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ORIGINAL ARTICLE

Pleistocene glacial and interglacial ecosystems inferred from ancient DNA analyses of permafrost sediments from Batagay megaslump, East Siberia

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Abstract

Pronounced glacial and interglacial climate cycles characterized northern ecosystems during the Pleistocene. Our understanding of the resultant community transformations and past ecological interactions strongly depends on the taxa found in fossil assemblages. Here, we present a shotgun metagenomic analysis of sedimentary ancient DNA (*seda*DNA) to infer past ecosystem-wide biotic composition (from viruses to megaherbivores) from the Middle and Late Pleistocene at the Batagay megaslump, East Siberia. The shotgun DNA records of past vegetation composition largely agree with pollen and plant metabarcoding data from the same samples. Interglacial ecosystems at Batagay attributed to Marine Isotope Stage (MIS) 17 and MIS 7 were characterized by forested vegetation (*Pinus*, *Betula*, *Alnus*) and open grassland. The microbial and fungal communities indicate strong activity related to soil decomposition, especially during MIS17. The local landscape likely featured more open, herb-dominated areas, and the vegetation mosaic supported birds and small omnivorous mammals. Parts of the area were intermittently/partially flooded as suggested by the presence of water-dependent taxa. During MIS 3, the sampled ecosystems are identified as cold-temperate, periodically flooded grassland. Diverse megafauna (*Mammuthus*, *Equus*, *Coelodonta*) coexisted with small mammals (rodents). The MIS 2 ecosystems existed under harsher conditions, as suggested by the presence of cold-adapted herbaceous taxa. Typical Pleistocene megafauna still inhabited the area. The new approach, in which shotgun sequencing is supported by metabarcoding and pollen data, enables the investigation of community composition changes across a broad range of taxonomic groups and inferences about trophic interactions and aspects of soil microbial ecology.

KEYWORDS
Batagay megaslump, metabarcoding, Pleistocenepollen, sedaDNA, shotgun sequencing,

1 | INTRODUCTION

Ongoing climate change affects biota at the ecosystem level (Pecl et al., 2017; Tylianakis et al., 2008; Walther, 2010). Amplified arctic warming causes northern boreal and arctic ecosystems to experience more extreme climate change than other ecosystems in the Northern Hemisphere (Miller et al., 2010) fostering the northward extension of shrubs and trees into tundra ecosystems (Myers-Smith et al., 2019; Tape et al., 2006). Due to the complexity of biotic and abiotic interactions, predictions of species distribution patterns at ecosystem scale are difficult to establish (HilleRisLambers et al., 2013; Walther et al., 2002).

Understanding past ecological changes enhances our ability to understand recent and future warming effects on ecosystems (Botkin et al., 2007). The Pleistocene Epoch (~2600–11.7 thousand years (kyr)) before present was characterized by alternating glacial and interglacial stages (Mix & Ruddiman, 1984; Shackleton, 1967),which involved major changes in past climate, ecosystems, and genetic diversity patterns (Hewitt, 2000; Jackson & Blois, 2015). Continuous environmental records spanning several glacial–interglacial cycles are rare in boreal and arctic regions of Eurasia, being primarily represented by two deep sediment cores from Lake Baikal and Lake El'gygytgyn (Andreev et al., 2014; Brigham-Grette et al., 2013; Demske et al., 2002; Melles et al., 2012; Tarasov et al., 2005). Other Eurasian palaeoecological records older than Marine Isotope Stage (MIS) 3 are discontinuous, being derived from permafrost sediment sequences, but some date to the MIS 7 (e.g., Andreev et al., 2011; Wetterich et al., 2019, and references therein). The Batagay megaslump in East Siberia provides access to ecological information since the Middle Pleistocene (Ashastina et al., 2017; Murton et al., 2017, 2021; Opel et al., 2019).

A particular challenge is to retrieve information on the past biota from such environmental records. Beyond the palaeofaunas that have been well documented from bone assemblages; plant macrofossil, pollen analysis (e.g., Andreev et al., 2011, 2012), and insect remains (e.g., Kuzmina et al., 2011) are the traditional methods for retrieving information on terrestrial ecological changes from permafrost sediments. Recently, the investigation of sedimentary ancient DNA (*seda*DNA) has become a promising approach (e.g., Willerslev et al., 2003, 2014). In particular, several plant metabarcoding studies have focused on vascular plants (Courtin et al., 2021; Epp et al., 2015; Liu et al., 2020; Sonstebo et al., 2010; Willerslev et al., 2014; Zimmermann, Raschke, Epp, Stoof-Leichsenring, Schirrmeister, et al., 2017; Zimmermann, Raschke, Epp, Stoof-Leichsenring, Schwamborn, et al., 2017). Other groups have also been investigated, such as fungi (Bellemain et al., 2013; Epp et al., 2012; Lydolph et al., 2005), bacteria (Wagner et al., 2007; Willerslev et al., 2004), invertebrates (Epp et al., 2012), birds (Epp et al., 2012), and mammals (e.g., Arnold et al., 2011; Haile et al., 2009; Wang et al., 2021; Willerslev et al., 2003, 2014). Recent advances in computational analysis and the development of new bioinformatic tools (such as Kraken2) and pipelines (Hübler et al., 2019; Piro et al., 2020; Wood et al., 2019) pave the way for shotgun sequencing of environmental DNA (Bálint et al., 2018), which allows the investigation of a broad range of organisms, from non-living viruses to mammals and plants within one single experiment (Ahmed et al., 2018; Murchie et al., 2021; Pedersen et al., 2016; Wang et al., 2021). Applied to *seda*DNA, it has the potential to provide, at least partially, an ecosystem view and snapshots of past glacial and interglacial environments.

Macrofossils mostly originate from plants growing at the study site and represent local conditions. However, the number of identifiable macrofossils is affected by taphonomy and preservation, which can vary strongly among taxa (e.g., Foote & Raup, 1996; Kienast et al., 2001; Tomescu et al., 2018). Similar issues affect vertebrates (Behrensmeyer, 1988; Gardner et al., 2016). Pollen records reflect regional ecological changes well, but information is constrained by different pollen productivities and transportability (Birks et al., 2012; van der Knaap, 1987). Moreover, the taxonomic resolution of pollen is limited, typically in trees and shrubs to genus/subgenus, but more variably from species to family in herbaceous taxa (Beug, 1961; Moore et al., 1991). In a rigorous comparison, pollen and sedaDNA (metabarcoding) from a lake-sediment core (Polar Urals; Clarke et al., 2019) yielded a similar floristic richness, 114 and 137 vascular plant taxa, respectively, but each method featured a different configuration of taxa. How sedaDNA represents vegetation at a sampling site requires further work as both taphonomy, for which investigation methods are still in their infancy, and geomorphological processes, which are site dependent, impact the scale of the sedaDNA signal (Alsos et al., 2018; Giguet-Covex et al., 2019). However, there is increasing evidence that DNA from lacustrine sediments reflects the catchment vegetation, while DNA from terrestrial sediments (e.g., palaeosols) provides a highly local signal (Edwards, 2020). *Seda*DNA is particularly well preserved in permafrost sediments as persistent subzero temperatures reduce microbial and enzymatic degradation (Zimmermann, Raschke, Epp, Stoof-Leichsenring, Schwamborn, et al., 2017) and because *seda*DNA binds strongly to minerogenic sediments (Edwards, 2020).

Here, we present data showing ecosystem-wide changes between Pleistocene glacial and interglacial intervals in East Siberia inferred from *seda*DNA shotgun sequencing, plant metabarcoding, and pollen analysis. Five sediment samples from the Batagay megaslump are analyzed. The dating of samples is imprecise: the oldest dates from the period relating to MIS 17 to MIS 16 (~650 kyr), another from MIS 16 to MIS 7 (~600 to ~200 kyr), the third to MIS 3 (~50 kyr), and the two youngest samples date to MIS 2 (~27 and ~23 kyr).

1.1 | Site description

The Batagay megaslump (67.58°N, 134.77°E) is located on a hillslope underlain by permafrost just of the Yana River in northern Yakutia, near the town of Batagay (Figure 1). It is the world's largest known retrogressive thaw slump. The slump formed during recent decades

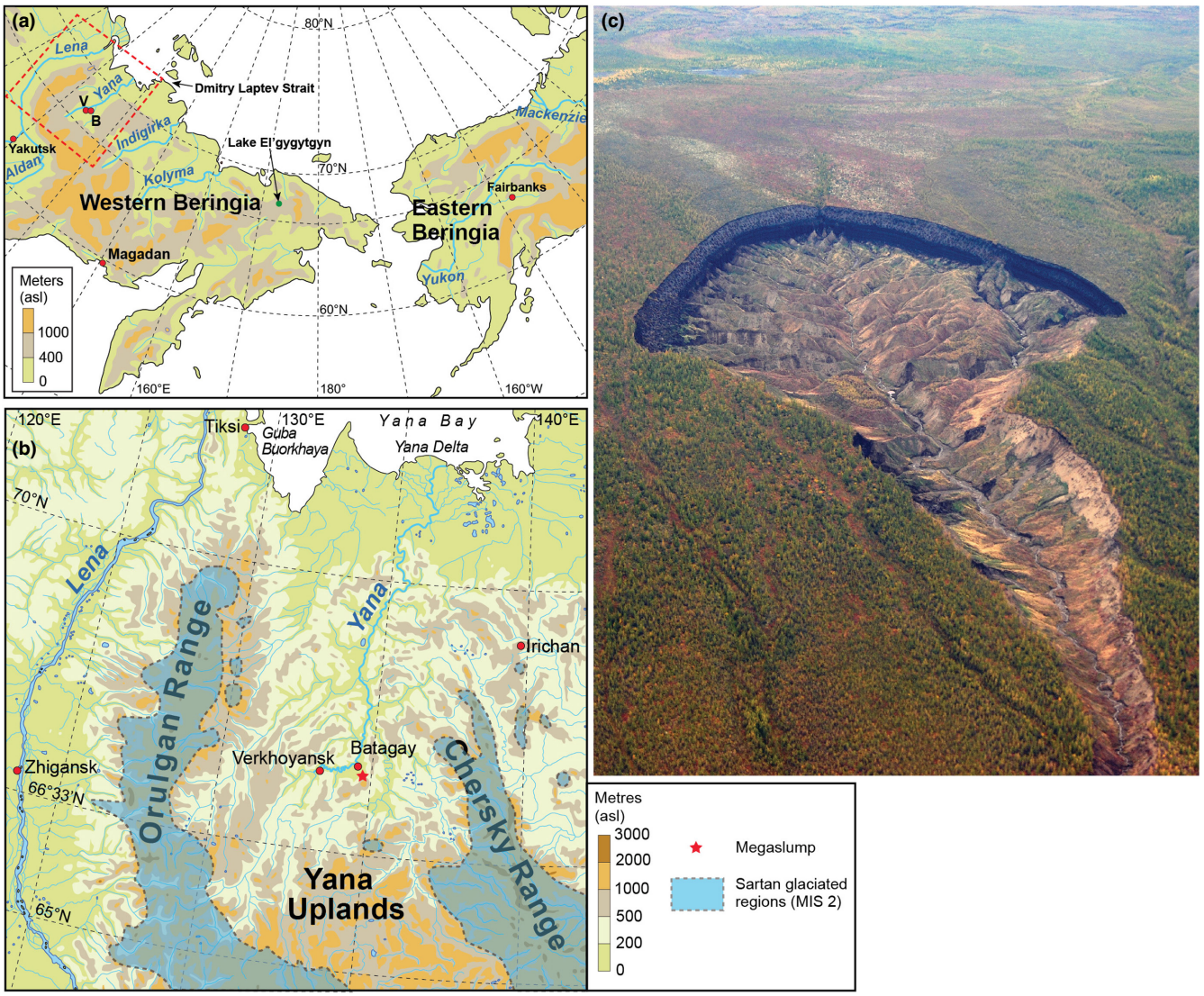

FIGURE 1 Map of the study site and picture of the Batagay megaslump. (a) Location of the study area in Beringia. (b) Location FFI of the Batagay megaslump in the Yana Uplands. (c) Aerial picture of the Batagay megaslump. Adapted from Murton et al. (2017).

and was ~1.8 km long and >0.8 km wide in 2019 (Kunitsky et al., 2013; Murton et al., in revision). More than 60 m deep, it exposes a discontinuous sequence of Middle Pleistocene to Holocene permafrost deposits ranging in age from >MIS 6 (Ashastina et al., 2017) and even >MIS 17 to 16 (Murton et al., 2021) to MIS 1.

The climate in the region of the Yana Highlands is continental subarctic (defined by Ḱoppen et al., 2011) and characterized by low precipitation and the largest seasonal temperature range globally. The mean air temperature between 1988 and 2017 was −40°C in winter (December to February) and 13.7°C in summer (June to August). The mean annual precipitation was 203 mm. Both air temperature and precipitation have increased at Batagay since the mid-twentieth century (Murton et al., in revision).

The Batagay megaslump is located within the northern taiga vegetation zone of the upper Yana floral district (Isaev et al., 2010). The modern vegetation is dominated by larch (*Larix gmelinii*) taiga. Siberian dwarf pine (*Pinus pumila*) is also common around the study site. The local flora includes shrubs such as *Alnus*, *Betula*, and *Salix* and dwarf shrubs such as *Ledum*, *Vaccinium*, *Arctous*, and *Empetrum*. The ground is moist and covered with a thick layer of lichens and mosses. Only a few grass species and herbs such as *Polygonum*, *Corydalis*, and *Pedicularis* occur (Ashastina, 2018). The local fauna is typical for Yakutian taiga comprising small mammals such as arctic hare (*Lepus arcticus*) and Siberian chipmunk (*Eutamias sibiricus*), which are prey for birds such as the northern goshawk (*Accipiter gentilis*) and the golden eagle (*Aquila chrysaetos*). Other predators include brown bear (*Ursus arctos*), lynx (*Lynx lynx*), and smaller species such as mink and wolverine (Mustelidae family). Wolves (*Canis lupus*) predate bigger prey such as reindeer (*Rangifer tarandus*) and elk (*Alces alces*). Insect-eating birds come to the taiga to breed, whereas seedeaters or omnivorous birds such as finches (Fringillidae), sparrows (Passeridae), and crows (Corvidae) are present all year round.

2 | MATERIALS AND METHODS

2.1 | Fieldwork and subsampling

During fieldwork in late July and early August 2017, we studied several cryostratigraphic units of the Batagay megaslump and took permafrost sediment and ground-ice samples for cryostratigraphic, sedimentological, geochemical, stable-isotope, chronological, and palaeoecological analyses. Before sampling, the exposure was cleaned and described. Ten samples were taken for *seda*DNA analysis using a battery-driven hand-drill or hammer and axe cleaned with bleach between each sample. Samples were placed in two whirlpack bags, stored for several hours in a plastic container placed directly on the permafrost table, covered by a thick moss layer, and then moved to a freezer. Samples were kept frozen during transportation to the DNA laboratory in Potsdam.

Five samples were selected for this study based on the cryostratigraphic and chronological data (Table 1; Figure S1). Subsampling took place in a climate chamber, in a different building than that housing the molecular genetics laboratories. The temperature was set to −10°C to prevent thawing of the core material. The subsampling procedure was carried out following the protocol described by Zimmermann, Raschke, Epp, Stoof-Leichsenring, Schwamborn, et al. (2017) using the same equipment, treatments, and facilities. Ten subsamples were collected: two per sample for both *seda*DNA and palynological analyses and stored at −20 and +4°C, respectively.

2.2 | Chronology

The ancient permafrost exposed at the Batagay megaslump is the oldest known in northern Eurasia (Murton et al., 2021). The pilot chronology of the exposed permafrost sediments is based on four dating techniques: radiocarbon dating of organic material, optically stimulated luminescence (OSL) dating of quartz grains, post-infrared infrared (pIRIR) luminescence dating of K-feldspar grains, and ^{36}Cl dating of ice wedges. Estimated ages for the sampled cryostratigraphic units are provided in Table 1 (for details, see Murton et al., 2021). The lower ice complex, hosting the sample B17-D3, developed at least 650 kyr ago, likely at some time within MIS 17 or 16. The lower sand unit, discordantly overlying the lower ice complex, was formed at some time between MIS 16 and MIS 6 but is likely nearer in age to the latter (Ashastina et al., 2017). Sample B17-D5 was taken from the lowermost part of this unit and may be partially reworked, and thus it may represent a transition or a remnant of older interglacial deposits rather than the environmental conditions associated with deposition of the main body of the lower sand. The upper ice complex overlies a wood-rich layer attributed to the Last Interglaciation (Ashastina et al., 2017). It formed mainly during MIS 3 and includes sample B17-D10. The two uppermost samples (B17-D7 and B17-D6) from the upper sand unit date to 22.4 and 27.3 kyr (MIS 2; Figure S1). Sample names within the manuscript consist of the sample ID and its associated MIS. In this way, we provide age context to the reader, even if we constrain the age information later on.

2.3 | Pollen analysis

For each sample, 2.85–4.58 g of wet sediment was taken for pollen preparation. A standard preparation method including KOH, HCl, HF, and acetolysis was used (Faegri et al., 1989). Palynomorphs were identified using a light microscope (Zeiss Axioskop 2) under 400–600× magnification. Pollen atlases (Beug, 2004; Kupriyanova & Alyoshina, 1972, 1978) were used for the identification of pollen and spores. Non-pollen palynomorphs (NPPs) were determined according to van Van Geel et al. (1983) and Van Geel and Aptroot (2006). Freshwater algae were determined using Jankovská and Komárek (2000) and Komárek and Jankovská (2001). At least 300 pollen grains and spores were counted in each sample except for B17-D3_MIS17-16, where only 178 palynomorphs were counted. The complete pollen record is available in Table S1.

2.4 | Isolation of sedimentary ancient DNA

DNA isolation, polymerase chain reaction (PCR) setup, and library preparations were performed in the palaeogenetic laboratory of the Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research in Potsdam, Germany. This laboratory is dedicated to ancient DNA isolation and PCR setup (see Supplementary Material). Precautions to reduce contamination were taken following the recommendations of Champlot et al. (2010).

All samples, 4.74–10.65 g, were prepared for DNA isolation within one extraction procedure including one extraction blank. Total DNA was isolated using the DNeasy PowerMax Soil kit (Qiagen, Hilden, Germany; see Supplementary Material for a detailed procedure). The extracts with a concentration lower than 3 ng/µl were concentrated with GeneJet PCR purification kit (Thermo Fisher) and samples were diluted to a final concentration of 3 ng/µl.

2.5 | Metabarcoding approach

The PCRs were performed with modified trnL g and h primers (Taberlet et al., 2007; see Supplementary Material) using the following cycle: initial denaturation at 94°C for 5 min, followed by 50 cycles of 94°C for 30 s, 50°C for 30 s, 68°C for 30 s and a final extension at 72°C for 10 min. To monitor for potential contamination, one no template control (NTC) was included in each PCR. For each extraction, three PCR replicates with different tag combinations were performed. The PCR products were purified using the MinElute PCR Purification Kit (Qiagen), following the manufacturer's recommendations (see Supplementary Material). All replicates were pooled in equimolar concentrations. The amplified extraction blank and PCR NTCs were included in the sequencing run, using a standardized

TABLE 1 Stratigraphic units of the permafrost sequence exposed in the Batagay megaslump

Unit and approximate thickness (m)	Description	Interpretation	Preliminary age (Marine Isotope Stage, MIS) Dating method	*seda*DNA sample number, depth below ground surface, and estimated age of each sample
7. Near-surface sand (1–3)	Ice-cemented sand	Colluvium	MIS 1 (Holocene) Radiocarbon dating	
6. Upper sand (20–30)	Ice-cemented fine sand with narrow syngenetic ice wedges and composite wedges. Unit thickens downslope	Aeolian sand sheet with reworking by slopewash	MIS 3-2 Radiocarbon dating OSL dating	B17-D-6 (2.15 m) - ~27 kyr BP B17-D-7 (2.3 m) - ~23 kyr BP
5. Upper ice complex (20–25)	Yedoma containing large syngenetic ice wedges	Growth of large syngenetic ice wedges	MIS 4–3 Radiocarbon dating OSL dating	B17-D-10 (25.8 m) - ~50 kyr BP
4. Woody debris (≤3)	Discontinuous lenses of woody debris cut down into lower sand unit.	Forest bed above erosional surface (disconformity)	MIS 5e (Last Interglacial) Palaeoecological interpretation	
3. Lower sand (≤20)	Ice-cemented fine sand with narrow syngenetic ice wedges and composite wedges	Aeolian sand sheet with forest bed near top	MIS 6 or older IRSL dating	B17-D-5 (~47.6 m) - ~200 kyr BP
2. Lower ice complex (3–7)	Contains ice wedges, angular clasts of slate, in situ tree stumps, and woody debris. Thaw unconformity along top.	Growth of large syngenetic ice wedges; forest bed near top; disconformity along top of unit	MIS 17-16 pIRIR luminescence dating ^{36}Cl dating	B17-D-3 (~49.5 m) - ~650 kyr BP
1. Diamicton (≥0.5)	Contains abundant clasts of slate and overlies slate bedrock	Colluvium, locally derived from bedrock	MIS 17-16 or older (at least 650 ka)	

Abbreviations: IRSL, infrared stimulated luminescence; pIRIR luminescence, post-infrared infrared luminescence; OSL, optically stimulated luminescence.
Modified from Murton et al. (2021).

TABLE 2 Summary of reads assigned with kraken2 to main taxonomic groups

	Total samples	B17-D3_MIS17-16	B17-D5_MIS16-6	B17-D10_MIS3	B17-D6_MIS2	B17-D7_MIS2	Total blanks	Extraction blank	Library blank
Total reads	771139326	166860854	156036581	138057439	155656957	154527495	1265153	404162	860991
custom database assigned	1033627	38384	373315	247415	175676	198837	1238	407	831
Viridiplantae	415593 (40.21%)	840 (2.19%)	4466 (1.2%)	156242 (63.15%)	121110 (68.94%)	132935 (66.86%)	765 (61.79%)	271	494
Fungi	317836 (30.75%)	17259 (44.96%)	281344 (75.36%)	10827 (4.38%)	3155 (1.80%)	5251 (2.64%)	56 (4.52%)	7	49
Mammalia	243 (0.02%)	6 (0.02%)	55 (0.01%)	70 (0.03%)	35 (0.02%)	77 (0.04%)	0 (0%)	0 (0%)	0 (0%)
Aves	17 (0%)	1 (0%)	2 (0%)	1 (0%)	8 (0%)	5 (0%)	0 (0%)	0 (0%)	0 (0%)
Insecta	1122 (0.11%)	73 (0.19%)	385 (0.1%)	374 (0.15%)	153 (0.09%)	137 (0.07%)	0 (0%)	0 (0%)	0 (0%)
NCBI nt database assigned	94940445	14963330	21842470	19540932	18773725	19819988	147770	62056	85714
Bacteria	28014036 (29.51%)	5114286 (29.51%)	6183906 (28.31%)	7416218 (37.95%)	4560976 (24.29%)	4738650 (23.91%)	39999 (27.07%)	11238 (18.11%)	28761 (33.55%)
Archaea	303556 (0.32%)	35333 (0.32%)	22004 (0.1%)	60196 (0.31%)	130211 (0.69%)	55812 (0.28%)	570 (0.39%)	213 (0.34%)	357 (0.42%)
Virus	37976 (0.04%)	4282 (0.04%)	5271 (0.02%)	6913 (0.04%)	13561 (0.07%)	7949 (0.04%)	74 (0.05%)	30 (0.05%)	44 (0.05%

volume of 5 µl. Fasteris SA sequencing service (Switzerland) performed the paired-end sequencing on one-tenth of a full lane of the Illumina HiSeq 2500 platform (2 × 125 bp).

2.6 | Shotgun approach

The DNA libraries were prepared following the single-stranded DNA library preparation protocol of Gansauge et al. (2017) except that the ligation of the second adapter (CL53/CL73) took place in a rotating incubator (Schulte et al., 2021; detailed procedure in Supplementary Material). The five libraries were pooled in equimolar ratios to a final pool of 10 nM, with the blanks accounting for a molarity of 20% compared to the samples. The sequencing of the pool was performed twice using a modified forward sequencing primer CL72, as described in Gansauge and Meyer (2013) by Fasteris SA sequencing service (Switzerland). After the first sequencing on one lane of an Illumina HiSeq 2500 platform (2 × 125 bp), we decided to increase the sequencing depth—especially for the deepest samples—and sequenced the pool a second time on an Illumina NovaSeq SP platform (2 × 100 bp).

2.7 | Bioinformatic processing

2.7.1 | Metabarcoding

Filtering, sorting, and taxonomic assignments of the metabarcoding sequences were performed with the OBITools package (Boyer et al., 2015). With the *illuminapairedend* function, we merged forward and reverse reads, and demultiplexing and sample sorting were performed with the *ngsfilter* function. With the *obigrep* command, sequences shorter than 10 bp and less than 10 read count were excluded. Duplicated sequences were merged with *obiuniq* and cleaning of potential PCR or sequencing errors was performed with the *obiclean* function. Two reference databases were used for taxonomic assignments. The first one, ArctBorBryo, is based on the quality-checked and curated Arctic and Boreal vascular plant and bryophyte reference libraries (Soininen et al., 2015; Sonstebo et al., 2010; Willerslev et al., 2014). The second is based on the European Molecular Biology Laboratory (EMBL) Nucleotide Sequence Database standard sequence release 138 (Kanz et al., 2005; http://www.ebi.acuk/embl/).

Final taxonomic assignments were determined by selecting the best identity given by EMBL or the ArctBorBryo database; if both assignments showed the same sequence identity, the taxonomic name was selected from ArctBorBryo database because of its specificity to arctic and boreal vegetation. Only sequences assigned with a best identity of 100% and present in at least two replicates of a sample or two different samples were used for this study. The sequences assigned to a taxonomic level higher than family were removed from the dataset. Sequences were also removed if more than 0.2% of their total read counts were present in the extraction blanks

and PCR NTCs (Table S2). Sequence data of the PCR replicates were merged to represent samples and sequences assigned to the same taxon were merged.

Before proceeding to the data analysis, a rarefaction analysis was performed based on the minimum number of sequence counts (n = 84,348, for sample B17-D3_MIS17-16) to normalize the total counts of each sample (https://github.com/StefanKruse/R_Rarefaction). The complete metabarcoding record, before and after rarefaction, is available in Table S2.

2.7.2 | Shotgun

Demultiplexed FASTQ files obtained from the two sequencing runs were quality checked, trimmed, and paired-end merged using fastp (Chen et al., 2018). Statistics of both sequencing runs are reported in Table S3. We then merged both sequencing run files and proceeded to taxonomic classification using kraken2 (v. 2.1.2, Wood et al., 2019) against two different databases.

The first database used was the non-redundant nucleotide database (nt) from the National Center for Biotechnology Information (NCBI) (ftp://ftp.ncbi.nlm.nih.gov/blast/db/FASTA/nt.gz; downloaded in May 2020), and the NCBI taxonomy (retrieved via the kraken2-build command). It was used with a confidence threshold of 0.05, first, to differentiate Eukaryota from Prokaryota and Viruses and, second, for the taxonomic assignment of Prokaryota (Bacteria and Archaea) and Viruses.

The sequences previously unassigned and assigned to Eukaryota against the nt database were extracted and taxonomically reassigned using kraken2 with default confidence parameters against a second, customized, database of curated full chloroplast and mitochondrion genomes from the NCBI Reference Sequence Database (Refseq, https://www.ncbi.nlm.nih.gov/refseq/, 10,010 sequences downloaded in December 2020).

Using the nt database to taxonomically assign our reads to Prokaryota and Viruses and the curated Refseq database for Eukaryota, we created different subsets for working taxonomic groups. We included bacterial families (34,521,704 reads assigned to a family), Archaea (317,626 reads), and Viruses (42,112 reads). For the Eukaryota, we investigated families from Viridiplantae (435,733 reads), Fungi (348,619 reads), and selected terrestrial classes representing Metazoa with at least 0.5% of total metazoan reads (6887 total reads): Insecta (1493 reads), Mammalia (294 reads), and Aves (29 reads). Only families that represented at least 0.5% of the total reads assigned to each subset were kept. The only exception was for the Viridiplantae, where we kept all families that represented at least 0.2% of total reads assigned, the rationale being to capture as many as possible of the families detected via the other methods. An overview of the read count and relative proportion of the selected families is summarized in Table 2.

If the abundance of reads detected in the blanks for an assigned family exceeded 1% of its total reads, this family was removed from the dataset. Furthermore, Primate reads were removed from the dataset as probable human contaminants. Finally, as shotgun sequencing almost inevitably yields "unlikely" taxa, only the families likely to be present in the study area were kept; therefore, we excluded metazoan aquatic classes and aquatic families of Mammalia. Detailed information about reads present in the blanks and the families excluded is reported in Table S3.

Resampling to the sample with the minimum read counts was performed with the rarefy function of the "vegan" R package (v. 2.5-7 Oksanen et al., 2020) using R v. 3.6.1 (R Core Team, 2019) for all the subsets, except the metazoan ones. Because few reads were assigned to the selected metazoan classes, differences in families detected per sample are reported instead of relative abundance, as was done in other phyla. Detailed information about lower taxonomic-level taxa detected from selected families is reported in Table S3.

2.7.3 | Damage pattern analysis

When performing data analysis on a shotgun sequenced dataset, we were particularly careful that the investigated signal comes from the ancient DNA of past organisms and not from modern contamination. Higher nucleotide misincorporation (cytosine deamination or C to T substitution) rates or DNA damage patterns can be observed on the 5′-overhang and statistically estimated. Here, we used the mapDamage 2.0 tool using a Bayesian framework to investigate the presence of such damage by comparing our sequenced reads to several reference genomes from each of our investigated taxonomic groups: Viridiplantae, Bacteria, Archaea, Virus, Metazoa, and Fungi (Jónsson et al., 2013). We used entire genomes as reference sequences for the Bacteria, Archaea, and Viruses; chloroplast entire genomes for the Viridiplantae and mitochondrion entire genomes for the Metazoa and Fungi. Detailed methodological information and examples of damage pattern plots are reported in Figure S2.

2.7.4 | Composition analysis

From the rarefied shotgun datasets, each studied taxonomic group is plotted as positive mean-centered. This transformation compares the distance of each sample's read counts to the average read counts and sets the minimum value to zero, adding this value to the other samples to remove noise and facilitate the inspection of biodiversity compositional patterns and trends. Using these intermediate plots, a final selection of target families was undertaken, and below we describe only the most represented families likely to appear in the study area (Figure S3).

Bubble charts were created to visualize relative abundance extracted from shotgun data of selected families recovered within larger taxonomic groups including Viruses, Bacteria, Archaea, Fungi, and Viridiplantae as well as read counts of selected families of Mammalia, Insecta, and Aves. Along with the plant shotgun data, we present relative abundance resulting from the pollen and metabarcoding analyses. We also compared the relative abundance of fungal

spores identified in the palynological record with fungal shotgun data.

3 | RESULTS

3.1 | General results of the three approaches: pollen, metabarcoding, and shotgun sequencing

In total, 1520 pollen and NPPs were counted in the five permafrost samples. *Seda*DNA analyses yielded 650,985 reads assigned with a 100% match to plant families with metabarcoding and 559,922 reads retrieved by the metagenomic approach (415,593 reads assigned to the selected families). The oldest sample (B17-D3_MIS17-16) had the lowest read count (84,348 reads for the metabarcoding and 840 reads for the shotgun dataset) and the lowest pollen concentration (460 grains per gram). Fungi were detected, both among the NPPs and in the shotgun data. In total, 317,836 reads were assigned to selected fungus families with the shotgun method, 88.5% of which (281,344 reads) came from the B17-D5_MIS16-6 sample. B17-D7_MIS2 had the lowest fungi read count with 3155. For the three selected terrestrial classes of Metazoa, in total 1122 reads were assigned to selected insect (Insecta) families, 243 reads to mammal (Mammalia) families, and 17 to bird (Aves) families. Finally, 28,355,568 reads were assigned to selected families from microbial communities and viruses; mostly Bacteria (28,014,036), whereas reads of Archaea (303,556 reads) and Viruses (37,976 reads) were 2–3 orders of magnitude lower.

The data indicated that two samples (B17-D3_MIS17-16 and B17-D5_MIS16-6) represent interglacial ecosystems and the three other samples (B17-D10_MIS3, B17-D7_MIS2, and B17-D6_MIS2) represent glacial ecosystems.

Finally, the damage pattern investigation confirmed the ancient origin of our shotgun sedaDNA signal. Indeed, we identified damage patterns for all investigated subsets except Metazoa as not enough reads were aligning to the reference genomes and no damage pattern investigation was possible. A detailed description of the tested signal is available in Figure S2.

3.2 | Plants (Viridiplantae)

All the palynological, *seda*DNA metabarcoding, and shotgun proxies similarly showed higher relative proportions of trees and woodland taxa in the interglacial samples (B17-D3_MIS17-16 and B17-D5_MIS16-6) compared to the glacial samples (B17-D10_MIS3, B17-D7_MIS2, and B17-D6_MIS2; Figure 2a).

The interglacial pollen spectra were characterized by the presence of Pinaceae (*Larix*, *Picea*, *Pinus*) in the pollen record (23.2% in B17-D3_MIS17-16, 18.2% in B17-D5_MIS16-6). With metabarcoding, *Cedrus* (Pinaceae) reached 6% in sample B17-D3_MIS17-16. Other woody taxa, such as Betulaceae (*Alnus*, *Betula*) in the pollen record and sequences assigned to Menispermaceae in the shotgun data, were also abundant in both interglacial spectra. The three methods also detected numerous herbs including Poaceae and other families typical of steppe communities, such as Asteraceae (*Artemisia*). Rosaceae was also detected with the metabarcoding approach and Solanaceae with the shotgun approach in B17-D3_MIS17-16 and B17-D5_MIS7.

The three methods used revealed that the glacial spectra had a high proportion of non-woody taxa, in particular, a high relative abundance of Asteraceae and Poaceae. B17-D10_MIS3 was particularly rich in Plantaginaceae (*Plantago*) and Boraginaceae (*Eritrichium*) detected both using metabarcoding (13.4% of Plantaginaceae, 6.7% of Boraginaceae) and shotgun (13.5% of Plantaginaceae, 2.5% of Boraginaceae). Both B17-D7_MIS2 and B17-D6_MIS2 samples were characterized by a high abundance of Cyperaceae, Caryophyllaceae, and Fabaceae (including *Astragalus* and *Oxytropis*).

3.3 | Fungi

Both palynological and *seda*DNA shotgun proxies provided information on fungi. Among the NPPs, *Glomus* (Glomeraceae) spores were numerous in all samples (Figure 2a).

B17-D5_MIS16-6 had the highest number of reads assigned to fungi, mainly to the saprophytic and commonly psychrophilic Pseudeurotiaceae family (*Pseudogymnoascus* genus, 72.5% of all fungal reads for this sample).

The palynomorph assemblage contained spores of coprophilous fungi from Sordariaceae (*Sordaria*, 10.6%) in B17-D10_MIS3, where both Glomerellaceae (12%) and Aspergillaceae (11.1%) were abundant in the shotgun dataset. Furthermore, high relative abundance of lichens (Parmeliaceae), saprophylic Dermateaceae, and the plant-pathogen Pleosporaceae was detected in the three glacial spectra by the *seda*DNA shotgun approach.

3.4 | Mammals (Mammalia)

Overall, this class had few reads assigned to family level, especially for the deepest and oldest sample (B17-D3_MIS17-16): six reads assigned to selected families. Selected families were detected even at higher confidence thresholds (--confidence 0.5 with kraken2), to taxa such as *Rangifer* (Cervidae), *Bison* (Bovidae), *Equus* (Equidae), *Prionodon* (Viverridae), and even *Mammuthus* (Elephantidae) but almost no exotic terrestrial taxa were found (Table S3). In addition, among the few metazoan selected families detected, three (out of 23) were representative of the extinct Pleistocene megafauna: Elephantidae (mammoths), Rhinocerotidae (woolly rhinoceros), and Megalonychidae (giant ground sloths).

Overall, the B17-D10_MIS3, B17-D7_MIS2, B17-D6_MIS2, and B17-D3_MIS17-16 samples provided evidence mostly of larger herbivores and predators, whereas the B17-D5_MIS16-6 provided evidence mostly of smaller and more forest-adapted mammals in general (Figure 2b).

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FIGURE 2 Bubble chart illustrating compositional changes between the five studied samples for each family in relation to their respective groups. (a) Relative vegetation changes per sample and method (P – Pollen, M – Metabarcoding, S – Shotgun). (b) Relative metazoan changes per sample. A selection of families from three metazoan classes are represented here. (c) Relative microorganism changes per sample

In the oldest sample (B17-D3_MIS17-16), very few reads were assigned to Mammalia but we detected the presence of larger mammals such as the giant ground sloth family (Megalonychidae) and bovids (Bovidae). Two families of bats from Pteropodidae and Vespertilionidae also occurred in the oldest sample.

In B17-D5_MIS16-6, reads of smaller forest carnivores from Viverridae (45 reads) were quite abundant given to the small number of reads assigned overall (55 reads assigned to all families). Other families detected in this sample included the squirrel family (Sciuridae), the wild boar family (Suidae), and three families of bats (Pteropodidae, Vespertilionidae, and Molossidae). Among the larger mammals, only the mammoth family (Elephantidae) was detected.

B17-D10_MIS3's mammalian community was represented mainly by larger species such as mammoths (Elephantidae, 25 reads assigned), horses (Equidae, 20 reads assigned), and ruminants (Bovidae and Cervidae, six and five reads, respectively). Camelids (Camelidae,

three reads assigned) were detected only in this sample. Predators from the Canidae were also detected in this sample. In addition to this community of large mammals, families of smaller mammals were detected such as rodents (Spalacidae, Octodontidae, Echimyidae) and insectivores in the hedgehog family (Erinaceidae).

Evidence of larger mammals, including horses (Equidae) and cervids (Cervidae), occurred again in the two samples dated MIS2. Predators such the Canidae and even the Felidae were detected. Smaller mammals occur in B17-D6_MIS2 but not in B17-D7_MIS2 including squirrels (Sciuridae), insectivores (Erinaceidae), and rodents (Cricetidae and Octodontidae), as well as the wild boar family (Suidae), bat families (Phyllostomidae and Pteropodidae), and the giant ground sloth family (Megalonychidae). In B17-D7_MIS2, however, there was even greater megafaunal representation: mammoths (Elephantidae), bovids (Bovidae), and the woolly rhinoceros family (Rhinocerotidae). Some smaller mammals occurred in B17-D7_MIS2, including rodents (Echimyidae), moles (Spalacidae), and viverrids (Viverridae).

3.5 | Birds (Aves)

Only very few reads were assigned to this class and no representative of the selected families was present in all five samples (Figure 2b).

In the two lowermost samples, woodland families were detected, with reads assigned to tits (Paridae), asian barbets (Megalaimidae), and warblers (Sylviidae) detected in B17-D3_MIS17-16, and to the arboreal cuckoo family (Neomorphidae) detected only in B17-D5_MIS16-6. The ground-dwelling bird family of pheasants (Phasianidae) was also detected in B17-D5_MIS16-6.

In B17-D10_MIS3, the woodland tit family of Paridae was detected. We also detected sandpipers (Scolopacidae) which, like Paridae, need an availability of invertebrates as a food source in addition to water; a need which is shared by herons (Ardeidae) that were also detected. Ground-dwelling rails (Rallidae) were detected too.

In B17-D7_MIS2 and B17-D6_MIS2, the avian community was similar, characterized by ground-dwelling birds from Phasianidae. Birds of prey such as falcons (Falconidae) in B17-D7_MIS2 and eagles (Accipitridae) in B17-D6_MIS2 were also detected. In addition, some bird families that need water habitat such as sandpipers (Scolopacidae) or cranes (Gruidae) were detected in B17-D6_MIS2. Lastly, families that typically need proximity to woodlands were detected in both samples, such as Paridae in B17-D7_MIS2 or doves and pigeons (Columbidae) in B17-D6_MIS2.

3.6 | Insects (Insecta)

Reads were assigned to Insecta in every sample, even at higher thresholds (--confidence 0.5). For the three most recent samples (B17-D7_MIS2, B17-D6_MIS2, and B17-D10_MIS3), between 137 and 374 reads were assigned to selected Insecta families, while only 73 were assigned to the oldest sample, B17-D3_MIS17-16, and 385 to B17-D5_MIS16-6. Culicidae, the mosquito family, and Tettigoniidae, bush crickets, were the only two families (out of the 31 selected) to be detected in all samples. In terms of insect communities, a clear difference was observed between the three most recent and the two most ancient ones (Figure 2b).

In the two oldest samples (B17-D3_MIS17-16 and B17-D5_MIS16-6), more parasitoids (Trichogrammatidae) and mosquitoes (Culicidae) were detected than in the three other samples. In B17-D5_MIS16-6, we also observed a high abundance of skippers (Hesperiidae), butterflies (Nymphalidae), and moths (Yponomeutidae). Several families that were only present in this sample indicate the local presence of dead wood, for example, flat bark beetles (Cucujidae) and termites (Rhinotermitidae), while the abundance of mayflies (Siphlonuridae) suggests a wetter environment. This sample is also the only one in which we detected skin beetles (Dermestidae).

In the three uppermost samples (B17-D7_MIS2, B17-D6_MIS2, and B17-D10_MIS3), several beetle families were detected, including lady beetles (Coccinellidae) and rove beetles (Staphylinidae; Table S3). Several families indicative of herb and shrub habitats were present, such as grasshoppers (Acrididae), grasshopper parasites (Meloidae), bush crickets (Tettigoniidae), leaf hoppers (Cicadellidae), and weevils (Curculionidae). Moths and butterflies were represented by three families: Nymphalidae, Lycaenidae, and Papilionidae. Some scavengers (Sarcophagidae) occurred in the three uppermost samples, alongside ants (Formicidae) and termites (Termitidae). Wasps were only detected in B17-D10_MIS3 but were abundant (251 reads) in this sample.

3.7 | Prokaryotes (Bacteria, Archaea) and Viruses

We identified a core community consisting of Actinobacteria (especially the soil-associated families Nocardioidaceae, Streptomycetaceae, Microbacteriaceae, and Mycobacteriaceae) and Haloarchaea (Figure 2c). Beside this shared community, we detected several distinctive patterns along the depositional sequence that likely reflect changes to the microbial community in response to environmental conditions.

In the most ancient sample (B17-D3_MIS17-16), Actinobacteria (Micrococacceae; 8.5% of all selected families of bacteria), particularly the genera *Arthrobacter* and *Pseudoarthrobacter* (Table S3), and Sphingomonadaceae (4.5%), had a higher relative abundance when compared to younger samples. Halophilic archaea were also well represented in the oldest samples, especially by Natrialbaceae (23.7% of all selected archaea families), Haloarculaceae (18.3%), Haloferacaceae (8.5%), Halorubraceae (13.7%), and Halobacteriaceae (9.9%), which shared a similar age-related distribution.

In B17-D5_MIS16-6, all major changes in community composition reflected an increase of taxa related to the degradation and cycling of organic matter in soil. These taxa included the biopolymer-degrading bacteria *Clostridium* (Clostridiaceae; 3.5%), *Nocardioides*

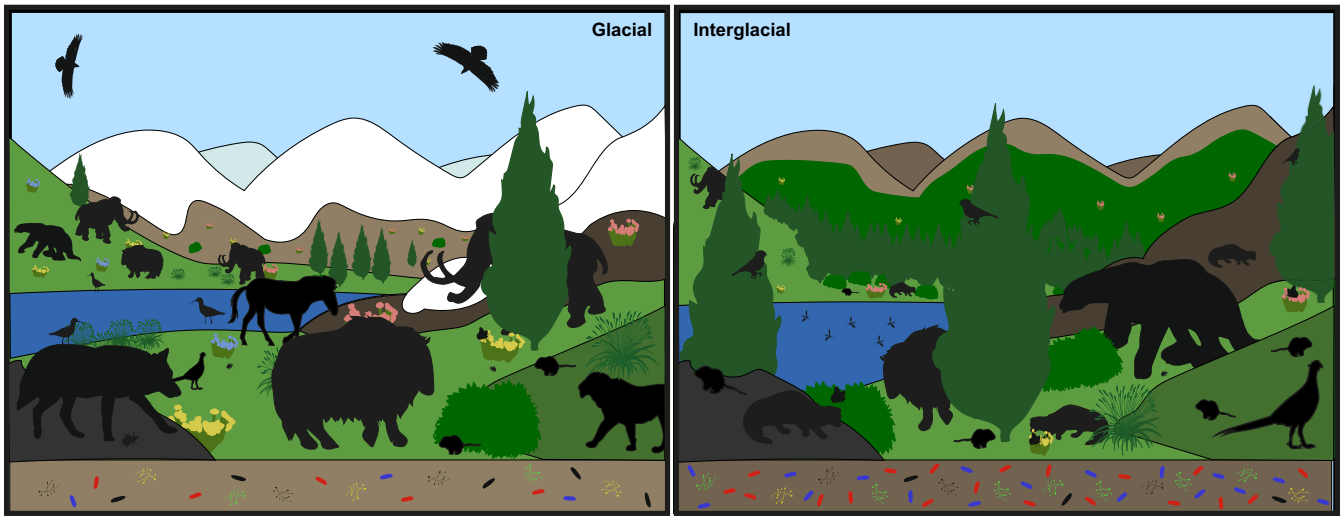

FIGURE 3 Schematic comparison of Pleistocene glacial and interglacial landscapes and their respective biodiversities. Using the silhouettes highlighted in Figure 2, we made a schematic comparison ecosystem reconstructed from our datasets. Left: glacial, right: interglacial

and *Pseudonocardia* (Nocardioidaceae; 24.2%), and the fermenting *Propionibacterium* (Propionibacteriaceae; 2.2%).

In B17-D10_MIS3, Flavobacteriaceae were particularly abundant (47.5% of the entire selected bacteria families) together with Oxalobacteraceae (2.6%). Overall, well-known cold-adapted bacteria, such as *Polaribacter* and *Chryseobacterium* (Flavobacteriaceae), *Cryobacterium* (Microbacteriaceae; 5.5%) and *Polaromonas* (Comamonadaceae; 2.1%) were found in variety in high numbers. Other minor taxa typically associated with algae (e.g., *Tenacibaculum*, *Formosa*, *Cellulophaga*, *Lacinutrix*, *Flavobacteriaceae* family) also showed a distinct increase.

The two most recent samples (B17-D6_MIS2, B17-D7_MIS2) had a particularly high degree of similarity in microbial community composition and only marginal differences with regard to bacteria assigned to Cellulomonadaceae (4.5% in B17-D6_MIS2) and Conexibacteriaceae (4.4% in B17-D7_MIS2 and 3.5% in B17-D6_MIS2). Instead, ammonia-oxidizing archaea (AOA) were highly dominant, in particular Nitrososphaeraceae (70.3% in B17-D6_MIS2), with the genus *Candidatus Nitrososocosmicus*, and Nitrosopumilaceae (10.8% in B17-D6_MIS2), including the genera *Nitrosopumilus*, *Nitrososphaera*, and *Candidatus Nitrosotalea*.

Sequences affiliated with methanogenic archaea represented 5%–13% of the archaeal community. In line with their known distribution and abundance in arctic soil ecosystems, Methanosarcinaceae and Methanobacteriaceae were the most abundant families. The two families followed an inverse distribution trend, with Methanobacteriaceae being dominant in the youngest samples (B17-D10_MIS3, B17-D6_MIS2, B17-D7_MIS2) and Methanosarcinaceae in the oldest ones (B17-D3_MIS17-16, B17-D5_MIS16-6).

Viral communities throughout the permafrost sequence were dominated by bacteriophages of the Siphoviridae, Mimiviridae, and Myoviridae families, which together accounted for 56%–76% of the entire viral community. Overall, there was a high representation of giant viruses (e.g., *Mimivirus*, *Pandoravirus*, *Klosneuvirus*, and *Tupanvirus*) that generally infect protozoa (Table S3). In some samples, virus presence correlated well with that of plants or animals acting as either host or vector, most notably, *Mastrevirus* (Geminiviridae, 15.4% in B17-D7_MIS2), which infects monocotyledon plants via grasshopper transmission, was characteristic in B17-D7_MIS2.

4 | DISCUSSION

The Batagay megaslump data date back discontinuously to MIS 17-16 (~650 kyr) or older. Hence, this study presents one of the few records from northern Eurasia that reach back to the Middle Pleistocene (Andreev et al., 2011; Ashastina et al., 2018; Tarasov et al., 2005; Wetterich et al., 2019). It is also one of the first to successfully investigate *sedaDNA* as old as MIS 17 (Parducci, 2019) and identify ancient DNA damage patterns for several representatives of investigated phyla supporting the signal's authenticity (Figure S2). Furthermore, we provide unique snapshots of Pleistocene glacial and interglacial biota that include all biological kingdoms. Previous shotgun sequencing investigations have been limited to fewer kingdoms (e.g., Murchie et al., 2021; Pedersen, 2016). Our results indicate distinct ecosystem-wide differences between forested (interglacial) and non-forested (glacial) intervals (Figure 3).

Our results for the samples B17-D3 and B17-D5 clearly point to interglacial ecosystems. Hence, our palaeoecological information contributes to constraining the ages of the respective samples. In the case of B17-D3, pilot dating results suggest an age of MIS 17-16 (Murton et al., 2021; Table 1). The reconstructed interglacial ecosystem rules out the glacial MIS 16 and hence, we attribute this sample to MIS 17. Similarly, we can exclude a glacial environment for sample B17-D5. Based on the existing dating results of the lower sand unit suggesting a MIS 7 to MIS 6 as the age of this unit (Ashastina

et al., 2017), we attribute this sample tentatively to MIS 7, even though an earlier interglaciation such as MIS 9 and up to MIS 15 cannot be excluded yet. The transition between the lower ice complex (MIS 17 sample) and the lower sand unit (MIS 7 sample) is marked by an erosional surface. Such erosional features can be caused during warm stages (i.e., interglaciations) by intense permafrost degradation with widespread thermokarst (e.g., Kienast et al., 2011; Reyes et al., 2010). This suggests that other interglacial and glacial deposits between MIS 17 and MIS 7 may not have been preserved. Further dating is needed to constrain the chronology of the sediments exposed in the Batagay megaslump.

4.1 | Interglacial communities

The two lowermost samples, attributed to MIS 17 and MIS 7, reflect forest vegetation with *Larix*. Understory shrubs (*Salix*, *Alnus*, *Betula*) and typical temperate families represented by herbs and low shrubs (e.g., Rosaceae, Orchidaceae, Poaceae, Cyperaceae, Ericaceae) were abundant. This assemblage aligns with data from the other kingdoms. These two samples should not be considered representative of the entire MIS 17 or MIS 7 stages, but they do provide snapshots of those two past interglacials, which featured an organic-rich soil and were probably intermittently flooded and/or characterized by wetlands.

Our record reveals that the MIS 17 forests were probably rather open, as indicated by the presence of grassland taxa such as Asteraceae and Poaceae, plus bovids and giant sloths. The presence of *Cedrus* DNA in this sample is surprising as *Cedrus* distribution today is only as far North as the Tibetan Plateau and the Himalayas, and it disappeared from southwestern China after the Late Pliocene, ~2.5 Myr ago (Su et al., 2013). Similar unlikely taxa were found in other records (e.g., *Juglans* in Jørgensen et al., 2012), but these finds could well be explained by reworking of older sediments or a misleading taxonomic identification, for instance, due to incomplete databases or the use of loose classification thresholds. Alternatively, *Cedrus* can be a rare contaminant already detected in other studies using the same marker (Voldstad et al., 2020). The occurrences of Menispermaceae and Solanaceae are similarly unexpected, though in the case of these two families, representative species are reported from north-central Asia, closer to the study site than the modern *Cedrus* distribution.

During MIS 7, fewer megafauna and more smaller mammals were detected. The data are in accordance with an inference of dense forest vegetation, as this favors smaller taxa while megafauna co-occur with open vegetation (Bakker et al., 2016; Smith et al., 2016). This confirms the hypothesis that large herbivores play a role in maintaining forests as a mosaic landscape composed of closed canopy woodland and open areas (Vera, 2000). Our data indicate that forest-dwelling taxa including viverrids (Viverridae) were common during MIS 7, as well as smaller taxa from the squirrel family (Sciuridae). The high proportion of insects in this sample includes forest-specific families such as flat bark beetles (Cucujidae) and termites (Rhinotermitidae) supporting the inference of forest conditions. Furthermore, the presence of parasitic insects such as Trichogrammatidae suggests a high host density, that is, other insects or mammals (Abrams, 2000).

In the MIS 7 sample, the microbial community is rather distinctive, owing to the marked increase of taxa involved in the degradation and cycling of organic matter in soil, which include the fungus *Pseudogymnoascus* sp. and bacteria such as Nocardioidaceae and *Clostridia*. Members of *Pseudogymnoascus* are associated with decaying roots or plants, especially in boreal forests (Sigler et al., 2000). They can form mycorrhizal associations with arctic shrubs, which align with the increased presence of shrubs and woody taxa in MIS 7 (Semenova et al., 2015). Similarly, Nocardiodaceae of the genera *Nocardioides*, *Corynebacterium*, and *Pseudonocardia* are aerobic saprophytic bacteria, considered to be consumers of organic material. Many, especially *Nocardioides*, secrete extracellular enzymes, and degrade and metabolize a wide range of natural organic compounds, thus being important players in the turnover of organic matter in ecosystems, including cold ones (Babalola et al., 2009; Perez-Mon et al., 2020). They have a copiotrophic lifestyle, implying that they are fast-growing and metabolically versatile, and are consequently fast responders to increased nutrient availability (Perez-Mon et al., 2020). *Clostridium* is a dominant bacterium of the permafrost subsurface environment and related DNA sequences have been detected in ancient samples up to ~1 Myr (Burkert et al., 2019; Liang et al., 2019). It can hydrolyze biopolymers such as cellulose, lignocellulose, and lignin, in deep sediments under anoxic conditions and produce alcohols, organic acids (e.g., lactate, acetate, butyrate), and hydrogen as metabolic by-products (Ueno et al., 2016). These compounds—especially lactate—can then be fermented by *Propionibacterium* spp. to propionate, acetate, and carbon dioxide, which can be assimilated by methanogenic archaea (Methanosarcinaceae) with a final synthesis of methane (Drake et al., 2009). Thus, these data suggest high organic matter turnover going on during MIS 7, both at the surface and subsurface. In permafrost and cold ecosystems generally, this is directly related to an increase in dissolved organic carbon mobilized through soil horizons, which is a consequence of relatively high ambient temperature (Bracho et al., 2016; Selvam et al., 2017). This may well mean that climate and environmental conditions were mild during this period.

4.2 | Glacial communities

Our data clearly indicate that herb communities (mostly Asteraceae, Poaceae, Cyperaceae) dominated the vegetation in the Batagay area under glacial conditions during MIS 3 and MIS 2. This is in line with the palaeo-vegetation reconstruction based on plant macrofossils and pollen previously reported for this site (Ashastina et al., 2018). It also confirms the open steppe–tundra landscape that has been described by other *sed*aDNA and pollen records from north-eastern Siberia (Andreev et al., 2011; Binney et al., 2017; Lozhkin et al., 2007; Willerslev et al., 2014; Zimmermann, Raschke, Epp, Stoof-Leichsenring, Schirrmeister, et al., 2017; Zimmermann, Raschke, Epp,

Stoof-Leichsenring, Schwamborn, et al., 2017; Wetterich et al., 2011; Zimov et al., 2012).

This open steppe–tundra landscape (Dale Guthrie, 2001; Zimov et al., 2012) spanned much of unglaciated northern Eurasia and Beringia during the Late Pleistocene (Johnson, 2009). Coprophilous fungi found in the pollen assemblage, such as *Sordaria*, indirectly point to the presence of herbivorous megafauna during the Late Pleistocene glacial interval. The presence of the latter is confirmed by the *seda*DNA mammal record, which shows the traces of megaherbivores such as mammoths (Elephantidae), woolly rhinoceroses (Rhinoceritadeae), camels (Camelidae), horses (*Equus*), bovids (Bovidae), and deer and reindeer (Cervidae). This availability of prey supported large predators, for example, taxa within Canidae and Felidae. Our shotgun data support the macrofossil remains (*Equus* sp., *Mammuthus primigenius*, *Bison priscus*, *Panthera leo spelaea*) found in the Yana River valley region (Novgorodov et al., 2013) and agree well with previous ancient DNA data (Seersholm et al., 2020).

The data can represent trophic interactions (Krebs et al., 2014). For example, smaller mammals such as hedgehogs (Erinaceidae) and rodents are detected in the MIS 3 sample. They feed on vegetative material, seeds, and arthropods and are in turn consumed by predators. The increased proportion of Plantaginaceae (*Plantago*) during MIS 3 is correlated with the presence of beetles and predators such as wasps (Vespidae), grasshoppers (Acrididae) and parasites (Meloidae), small insectivores—mammals (Erinaceidae) and birds (Scolopacidae)—and their predators in the Canidae. This increased proportion of *Plantago*, and the related trophic links suggest that the MIS 3 interstadial was milder than the MIS 2 stadial but the high abundance of psychrophilic bacteria, a typical inhabitant of glacial environments (Boetius et al., 2015), provides further evidence for a glacial climate. The presence of sand pipers (Scolopacidae), herons (Ardeidae), mayflies (Siphlonuridae), and mosquitoes (Culicidae) indicates the availability of water in the area, which points to at least an intermittent but substantial moisture supply. This is in accordance with an increased presence of *Flavobacterium* spp. and other minor taxa (*Tenacibaculum*, *Formosa*, *Cellulophaga*, *Lacinutrix*), which are considered as "first responders" to phytoplankton blooms due to their activity in degrading algal cells and detritus (Williams et al., 2013; Zeder et al., 2009). This may relate to spring bloom of snow algae or algae in permafrost polygonal or thaw ponds during the cold MIS 3.

The reconstructed glacial mammoth steppe ecosystems lack modern analogs. It is hypothesized that the extinct megaherbivores played a critical role in Pleistocene ecosystems. They directly affected the abiotic component of the system by functioning as "nutrient pumps" (Gross, 2016), allowing distribution of nutrients in the ecosystem. The community structure of the mammoth steppes can be compared to that of the modern savannah (Zimov et al., 2012). Indeed, the megafauna taxa, by their trampling and sapling consumption, may, during milder climatic intervals, have suppressed colonization by trees, keeping the open vegetation largely intact (Bakker et al., 2016; Dale Guthrie, 2001), with the exception, perhaps of woodland patches in habitats with a favorable mesoclimate (Chytrý et al., 2019; Edwards et al., 2014). In our record, low abundance of tree taxa and relatively more abundant megafauna such as Equidae, Elephantidae, Rhinocerotidae, and Megalonychidae and bigger predators such as Canidae and Felidae were detected during Pleistocene glacial time, in contrast to the Pleistocene interglacial time, when megafauna were less abundant and forest taxa more abundant. Modern vegetation in the Altai region (Chytrý et al., 2019) is the most similar analog yet identified for steppe–tundra of the Pleistocene, being characterized by forest patches within grassland and shrub areas. Such a mosaic may explain the presence of sequences related to small- and medium-sized birds such as rails (Rallidae) or tits (Paridae), typical inhabitants of wooded areas, and of cellulose-degrading bacteria (Cellulomonadaceae and Conexibacteriaceae families) during MIS 2 and MIS 3.

4.3 | Potential and limitations of the sedaDNA shotgun approach applied to ancient permafrost sediments

Using the *seda*DNA shotgun approach, we were able to identify taxa from multiple kingdoms and infer trophic structure and biotic interactions at the ecosystem level. Information about vegetation was in some cases confirmed by the more established methods of metabarcoding and pollen. However, we faced some challenges, in particular the handling of taxonomic assignment to optimize the ratio between false negative and false positives (Guillera-Arroita et al., 2017). Highly curated databases are often used to limit the biases of false positives during taxonomic assignments via filtering out unlikely taxa from the database (Pedersen, 2016). In our opinion, such methods are too sensitive to false negatives, particularly with respect to groups where we have only limited knowledge on the past biota and which are poorly presented in the databases. Therefore, we used non-curated databases and worked with likely families to allow the reconstruction of communities to be as broad as possible, limiting the false negatives but reducing the impact of false positives with a taxonomic assignment at higher taxonomic levels. Furthermore, we opted for a more stringent taxonomic assignment using kraken2 v.2.1.2, which requires 2 hit groups (set of overlapping k-mers with the same minimizer) in comparison to the previous version kraken2 v.2.0.7-beta requiring only 1 hit group. While this leads to fewer reads assigned especially for very short DNA fragments (i.e., as those originating from old sediment up to MIS17), it minimizes the rate of false positive, thus increasing the overall confidence that the taxonomic assignments correspond to real signals. This would also provide a stronger support to the reads assigned, for example, to Metazoa, which are known to be underrepresented in *seda*DNA metagenomics when compared to other taxonomic groups as reported also by others (Pedersen et al., 2016).

To overcome the technical challenges of the metagenomics analyses of *seda*DNA and further reduce taxonomic assignment biases such as false positive rates, one could improve the quality of metagenomic reference databases that are incomplete, contaminated,

biased toward modern taxa, domain specific and have a skewed taxonomic representation (Breitwieser et al., 2019; Steinegger & Salzberg, 2020). The use of other tools is also advised, such as alignment-based classification with MALT (MEGAN Alignment Tool) or de novo assembly of contigs and investigation of metagenome-assembled genomes (MAGs; Herbig et al., 2016; Hübler et al., 2019; Sangwan et al., 2016) but they are still computationally very intense and will need further optimization for use with large databases.

5 | CONCLUSIONS

We reconstructed the palaeoecology associated with permafrost sediments from the Batagay megaslump in eastern Siberia using *sedaDNA* shotgun sequencing, metabarcoding, and pollen analyses. We inferred ecosystem-wide information on glacial and interglacial biotic assemblages including Bacteria, Archaea, Viruses, Fungi, Viridiplantae, Mammalia, Aves, and Insecta. All methods indicate that samples at Batagay attributed to interglacial ecosystems in MIS 17 and MIS 7 were characterized by forested vegetation. The local landscape from the MIS 17 snapshot likely featured more open, herb-dominated areas, probably maintained by large herbivores. Around the study site, during MIS 3, diverse megafauna coexisted with smaller mammals on cold grassland. The MIS 2 ecosystems existed under harsher conditions, as suggested by the presence of cold-adapted taxa, while typical Pleistocene megafauna still inhabited the area.

AUTHOR CONTRIBUTIONS

J. C. designed and performed research, analyzed and interpreted the data, and wrote the first draft of the manuscript. A. P. interpreted the data, contributed in detail to the microorganisms' and viruses' interpretation, and wrote the microbial part of the manuscript. A. A. A. analyzed and interpreted the data and contributed in detail to the pollen analysis and the vegetation reconstruction. T. O. and J. B. M. performed the fieldwork and contributed details on regional background, cryostratigraphy, and chronology of the Batagay megaslump. K. R. S. L. supervised palaeogenetic laboratory work and contributed to the interpretation of both metabarcoding and shotgun sequencing results. M. E. reviewed the manuscript and contributed in detail to the ecological interpretation. U. H. designed and supervised the research and contributed in detail to the interpretation of the data and co-wrote the first draft of the manuscript. All co-authors contributed to the final discussion of results and commented on the draft of the manuscript.

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CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

DATA AVAILABILITY STATEMENT

Pollen datasets are uploaded to PANGAEA under: https://doi.org/10.1594/PANGAEA.945257. DNA sequences after shotgun sequencing are uploaded to the European Nucleotide Archive under Project Number PRJEB43506 (Courtin, 2022). DNA metabarcoding sequencing and DNA datasets after metabarcoding are uploaded to DRYAD under doi:10.5061/dryad.xpnvx0kj1.

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