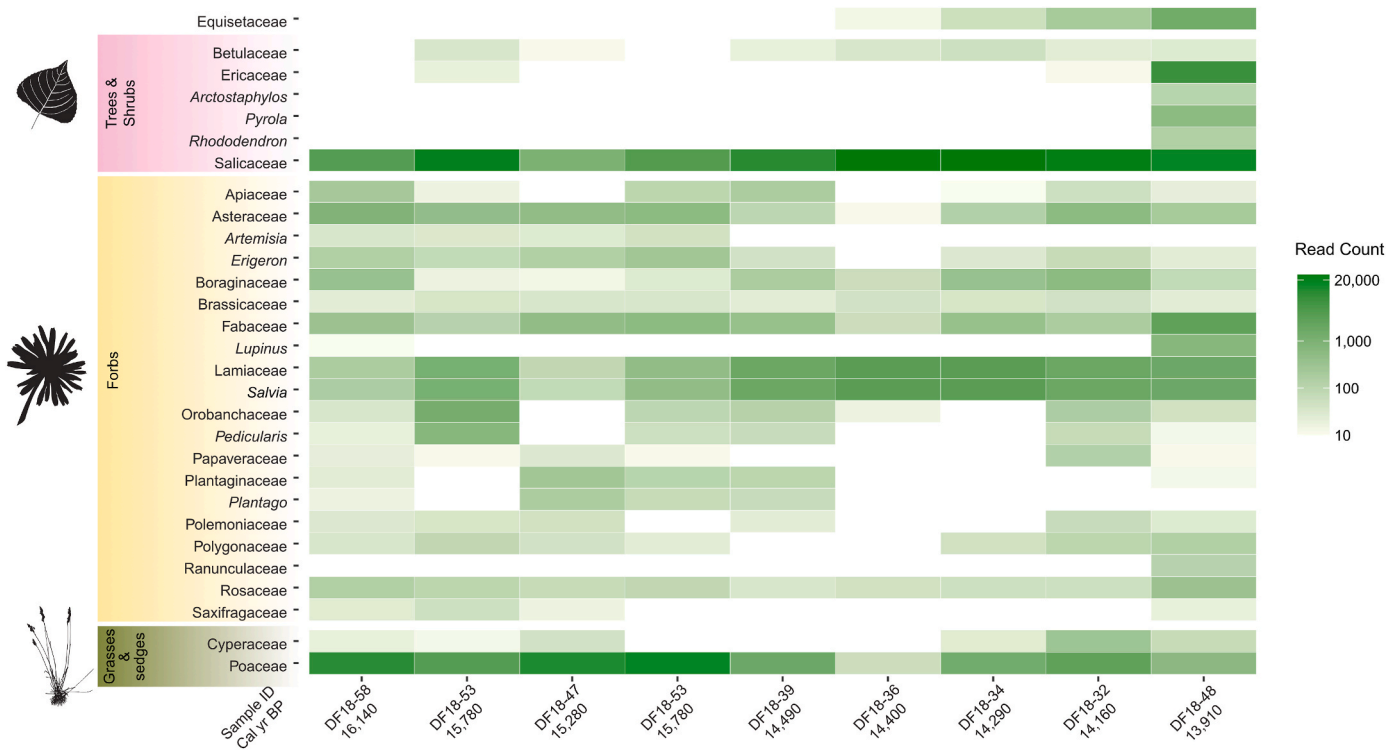

Fig. 5. A heatmap of the most common plant taxa based on read counts.

3.4. Macrofossils

We collected and analysed macrofossils from a total of five samples (Table 3). The most diverse and abundant source of macrofossils was the Arctic ground squirrel midden DF18-37 which was analysed in detail by Cocker et al. (2025c). Additionally, core samples (DF18-31/32 & 54) and grab samples (DF18-48 & 49) were analysed to supplement the record from midden DF18-37.

From Unit 1, the oldest sample analysed for macrofossils, DF18-54 (ca. 15,950 cal yr BP), is dominated by Arctic ground squirrel faecal pellets. Moving up the sequence, the Arctic ground squirrel midden, DF18-37 (ca. 13,680 cal yr BP), contained an abundance of invertebrates including mammal-associated ectoparasitic mites (cf. *Haemogamasus* sp.; MNI = 1) and a flea (*Oropsylla alaskensis*; MNI = 1), glycyphagid

Fig. 6. Macrofossil remains. Scale bars are 1 mm. (1) *Tachinus* cf. *brevipennis* right elytra (DF18- 49); (2) Indet. Carabidae head (DF18-49); (3) Succineidae gen. indet (DF18-48); (4) *Morychus* sp. elytra apex fragment (DF18-31/32); (5) Unidentified fruit. UK1 (DF18-48); (6) Shrub fragment (DF18-49); (7 & 8) Indet. Amblystegiaceae (DF18-31/32 & DF18-49).

mites (*Fusacarus* sp.; MNI = 31) (Cocker et al., 2025b), the weevil *Lepidophorus lineaticollis* (MNI = 1), and the dung beetle *Aphodius* cf. *consentaneus* (MNI = 3). A similarly abundant plant macrofossil record is dominated by the achenes of cf. *Artemisia* sp. (RAI = 4), florets of a grass, likely *Elymus* sp. (RAI = 4), and seeds of the perennial herb, *Potentilla* cf. *glaucophylla* (RAI = 4). Additionally, the midden contained skeletal remains of Arctic ground squirrel (MNI = 1) and faecal pellets of an arvicoline rodent. Macrofossils from the palaeosol P1 (DF18-31/32, ca. 14,190 cal yr BP) preserve the remains of three beetles, the pill beetle *Morychus* sp. (MNI = 1; Fig. 6, no. 4) and the weevils *Connatichela artemisiae* (MNI = 1) and *Lepidophorus lineaticollis* (MNI = 2). Palaeosol P1 preserved little identifiable plant material with the exception of bryophytes (Amblystegiaceae) (RAI = 1). Gastropod fragments were also present, but poorly preserved.

From Unit 2, grab sample DF18-49 (ca. 13,185 cal yr BP) yielded four beetles including the weevils *C. artemisiae* (MNI = 1) and *L. lineaticollis* (MNI = 1), the rove beetle *Tachinus* cf. *brevipennis* (MNI = 1; Fig. 6, no. 1) and an indeterminate head of a ground beetle (Carabidae; MNI = 1; Fig. 6, no. 2). Woody material and bryophytes (Amblystegiaceae) dominate the sample (RAI = 3; Fig. 6, no. 6, 7 and 8), but remain largely indeterminate due to the lack of identifiable characteristics. Plants from sample DF18-48 (ca. 13,910 cal yr BP) remain compositionally the same as DF18-49 and both samples contain an additional unidentified fruit (Unknown 1, UK1; Fig. 6, no. 5) that appears for the first time in Unit 2. DF18-48 also preserves a complete terrestrial gastropod from the family Succineidae (Fig. 6, no. 3).

4. Discussion

4.1. Sedimentary ancient DNA at Mint Gulch

The sedaDNA record at Mint Gulch is dominated by forbs and grazing megafauna taxa from ca. 16,000 to 14,000 cal yr BP in Unit 1 with the arrival of shrubs, browsers, and shrub-dependent taxa by ca. 13,910 cal yr BP in Unit 2 (Figs. 4 and 5).

The youngest sample (DF18-48; ca. 13,910 cal yr BP), although recording the arrival of shrubs, largely comprises herbaceous and grassland taxa that are compositionally typical of other sedaDNA records of steppe-tundra environments from the region (Murchie et al., 2021a; Monteath et al., 2023). This sample appears to capture the

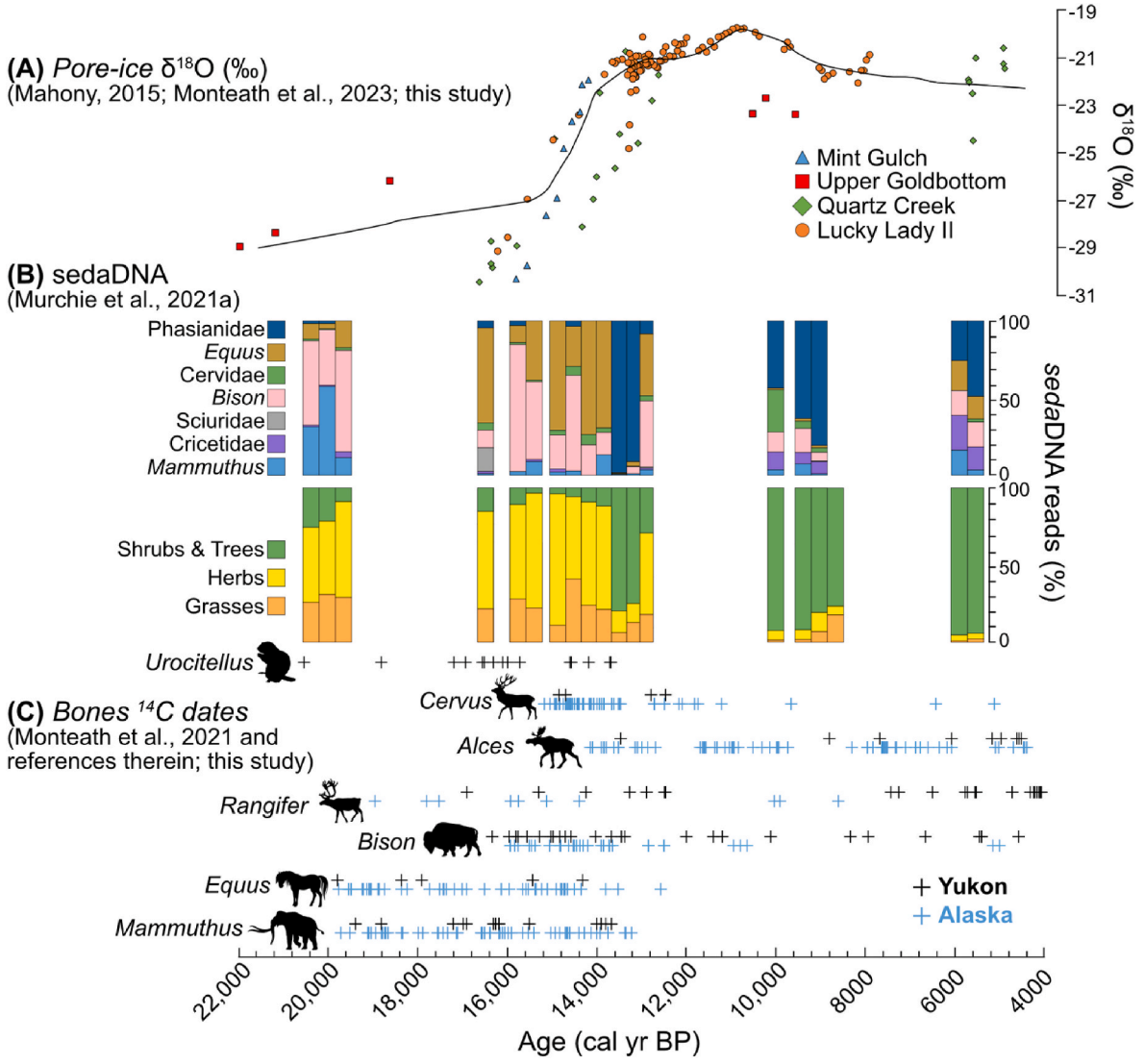

Fig. 7. Palaeoenvironmental data from the Klondike. (A) Pore-ice $\delta^{18}O$ isotope data from the Mint Gulch, Upper Goldbottom, Quartz Creek and Lucky Lady II sites (data from Mahony, 2015; Monteath et al., 2023, and this study). (B) Sedimentary ancient DNA (sedaDNA) from sites across the Klondike (Data from Murchie et al., 2021a). (C) Radiocarbon-dated fossil and midden vegetation (Arctic ground squirrels) from Yukon Territory and Alaska (data from this study and Monteath et al., 2021, references therein, with amendments from Bronk Ramsey et al., 2011).

initial transition with the co-occurrence of both steppe-tundra and shrub-tundra taxa without one dominating the signal over the other. In other Yukon sedaDNA records this transition has not been fully captured as it took place rapidly and sampling resolutions are often too coarse. Therefore, the Mint Gulch vegetation record provides improved insight into what was likely a period of ecosystem restructuring from steppe-tundra to shrub-tundra. A similar transition is recorded in the animal sedaDNA record. From ca. 16,140 to 14,160, large grazing megafauna (mammoth, bison, horse) dominates the assemblage with minor contributions from Caprinae (likely *Ovis* sp.; sheep), Cervidae (*Rangifer tarandus*; caribou), Arvicolinae (lemmings and voles), and minor contributions early in the record from the rock ptarmigan (*Lagopus muta*). Like the steppe-tundra vegetation, this fauna persists throughout the sequence with the additional arrival of browsing and shrub-associated taxa such as moose (*Alces alces*), and the read-dominant willow ptarmigan (*Lagopus lagopus*) in the youngest sample, DF18-48 (ca. 13,910 cal yr BP). Present-day willow ptarmigan populations are distributed from Newfoundland to NW Alaska and across into Eurasia (Hannon et al., 2020). This species typically inhabits wetter and low elevation sites, specifically in thickets of shrubs like birch (*Betula* spp.) or bordering ecotones of subarctic and alpine ecosystems (Wilson and Martin, 2008). Considering the ecology of willow ptarmigans, the arrival of this taxon supports the initiation of shrub development at the Mint Gulch site. The co-arrival of willow ptarmigans and shrubs has similarly been recorded by Murchie et al. (2021a) from additional Klondike sedaDNA records around ca. 13,335 cal yr BP (see Fig. 7B). Despite evidence for arvicoline rodents in the sedaDNA record, we only find macrofossil evidence of Arctic ground squirrels from midden DF18-37. It is unclear whether Arctic ground squirrels were not a common component of the fauna at the Mint Gulch site or if they were a minor component of the local fauna that contributed little DNA. This near absence in our sedaDNA data highlights that a combination of traditional and genetic palaeoecological approaches to sites like Mint Gulch captures the most holistic view of the local ecosystem.

4.2. A regional or local hydroclimate signal?

The Mint Gulch site captures a latest Pleistocene sedimentary sequence and preserves a pore-ice isotope record ($\delta^{18}O$) transitioning from cold-stage (−33.9‰ at ca. 16,000 cal yr BP) to warm-stage (−21.4‰ by 13,180 cal yr BP) conditions (Fig. 2B). Pore-ice isotope signatures in permafrost can be altered by active layer freeze–thaw cycles, which enable incorporation of meteoric waters during surface aggradation or active layer thinning. (Porter et al., 2016; Porter and Opel, 2020). This means that pore-ice can contain several years of precipitation until it is integrated into the permafrost table where it no longer experiences seasonal thaw. This integration can be influenced by active layer depth, which can be estimated through time (Supplementary material S2). In the case of Pleistocene sites like Mint Gulch, this aggradation occurs primarily through the deposition of loess (Porter et al., 2016; Monteath et al., 2023). In Fig. 7A, we compare the Mint Gulch pore-ice isotopes to other sites across the Klondike (Fig. 7A; Mahony, 2015; Monteath et al., 2023). In all cases, an enrichment of the regional $\delta^{18}O$ values (from ~ −30‰) initiates by ca. 15,000 cal yr BP and continues for the next ~2000 years before reaching Early Holocene values ranging between −19‰ and −22‰. Combined with an agreement between the Mint Gulch pore-ice isotopic record and the local meteoric water line (LMWL; Fig. 3) the data indicate that pore-ice waters at Mint Gulch originate from a meteoric source and therefore represent a regional hydroclimate signal and not a localised one.

4.3. Ecosystem response to a shifting regional hydroclimate

Pore-ice isotope, sedaDNA, and fossil records across the Klondike record the transition from steppe-tundra to shrub-tundra in response to a shifting regional hydroclimate (Mahony, 2015; Monteath et al., 2021, 2023; Murchie et al., 2021a,b). These data are illustrated in Fig. 7 which spans ca. 22,000 to 4000 cal yr BP. Pore-ice $\delta^{18}O$ isotope records from the Klondike increase from the lowest values of ~30.4‰ at ca. 16, 600 cal yr BP to ~19.7‰ by ca. 11,000 cal yr BP. In response to this hydroclimate shift, plant and animal sedaDNA across the Klondike records a transition from grass and herbs to trees and shrubs by at least ca. 13,300 cal yr BP and simultaneously record an increase in the presence of ground nesting birds like the willow ptarmigan (Fig. 7B). After ca. 13, 300 cal yr BP, Klondike sedaDNA records show the decline of herbs, grasses, and grazing megafauna (e.g., horse, bison, mammoth) for which the faunal response is also recorded in the bone record. Except for bison, which were extirpated and subsequently reintroduced, radiocarbon-dated fossils of horse and mammoth are last recorded at ca. 13,910 and 13,720 cal yr BP, respectively, from Yukon Territory (Guthrie, 2004; Kelly, 2022). With the decline of grazers, the bone data also records the arrival of browsing herbivores like moose by ca. 8810 cal yr BP in central Yukon (and ca. 13,430 cal yr BP in northern Yukon) (Fig. 7C). Our sedaDNA data from Mint Gulch indicate that moose had actually arrived in central Yukon ~4000 years earlier, but bone data to corroborate this does not yet exist.

Data from Mint Gulch are consistent with these regional trends, capturing the early transitionary phase from steppe-to shrub tundra. Our data agree with the interpretation that hydroclimate-driven ecosystem restructuring occurred at a regional scale in the Klondike. Additionally, the Mint Gulch record highlights that a site-specific approach is important for resolving the heterogeneous and site-specific ecological responses that are not captured in broader regional summaries.

4.4. Steppe-tundra persistence at Mint Gulch

Although a regional transition from steppe-tundra to shrub-tundra was in motion by ca. 14,000 cal yr BP across east Beringia (Monteath et al., 2021), chronologically constrained palaeoecological data at Mint Gulch highlight the value of local records. In addition to our sedaDNA data that highlights the persistence of steppe-tundra taxa despite the arrival of shrub-tundra taxa, our plant and invertebrate macrofossils provide additional evidence to support the local persistence of steppe-tundra at the Mint Gulch site.

The middens of Arctic ground squirrels are another important source of macrofossil data from Beringia (Zazula et al., 2005, 2007, 2011; Lopatina and Zanina, 2006; Zanina et al., 2011; Gaglioti et al., 2011; Ashastina et al., 2018; Cocker et al., 2024, 2025a, 2025b, 2025c). The caching behaviours of Arctic ground squirrels are recognised as reliable sources of ecological data, and their extirpation from the Klondike at the end of the Pleistocene emphasises how this taxon was impacted by the collapse of steppe-tundra and the arrival of shrub-tundra (Buck and Barnes, 1999; Zazula et al., 2005, 2007, 2011; Lopatina and Zanina, 2006; Zanina et al., 2011; Gaglioti et al., 2011).

Midden DF18-37, dating to ca. 13,680 cal yr BP, is well poised to address steppe-tundra persistence at the Mint Gulch site. DF18-37 is isotopically anomalous relative to other Pleistocene middens when placed in the framework of Klondike pore-ice isotopes and additional midden records as it occupies a more enriched isotopic space typical of a post 15,000 cal yr BP shift in the regional hydroclimate (Fig. 8Table 4) (Monteath et al., 2023; Mahony, 2015). Additionally, DF18-37 is younger than sedaDNA sample DF18-48 (ca. 13,910 cal yr BP), the latter of which includes shrubs, ground nesting birds (willow ptarmigan), and browsing herbivores (e.g., moose). Despite this, midden DF18-37 also includes plant macrofossil taxa such as *Artemisia* sp. (likely *A. frigida*), *Potentilla* sp., and *Penstemon* cf. *gormanii*, which have routinely been recovered from Pleistocene middens (Zazula et al., 2007, 2011) and are important components of steep, dry, and south-facing grasslands in SW Yukon Territory today (Vetter, 2000). From the invertebrate fauna, the weevil *Lepidophorus lineaticollis* was present, although at low abundance (MNI = 1). This taxon is routinely recognised as an indicator of dry tundra environments across Pleistocene Beringia (e.g., Kuzmina et al.,

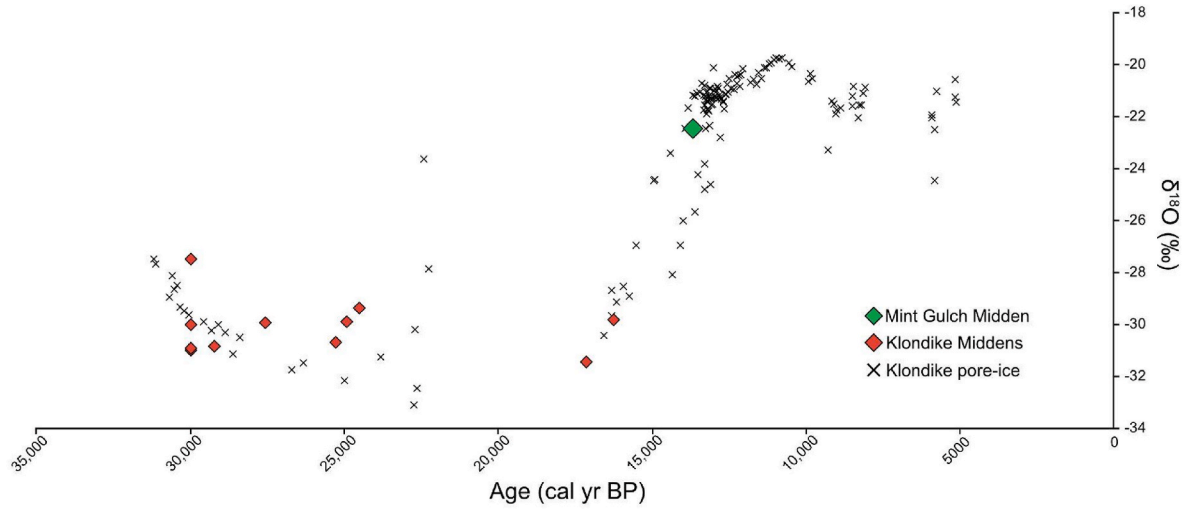

Fig. 8. Klondike pore-ice and Arctic ground squirrel midden $\delta^{18}O$ data plotted against age.

Table 4
$\delta^{18}O$ isotope data for Klondike Arctic ground squirrel middens used in Fig. 8.

Midden ID	Lab ID (UCIAMS)	Age	Age (cal. yr BP)	Site	$\delta^{18}O$ (‰)
DF18-37	215600	11,810 ± 25	13,680	Mint Gulch	−22.48
MM13-22	131096	13,510 ± 45	16,270	Upper Quartz Creek	−29.81
DF09-HC-29	67157	14,100 ± 40	17,155	Hunker Creek	−31.45
MM13-44	131093	20,310 ± 720	24,525	Upper Goldbottom	−29.39
MM13-42		Estimated	25,000	Upper Goldbottom	−29.9
MM13-41	131095	20,960 ± 150	25,285	Upper Goldbottom	−30.7
MM12-67	114718	23,360 ± 100	27,575	Black Hills	−29.92
SC22-HC-38	274808	25,030 ± 90	29,220	Hunker Creek Sailer's Property	−30.84
SC22-HC-37		Dawson tephra	ca. 30,000	Hunker Creek Sailer's Property	−30.99
SLC21-KL-8		Dawson tephra	ca. 30,000	Hunker Creek Sailer's Property	−30.03
SLC21-TM-1a		Dawson tephra	ca. 30,000	Eureka Creek (Treadstone Mine)	−27.48
SC22-HC-40		Dawson tephra	ca. 30,000	Hunker Creek Sailer's Property	−30.92

2008). To complement macrofossil data from midden DF18-37, additional analysis of grab sample DF18-49 yielded an individual of the weevil *Connatichela artemisiae*. This taxon is endemic to Yukon Territory, is regularly recovered from Pleistocene deposits, and is closely associated with prairie sage (*A. frigida*) (Anderson, 1984). Comparing the modern and Pleistocene records of *C. artemisiae* from east Beringia, it is apparent that this taxon had a wider distribution during the Pleistocene and that its present-day distribution is limited by availability of dry steppe environments (Anderson, 1984, 1997; Zazula et al., 2007; Kuzmina, 2025). The presence of *C. artemisiae* in grab sample DF18-49 provides an additional line of evidence supporting the local persistence of steppe-tundra at Mint Gulch.

One data point that contrasts with the interpretation of xeric environments is the occurrence of willow (*Salix* sp.) in sample DF18-37. However, willow is one of the key taxa that separates steppe-tundra from steppe, and the capsules, twigs, and leaves of willows have routinely been recorded from Arctic ground squirrel middens throughout the late Pleistocene (Zazula et al., 2007, 2011), as well as from the ca. 21,500-year-old Kitluk palaeosol on the Seward Peninsula, Alaska (Goetcheus and Birks, 2001). Furthermore, willow macrofossils recovered in DF18-37 are most likely from the Arctic willow (*Salix arctica*), which is a prostrate to somewhat erect subshrub species of willow typically found in mesic to dry meadows, slopes, ridges, heath, and thickets in subalpine and alpine zones (Flora of North America Editorial Committee, 1993+).

The records from both midden DF18-37 and grab sample DF18-49 highlight the benefit of complementing sedaDNA and isotopic analyses with the analyses of plant and invertebrate macrofossils. Without macrofossils, sedaDNA analyses may fail to detect key taxa, such as *Connatichela artemisiae*, due to the underrepresentation of invertebrates in genetic reference databases. Importantly, this multiproxy evidence reflects local environmental conditions that are strongly mediated by factors such as aspect and topography which impact both which species can establish and persist on the landscape and the spatial source area of our sedaDNA signal.

Consequently, our data do not argue for the widespread persistence of steppe-tundra, but instead they capture localised conditions which are consistent with the sporadic distribution of azonal steppe communities on south-facing slopes in SW Yukon Territory today (Laxton et al., 1996; Vetter, 2000; Zazula et al., 2007).

4.5. Intra site consistency across east Beringia

Throughout this study, we discuss the timing of shrub expansion across east Beringia with a focus on sites in the Klondike goldfields of Yukon Territory. Our data from the Mint Gulch site indicate that slowing loess deposition (marked by formation of a palaeosol P1) correlates temporally with the initiation of shrub expansion around 14,000 cal yr BP. The formation of palaeosol P1 differs from the palaeosol at the nearby Lucky Lady II site (ca. 13,480 cal yr BP) but closely aligns with shrub expansion records from interior Alaska. Clarke et al. (2024) document the expansion of birch (*Betula*) DNA and reduction of grasses ca. 14,500 cal yr BP using sedaDNA from Chisholm Lake. The Chisholm Lake chronology was developed from an integrated Bayesian age-depth model that included radiocarbon data from both Chisholm Lake (Tinner et al., 2006; Clarke et al., 2024), nearby Birch Lake (Bigelow, 1997) and Jan Lake (Carlson and Finney, 2004). The model also constrains the regional *Betula* pollen rise to the same date. It is conceptually

challenging to understand why the Lucky Lady II site preserved a different record of shrub expansion in east Beringia compared with Mint Gulch, despite being only 20 km away, other than to highlight how site specific or local conditions may drive spatial differences. Monteath et al. (2021, 2023) discuss the delayed expansion of shrubs at higher elevation sites or in response to continental glaciation on the eastern margin of Beringia. However, it is not clear how the Laurentide or Cordilleran ice sheets, which were fully separated and in retreat by ca. 14,000 cal yr BP (Dalton et al., 2023), would impact the Lucky Lady II site but not Mint Gulch. Differences like those observed between Mint Gulch and Lucky Lady II might be attributable to characteristics like slope and aspect. A recent study of Chisholm Lake in interior Alaska by Clarke et al. (2024) addresses the influence of slope and aspect. The authors highlight that both incident radiation and soil moisture would have favoured dry tundra taxa on the steep north-facing slopes and boreal steppe taxa on the south-facing slopes. This variation alone may account for local scale variation in palaeoecological records.

Sediment accumulation may also alter how rapidly our proxies record ecosystem shifts. Unit 1 from the Mint Gulch site has a sedimentation rate of ~0.18 cm/yr (accumulating ~ 325 cm from ca. 15,950 – 14,185 cal yr BP) in comparison to the ~0.1 cm/yr at Lucky Lady II (accumulating ~350 cm from ca. 16,495 – 13,150 cal yr BP). This difference in sedimentation rate may account for increased sensitivity at the Mint Gulch site, but this is difficult to attribute this confidently.

Alternatively, we may consider differences in the number of radiocarbon dates obtained within and above each site's respective main palaeosol. At the Mint Gulch site, we were able to obtain one date from palaeosol P1 and two from the overlying sediments (due to inaccessibility of the upper section at the time of sampling). In comparison, Monteath et al. (2023) obtained four dates from their prominent palaeosol and an additional six dates from the 1 m of overlying sediments. This difference in chronological resolution may account for the apparent offset in the timing of shrub expansion between the Mint Gulch and Lucky Lady II sites. The reason we raise this point is not to suggest a definitive solution, but rather to highlight the uncertainty that remains in the literature, which we think is still the case. This reiterates the need for additional sites to help constrain the regional timing of shrub expansion.

Our analysis of the Mint Gulch site shows that palaeoecological records from east Beringia do not directly support a uniform expansion of shrubs in response to a shifting hydroclimate. Instead, our data analysed in the broader context of eastern Beringian palaeoecological records support the Brubaker et al. (2005) concept of glacial refugia, which highlights a mosaic of habitats and the gradual expansion of shrubs from isolated populations across east Beringia.

5. Conclusion

The Mint Gulch site documents a latest Pleistocene ecosystem preserved in relict permafrost and provides evidence for the local persistence of steppe-tundra despite a shifting regional hydroclimate. The site records an increase of pore-ice isotopes from ca. −32‰ at ca. 16,000 cal yr BP to −21.4‰ by ca. 13,180 cal yr BP and the initiation of transition from steppe-tundra to shrub-tundra ca. 13,910 cal yr BP in the sedaDNA record. However, a midden-focused macrofossil record indicates the local persistence of steppe-tundra.

Our data demonstrate the value of multiproxy analysis as, if any one proxy was analysed in isolation, a biased view of the Mint Gulch ecosystem would have been interpreted. Our multiproxy approach allows us to record an ecosystem in transition (beginning around 13,910 cal yr BP) and captures the co-occurrence of steppe-tundra taxa like mammoth and horse and later-arriving browsers and shrub-dependent taxa like moose, and willow ptarmigan. A similar co-occurrence is seen in the plant sedaDNA data. The persistence of steppe-tundra despite a shifting regional hydroclimate is further supported by macrofossil records that preserve steppe-tundra taxa like *Artemisia* sp. (likely *A. frigida*), *Potentilla* sp., and *Penstemon* cf. *gormanii*, the dry-tundra weevil *Lepidophorus lineaticollis*, and the steppe-tundra weevil *Connatichela artemisiae*.

To our knowledge, the Mint Gulch site presents the first record that effectively captures the initiation of this transitionary period. Latest Pleistocene data is also recorded from Lucky Lady II, a site located ~20 km from Mint Gulch (Monteath et al., 2023) but this transition occurs almost 500 years later. Despite the offset between Mint Gulch and Lucky Lady II, the Mint Gulch site chronology and ecosystem transition is in agreement with other records from interior Alaska like Chisholm Lake (Clarke et al., 2024). The Mint Gulch site highlights the value of local-scale palaeoecological records to understand the nuanced transition from steppe-tundra to shrub-tundra at the end of the Pleistocene in east Beringia.

Author contributions

Conceptualisation: Scott L. Cocker and Duane G. Froese. Sample collection and stratigraphy: Duane G. Froese and Beth Shapiro. Formal analysis: Scott L. Cocker, Evan Francis, Ciara Wanket, and Diana Tirlea. Investigation: Scott L. Cocker, Evan Francis, Ciara Wanket, Diana Tirlea, and Svetlana Kuzmina. Visualisation: Scott L. Cocker, Evan Francis, Ciara Wanket and Alistair J. Monteath. Writing – original draft: Scott L. Cocker and Evan Francis. Writing – review & editing: All

unsited references

Campbell, 1988,Forsyth, 2005,Halliday and Walter, 2006,Krell, 2024,Kuzmina, 2022,McLean, 2018,Nadler and Hoffmann, 1977, Whitaker Jr and Wilson, 1974

Declaration of competing interest

We confirm that none of this material has been published or is under consideration elsewhere, and all authors have approved submission.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.

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Data availability

All data and/or code is contained within the submission.

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