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University of Southampton

Faculty of Environmental and Life Sciences

Geography and Environmental Science

Late-Quaternary Dynamics of Northern Plant Communities: A Sedimentary Ancient DNA (*Sed*aDNA) Perspective

by

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Thesis for the degree of Doctor of Philosophy (Ph.D.)

September 2019

University of Southampton

Abstract

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Tundra plant communities in the northern high latitudes are expected to undergo large distributional changes, habitat loss and/or local species extinction with future climate changes. This is in part linked to the projected northward expansion of climate-sensitive, woody plant taxa of the boreal forest-tundra ecotone. Nevertheless, recurrent glacial-interglacial cycles throughout the Quaternary period (<2.6 million years BP) have repeatedly shifted the ranges of many northern plants. Knowledge of how the composition of plant communities changed in response to previous large-magnitude climate changes could therefore inform predictive modelling of the effect of global change on their distributions.

Ancient DNA recovered from sediments (*sed*aDNA) has proved a useful new tool for studying change in terrestrial ecosystems over time. The cold climate conditions in northern regions appear to be ideal for the long-term preservation of extra-cellular (i.e. "environmental") DNA. This thesis explores the potential of *sed*aDNA extracted from lake sediments for reconstructing changes in the composition and diversity of northern plant communities since the peak of last glacial interval (*ca*. 24,000 cal. years BP). It focuses on three specific northern regions: a previously glaciated area of northeast Norway and two regions (the Polar Ural Mountains of northern Russia and interior Alaska) which remained ice-free during the last glacial interval.

In each of the four papers presented in this thesis, the analysis of *sed*aDNA is shown to contribute new insights towards understanding the long-term dynamics of northern plant communities over the late-Quaternary interval. Many of these new insights arise from unique differences in the source and representation of *sed*aDNA compared to records derived from pollen. Reconstructions of community composition and floristic richness were improved by using *sed*aDNA as the *sed*aDNA signal is less sensitive to "swamping" by woody anemophilous taxa at the expense of insect-pollinated herbaceous taxa than pollen. *Sed*aDNA was also able to document fossil-silent plant taxa which are poorly represented and/or taxonomically resolved within pollen records, including the coniferous tree *Larix,* a diversity of arctic-alpine herbs and turnover amongst bryophyte and grass genera over time.

The findings presented in this thesis demonstrate that lake selection is an important consideration for plant *sed*aDNA studies, as it acts as a control on levels of DNA preservation,

detection success and representation of floristic composition. At a small lake (with <2 km^2 catchment area) located close to the *Pinus* treeline in northeast Norway, the *sed*aDNA record shows little change in floristic composition and diversity over the Holocene period investigated (*ca.* 10,700 and 3,300 cal. years BP), with high values of *Pinus sed*aDNA detected throughout the record. The high *Pinus* values could not be determined with certainty as representing past local presence of the tree. In contrast, at a large lake (with >200 km^2 catchment) in the Polar Urals, located north of a treeline comprising *Larix* and *Picea*, the *sed*aDNA record documents a range of different plant communities growing within its large and topographically complex catchment, including a period of establishment of coniferous forest taxa between *ca.* 9000 and 4000 cal. years BP. Contrary to the conclusions of earlier *sed*aDNA studies, the findings presented in Paper III demonstrate that when compared with a detailed pollen record counted with a high pollen sum, *sed*aDNA and pollen analyses can show considerable overlap in the pattern of occurrence of key plant taxa.

At the two sites which remained unglaciated during the last glacial interval, the *sed*aDNA records reveal a diverse full-glacial flora of what can best be called a herb-tundra vegetation, dominated by forbs (e.g. *Papaver*, *Draba*, *Saxifraga* sp., *Astragalus*, *Bistorta vivipara*) with some graminoids (e.g. *Puccinellia*, *Festuca*, *Juncus biglumis, Bromus pumpellianus*). Floristic richness based on *sed*aDNA at these sites showed a sustained increase until the early- to middle-Holocene, with the sequential addition of new plant taxa over time, before stabilising over the later Holocene. The *sed*aDNA record from the previously glaciated site in northeast Norway provides no evidence for increased floristic richness over time that might have been expected due to successional arrival and/or delayed immigration of species. Lastly, the *sed*aDNA record from the site in the Polar Urals provides robust evidence of persistence of arctic-alpine taxa through several large-magnitude climate changes over the past 24,000 cal. years BP, demonstrating the buffering capacity of a spatially heterogeneous mountain region. However, a distinct decline in their proportional abundance and diversity began as soon as woody taxa started to expand, suggesting that in a future warming scenario, local species loss may occur long before tree establishment occurs.

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Research Thesis: Declaration of Authorship

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I declare that this thesis and the work presented in it are my own and has been generated by me as the result of my own original research.

I confirm that:

1. This work was done wholly or mainly while in candidature for a research degree at this University;
2. Where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;
3. Where I have consulted the published work of others, this is always clearly attributed;
4. Where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;
5. I have acknowledged all main sources of help;
6. Where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself;
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Paper I: Clarke, C.L., Edwards, M.E., Brown, A.G., Gielly, L., Lammers, Y., Heintzman, P.D., Ancin-Murguzur, F.X., Bråthen, K-A., Goslar, T. and Alsos, I.G. 2018: Holocene floristic diversity and richness in northeast Norway revealed by sedimentary ancient DNA (*sed*aDNA) and pollen. *Boreas*, 48(2), 299-316, doi: 10.1111/bor.12357.

C.L.C, M.E., I.G.A., A.G.B. and K.A.B. developed the concept and design of the study. K.A.B. surveyed potential lake sites and C.L.C., I.G.A., A.G.B. and F.X.A-B. carried out the fieldwork and collected the lake sediment core. C.L.C. sub-sampled the core, extracted the DNA and generated the geochemical, lithological and pollen data. L.G. performed the amplifications and initial taxonomic assignment. C.L.C. performed the post-identification filtering of the data and picked plant macrofossils for radiocarbon dating: T.G. performed the radiocarbon dating in the Poznan Radiocarbon Facility. C.LC. constructed the age-depth models. M.E., I.G.A. K.A.B. and A.G.B. contributed ecological and geomorphological expertise. CL.C. created the visualizations and wrote the chapter, of which all co-authors commented on.

Paper II: Clarke, C.L., Edwards, M.E., Gielly, L., Ehrich, D., Hughes, P.D.M., Morozova, L.M., Haflidason, H., Mangerud, J., Svendsen, J-I. and Alsos, I.G. 2019: Persistence of arctic-alpine flora during 24,000 years of environmental change in the Polar Urals. Manuscript in review at *Scientific Reports.*

C.L.C., I.G.A. and M.E. developed the concept and design of the study. J-I.S., H.H. and J.M. carried out the fieldwork, collected the lake sediment core, constructed the age-depth model and provided geomorphological background. C.L.C. sub-sampled the core and extracted the DNA. L.G. performed the amplifications and initial taxonomic assignment. C.L.C. perfomed the post-identification data filtering, data analyses and classified taxa based on their present-day distributional range, with ecological expertise provided by M.E., I.G.A. and P.D.M.H. Botanical knowledge of the region was provided by L.M. and D.E. C.L.C. created the visualizations and wrote the chapter, of which all co-authors commented on.

Paper III: Clarke, C.L., Bjune, A.E., Edwards, M.E., Gielly, L., Paus, A., Hughes, P.D.M., Haflidason, H., Mangerud, J., Svendsen, J.I. and Alsos, I.G. 2019: Arctic plant community dynamics over the past 24,000 years in the Polar Urals: New insights from sedimentary ancient DNA (*sed*aDNA) and pollen. *Manuscript in preparation*.

C.L.C., A.B., I.G.A. and M.E. developed the concept and design of the study. J-I.S., H.H. and J.M. carried out the fieldwork, collected the lake sediment core, constructed the age-depth model and provided geomorphological background. C.L.C. sub-sampled the core and extracted the DNA. L.G. performed the DNA amplifications and initial taxonomic assignment. A.B. and A.P. generated the pollen data. C.L.C. perfomed the post-identification filtering of the DNA data and compared it to the pollen data, performed all of the data analyses and created the visualizations. M.E., I.G.A. and P.D.M.H. contributed their ecological expertise for interpreting the data. C.L.C. wrote the chapter of which all authors commented on.

Paper IV: Clarke, C.L., Bigelow, N.H., Edwards, M.E., Alsos, I.G., Heintzman, P.D., Lammers, Y. and Reuther, J.D. 2019: Insights into late Pleistocene vegetation of interior Alaska from sedimentary ancient DNA (*sed*aDNA). *Manuscript in preparation*.

C.L.C., M.E., N.H.B. and J.D.R. developed the concept and design of the study, carried out the fieldwork and collected the lake sediment core. C.L.C. sub-sampled the core, extracted the DNA, performed the DNA amplifications and generated the lithological and macroscopic charcoal data. C.L.C. successfully obtained a NERC Radiocarbon Facility research grant (allocation no. 2140.1018) which provided financial support for radiocarbon dating, picked plant macrofossils for dating and constructed the age-depth model. Initial sequence filtering and taxonomic assignment was performed by P.D.H. and Y.L., with post-identification filtering and data analyses performed by C.L.C. M.E. and I.G.A. contributed ecological expertise on the data interpretation. C.L.C. created the visualizations and wrote the chapter, of which M.E. and P.D.M.H. commented.

Signature:	Date:
Supervisor Signature:	Date:

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Chapter 1 Introduction

1.1 Preamble

The Arctic is warming at a rate almost twice as fast as the global average (Kattsov *et al*., 2005; Serreze and Barry, 2011; IPCC, 2013; Walsh *et al*., 2017) and the impacts of this warming on terrestrial ecosystems are already starting to become visible (Bhatt *et al*., 2010; CAFF, 2013; Hobbie *et al*., 2017; Bjorkman *et al*., 2019). Ongoing northward expansion of shrub-tundra has been observed in many northern regions in response to increasing summer temperatures over the past few decades, in a process known as 'greening' (Jia *et al*., 2003; Myers-Smith *et al*., 2011; Bjorkman *et al*., 2019). Projected longer growing seasons, increased thaw depth and altered snow regimes could lead to large changes in Arctic vegetation in the future, with implications for trophic interactions, ecosystem functioning, carbon cycling and subsequently, climate feedbacks (Chapin *et al*., 1995; Post *et al*., 2009; Pearson *et al*., 2013; van Gestel *et al*., 2018).

How individual species and entire ecosystems will respond to future climate change and whether they can respond quickly enough to a changing environment are among the most pressing questions facing the ecological research community today (Loarie *et al*., 2009; Nogués-Bravo *et al*., 2018). Palaeoecological records can offer a unique insight into floristic composition at a given time in the past and how it changed in response to climate and environmental changes over long timescales (e.g. Brubaker *et al*., 1995; Bigelow, 2013; Hjelle *et al*., 2018). Moreover, they can provide new perspectives for conservation strategies and knowledge of the ecological resilience of a given individual and/or plant community to environmental perturbation (Willis and Birks, 2006; Froyd and Willis, 2008; Willis *et al*., 2010; Greiser and Joosten, 2018).

1.2 The Last Glacial-Interglacial Transition

In the mid- to high latitudes, recurrent glacial-interglacial cycles throughout the Quaternary (<2.6 million years BP) period have resulted in repeated range expansions, fragmentations and reunions of previously isolated plant populations (Hultén, 1937; Murray, 1995; Comes and Kadereit, 1998; Abbott and Brochmann, 2003). Global ice volumes during the last glacial interval peaked between 30,000 and 15,000 cal. years BP in the Last Glacial Maximum (LGM), when mean annual temperatures over some parts of the Arctic were as much as 20 ^{o}C colder than at present (Cuffey *et al*., 1995; Elias *et al.,* 1996; Dahl-Jensen *et al*., 1998; Miller *et al*., 2010). At their LGM maxima (Figure 1.1), the last Eurasian (British-Irish, Scandinavian, and Svalbard-Barents-Kara Seas) and North American (Laurentide, Cordilleran, Innuitian) ice sheet complexes covered extensive parts of

the British Isles, northern Eurasia (including northern Fennoscandia) and North America, yet parts of northern Russia and Alaska remained ice-free (Dyke *et al*., 2002; Svendsen *et al.,* 2004; Clark *et al*., 2009; Hughes *et al*., 2016; Svendsen *et al*., 2019).

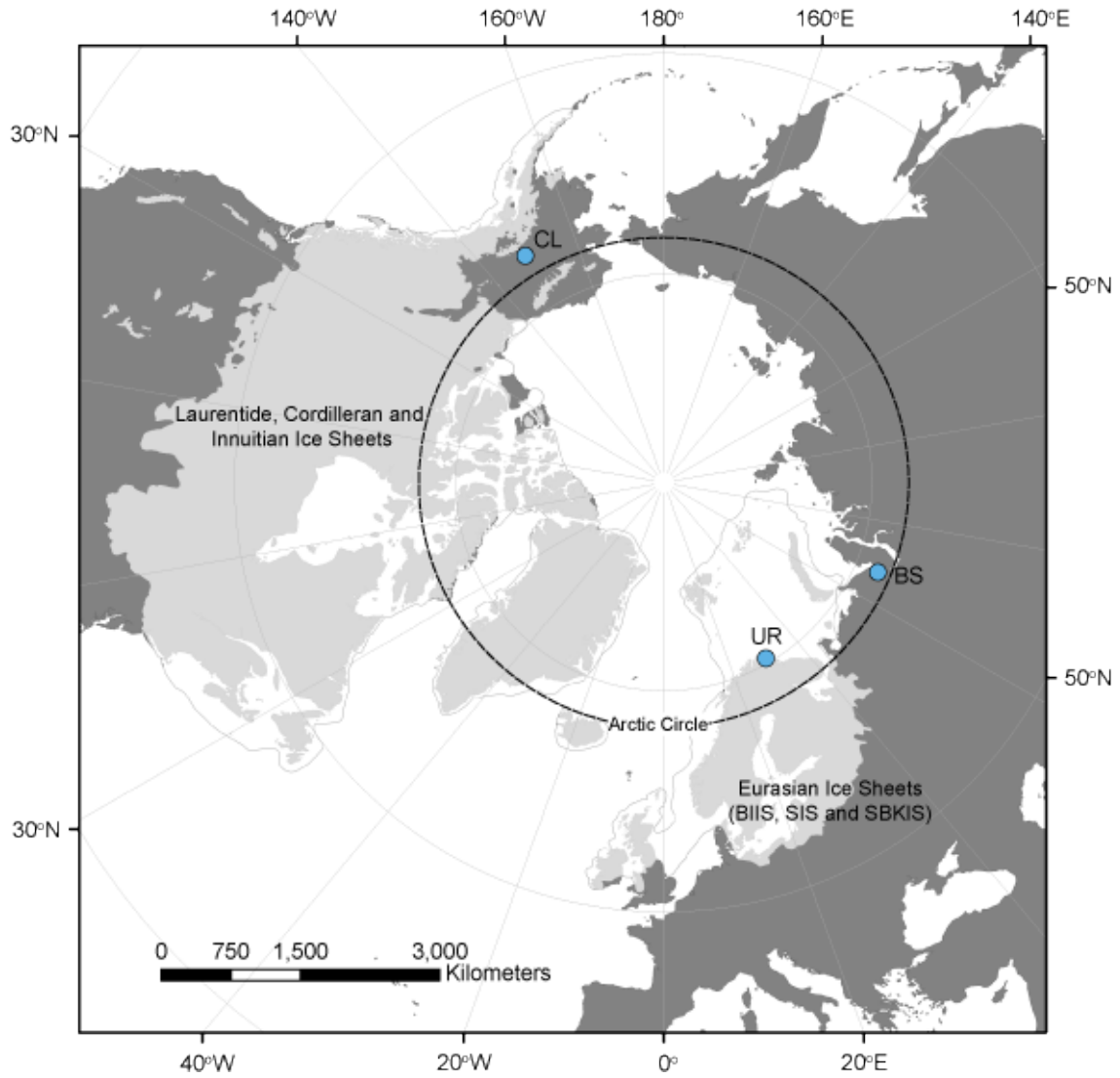

Figure 1.1 Maximum extent of the North American and Atlantic (Laurentide, Cordilleran and Innuitian; from Dyke *et al*., 2002) ice sheet complex and Eurasian (British-Irish (BIIS), Scandinavian (SIS) and Svalbard-Barents-Kara (SBKIS); from Hughes *et al*., 2016) ice sheet complex during the Last Glacial Maximum (LGM) at *ca.* 20,000 cal. years BP. Filled blue circles indicate the location of the three lake sites studied in this thesis: (1) Uhca Rohči' (UR) Lake, located on the Varanger Peninsula, northeast Norway, (2) Lake Bolshoye Shchuchye (BS), located in the Polar Ural Mountains of the Russian Arctic and (3) Chisholm (Lost) Lake (CL), located in the Tanana River Valley in interior Alaska.

By 20,000 cal. years BP, increasing summer insolation due to orbital forcing plus rising greenhouse gas concentrations initiated the recession of continental ice sheets, with a noticeable increase in recession rate around 16,000 cal. years BP, and by around 12,000 cal. years BP most coastlines became ice-free (Miller *et al*., 2010; Hughes *et al*., 2016). The time interval between the end of the LGM (*ca.* 15,000 cal. years BP) and the onset of the current Holocene interglacial (*ca.* 11,800 cal. years BP) is referred to as the Lateglacial interval, and several complex and variable climate changes characterise this interval (Walker, 1995a; Velichko *et al*., 2002; Rasmussen *et al*., 2015). The first half of the Holocene interval (*ca.* 11,800 - 5,000 cal. years BP) saw summer thermal maxima, but the timing and expression of this was geographically variable (Kaufman *et al*., 2004) according to the interaction of variation in summer insolation (Berger, 1978; Berger and Loutre, 1991) and ice sheet extent (Miller *et al*., 2010; Bartlein *et al*., 2015).

1.3 Compositional Changes in Northern Plant Communities Over the Last Glacial-Interglacial Transition

Knowledge of the postglacial colonisation of and development of plant communities within regions that were previously covered by ice during the last glacial interval could inform estimates of plant migration rates and an understanding of how the present-day flora has assembled over the course of the Holocene (<11,800 cal. years BP) interglacial period. Moreover, more information on the composition of plant communities which persisted within ice-free northern regions during the last glacial interval, and how they responded to climatic amelioration at the start of the Holocene, is crucial for understanding their resilience to large-magnitude climate changes.

Traditionally, the reconstruction of floristic history and vegetation dynamics over the Quaternary period (<2.6 million years BP) has relied on unique morphological characteristics to identify plant taxa from their fossil pollen and/or plant macrofossil remains preserved within ancient sediments (e.g. Moore *et al*., 1991; Birks, 2002; Birks and Berglund, 2018). This requires both an intensive sampling effort and an array of taxonomic expertise. Moreover, records of floristic history based on pollen and/or plant macrofossils are often constrained by limits of the proxy data (e.g. productivity, preservation and taxonomic resolution). Pollen records from tundra landscapes, in particular, often suffer from an over-representation of wind-pollinated and high pollen-producing woody taxa which mutes the signal from insect-pollinated, low pollen-producing, herbaceous plants (Sugita, 1994; Lamb and Edwards, 1988; Gajewski, 2015).

Although highly informative, pollen grains are often variably resolved taxonomically, particularly within the Arctic, with several diverse groups such as Poaceae and Cyperaceae barely distinguishable below family level. Common plant taxa which cannot be distinguished below family

or genus level based on their pollen (e.g. *Salix*, *Pinus*, *Potamogeton*, Cyperaceae, Poaceae, Ericaceae) can often be identified to genus or even species level based on macrofossil remains of their seeds and fruits (Birks and Birks, 2000). The preservation of plant macrofossil remains displays large variability between site and species, for example, in the Arctic, detailed reconstructions can be challenging due to low concentrations and/or poor preservation of identifiable material (Birks *et al*., 2003; Bigelow *et al*., 2013).

The analysis of sedimentary ancient DNA (*sed*aDNA) is rapidly emerging as a useful tool for reconstructing the composition and diversity of past floral assemblages and may be able to provide the fraction of the fossil plant community that is not readily preserved and/or well-represented as identifiable pollen or plant macrofossil remains (Jørgensen *et al.,* 2012; Alsos *et al*., 2016; Parducci *et al*., 2015; Pedersen *et al*., 2016; Sjögren *et al*., 2017; Zimmermann *et al*., 2017a). The success and applicability of *sed*aDNA analysis for reconstructing plant community composition can be assessed using qualitative measures such as its ability to detect taxa of the target group (i.e. vascular plants) within samples, the fidelity of the taxa identified by DNA to the local and/or regional flora, and whether it contributes new information as a proxy when compared with pollen analysis.

In ancient DNA studies, detection probability is often low due to the small quantities and degraded nature of starting aDNA molecules (Shapiro *et al*., 2019). To avoid missing detection of taxa which are present in a *sed*aDNA sample, at least eight separate PCR replicates should be performed (Ficetola *et al*., 2015). Quantitatively, the success of *sed*aDNA analysis can be assessed based on total DNA reads retrieved per sample and the proportion of PCR replicates (out of eight) a taxon was detected within, the latter of which is a function of the quantity of a taxon's DNA within the sediment sample. The detectability of a plant taxon growing on the landscape within the DNA signal of lake sediments depends on both its distance to the lake and its abundance in the vegetation (Sjögren *et al*., 2017; Parducci *et al*., 2017; Alsos *et al*., 2018). Typically, the dominant species growing nearby to the lake shore are often found with a high proportion of DNA reads and are present in a higher proportion of PCR replicates. However, when using PCR-based methods, there are several biases (e.g. related to primer binding site, amplicon length, polymerase and diversity of the DNA extract) which could occur in favour of certain plant taxa and/or growth forms (Yoccoz *et al*., 2012; Pornon *et al*., 2016; Nichols *et al*., 2018). Thus, for the time being, interpreting quantities of *sed*aDNA beyond rough estimates when using PCR-based methods, is discouraged (Parducci *et al*., 2017).

In a large-scale *sed*aDNA study of 242 sediment samples from 21 sites across the Arctic, Willerslev *et al*. (2014) concluded that Arctic vegetation during the last glacial interval consisted of dry steppe-tundra dominated by forbs: herbaceous vascular plants not including graminoids

(grasses, sedges, rushes). Moreover, they demonstrated an increase in floristic diversity based on *sed*aDNA between the LGM and present-day. The results of Willerslev *et al*. (2014) contributed new insights into the ongoing debate (described in more detail in Section 2.3.3) as to whether the last glacial flora in northern high latitude regions comprised a dry, productive grassland (e.g. Guthrie, 1982, 2001; Zimov *et al*., 2012), sparsely vegetated Arctic herb communities dominated by both forbs and graminoids, with some dwarf shrubs (e.g. Cwynar, 1982; Anderson and Brubaker, 1994; Bigelow *et al*., 2003), or a range of communities between these extremes (Anderson *et al*., 2004; Zazula *et al*., 2007). The sediment samples analysed by Willerslev *et al*. (2014), however, originate from a range of depositional settings (ice-complex deposits, modern soil, peat, loess and lake sediments) across the circumpolar Arctic region and individually represent only short snapshots in time. Whether similar patterns in floristic composition and diversity occur in a single landscape represented by a single site and through time using a high-resolution, continuous *sed*aDNA record remains untested.

This thesis explores the potential of *sed*aDNA analysis from the sediments of lakes within regions that were previously glaciated (northeast Norway) and those which remained ice-free (the Polar Ural Mountains of northern Russia and interior Alaska) during the last glacial interval, with the goal of reconstructing changes in plant community composition and diversity since the last glacial interval to the present-day. This time interval is an ideal natural experiment for understanding plant community dynamics through several large-magnitude climate changes, which is an essential component informing predictive modelling of global environmental change (Gavin *et al*., 2014).

1.4 Research Questions

This thesis aims to test the following research questions:

(1) What was the composition of the early postglacial plant community which colonised previously glaciated regions in northern Fennoscandia?

(2) What was the composition of plant communities during the LGM and early Lateglacial interval within two specific ice-free northern regions (the Polar Urals of northern Russia and interior Alaska) and how did they change through the transition to the Holocene interval?

(3) How has plant community composition and floristic richness in these northern regions changed over the course of the Holocene?

(4) What is the value and limitation of plant *sed*aDNA from lake sediments compared to pollen analysis for reconstructing past changes in plant community composition and floristic richness?

1.5 Research Aim and Objectives

The primary aim of this thesis is to characterise long-term changes in plant community composition and floristic richness in northern ecosystems (Arctic and Subarctic) since the last glacial interval using *sed*aDNA extracted from lakes. A further aim of this thesis is to assess the power and limitation of *sed*aDNA analysis as a palaeoecological tool for reconstructing past floras. These aims will be addressed through five main objectives:

- Identify suitable sites and retrieve sediment cores from lakes both within previously glaciated northern regions and those which remained ice-free (i.e. unglaciated) during the last glacial interval;
- Extract, amplify and sequence vascular plant *sed*aDNA from those lake sediment cores;
- Reconstruct changes in plant community composition and floristic richness over time at each site;
- Compare the results with high-resolution and detailed pollen analysis;
- Assess the success and applicability of *sed*aDNA for reconstructing plant community composition and floristic diversity using qualitative and quantitative measures (described in Section 1.3).

1.6 Thesis Structure and Author Contributions

This thesis adopts the 'paper approach', and I present its core works in the form of four manuscripts – one published (Clarke *et al*., 2018), one currently in review at *Scientific Reports* (Clarke *et al*., 2019), and two manuscripts which are in the pre-submission stage of the publication process.

Chapter 1 has outlined the rationale and provided background context for the principal aims and objectives of the research presented in this thesis.

Chapter 2 reviews the relevant literature concerning the processes and historical events which have helped to shape the present-day flora of northern regions and identifies the current gaps in knowledge concerning floristic composition over the late-Quaternary interval. The specific research questions identified above that are to be tested within this thesis are elaborated upon through a synthesis of existing literature. This chapter concludes with a review of the history, representation and potential of *sed*aDNA analysis from lake sediments for documenting past changes in plant community composition and diversity.

In **Chapter 3 (Paper I),** *sed*aDNA of vascular plants was extracted and analysed from Uhca Rohči Lake on the Varanger Peninsula, northeast Norway, to investigate the postglacial development of vegetation following deglaciation around 14,000 cal. years BP (Clarke *et al*., 2018)

This chapter also addresses the value of *sed*aDNA for estimates of floristic richness and discusses potential issues related to reconstructing conifer treeline dynamics from *sed*aDNA data.

The lake sediment samples used in this chapter were collected by myself, Professor Inger Greve Alsos, Professor Tony Brown and Mr Francisco Javier Ancin Murguzur, following a survey of potential lake sites conducted by Professor Kari Anne Bråthen. I subsampled the core and extracted the DNA and Dr. Ludovic Gielly performed the amplifications, initial sequence filtering and taxonomic assignment. I performed the post-identification filtering and analysis of the *sed*aDNA data and picked terrestrial macrofossils for dating: radiocarbon dating was performed by Professor Tomasz Goslar. I constructed the age-depth models and generated the geochemical and pollen data. Professor Mary Edwards contributed her expertise on the pollen analysis and, along with Professors Inger Greve Alsos, Kari Anne Bråthen and Tony Brown, provided ecological background and suggestions on the interpretation. Professor Tony Brown provided geomorphological background and compiled data on *Pinus* pollen influx rates. I created the visualizations and wrote the chapter, of which all co-authors commented on.

In **Chapter 4 (Paper II),** vascular plant *sed*aDNA was extracted and analysed from Lake Bolshoye Shchuchye in the Polar Ural Mountains, northern Russia, to evaluate the persistence of arctic-alpine plants through large-magnitude climate and environmental changes over the past 24,000 years (Clarke *et al*., 2019). In particular, it tests the fate of arctic-alpine plants during and after the establishment of shrubs and sub-shrubs from *ca.* 15,000 cal. years BP and later, the establishment of the coniferous tree taxa *Picea* sp. and *Larix* sp. in the vicinity of the lake between 9000 and 4000 cal. years BP.

The samples used in this chapter were collected by Professors John Inge Svendsen, Haflidi Haflidason and Jan Mangerud, who also constructed the age-depth model and provided the geomorphological background. I subsampled the core and extracted the DNA and Dr. Ludovic Gielly performed the amplifications, initial sequence filtering and taxonomic assignments. I performed the post-identification filtering, analysis of the *sed*aDNA data and classified taxa based on their present-day distributional range, with the use of ecological expertise provided by Professors Mary Edwards, Inger Greve Alsos and Paul Hughes. Professors Liudmila Morozova and Dorothee Ehrich contributed their botanical knowledge of the region. I created the visualizations and wrote the chapter, of which all of my co-authors commented on.

In **Chapter 5 (Paper III),** the vascular plant *sed*aDNA record from Lake Bolshoye Shchuchye, Polar Urals, is combined with a high-resolution and detailed pollen record from the same core (developed by Associate Professor Anne Bjune at the University of Bergen) to investigate long-term plant community dynamics over the past 24,000 years, including the composition of the last glacial

flora and how it changed over the last glacial-interglacial transition. This chapter also compares the main similarities and differences between the *sed*aDNA and pollen records and uses this to assess the power and limitation of *sed*aDNA analysis for reconstructing past floras.

This chapter focuses on the same sediment core from Lake Bolshoye Shchuchye as Chapter 4 (Paper II), which was collected by Professors John Inge Svendsen, Haflidi Haflidason and Jan Mangerud. I generated and interpreted the *sed*aDNA data and compared it with the detailed pollen record developed by Associate Professors Anne Bjune and Aage Paus on the same core. Professors Inger Alsos, Mary Edwards and Associate Professors Anne Bjune and Aage Paus contributed their ecological and methodological expertise and suggestions on the interpretation. I created the visualizations and wrote the chapter, of which all of my co-authors commented on.

In **Chapter 6 (Paper IV),** vascular plant *sed*aDNA is extracted and analysed from Chisholm Lake in interior Alaska to investigate the composition of the early Lateglacial flora (*ca*. 15,000 cal. years BP) and how it changed following the climate changes associated with the transition to the Holocene interglacial. The *sed*aDNA record is compared with an existing published pollen record from the same site by Tinner *et al*. (2006) to assess the success of *sed*aDNA analysis for resolving the composition of the early Lateglacial flora.

The lake sediment samples analysed in this chapter were collected by myself, Professor Mary Edwards, Dr. Nancy Bigelow and Dr. Joshua Reuther. I subsampled the core, extracted and amplified the DNA and generated the sediment and macroscopic charcoal data. I successfully obtained a NERC Radiocarbon Facility research grant (allocation no. 2140.1018) which provided financial support for radiocarbon dating. I sieved and picked plant macrofossils for dating and constructed the age-depth model. Initial sequence filtering and taxonomic assignment was performed by myself, Dr. Peter Heintzman and Mr Youri Lammers. I performed the post-identification filtering and analysis of the *sed*aDNA data, interpreted the results and created the visualizations. Professor Mary Edwards contributed her ecological expertise and suggestions on the interpretation. I wrote the chapter, on which my supervisors, Professor Mary Edwards and Professor Paul Hughes, commented.

Lastly, **Chapter 7** presents a synthesis of the new insights gained by *sed*aDNA analysis within Chapters 3 to 6 concerning changes in plant community composition and diversity over the late-Quaternary interval. It reviews the power and limitation of *sed*aDNA as a palaeoecological tool for reconstructing past floras and provides suggestions for the future direction of this research.

Plant taxonomy is changing rapidly, and older floras can be out of date for some plant genera. As with the local arctic and boreal DNA reference libraries (see Section 2.4.4; Sønstebo *et al*., 2010; Willerslev *et al*., 2014; Soininen *et al*., 2015), the works presented in this thesis follow the

taxonomy of the Panarctic Flora checklist of vascular plants (Elven, 2008) where possible. In cases when a plant taxon was identified following a match to a sequence within a global reference library (EMBL release 117), taxonomy follows the National Center for Biotechnology Information (NCBI; Sayers *et al*., 2009).

Chapter 2 Literature Review

2.1 Introduction

This literature review is divided into two parts; the first provides an overview of present-day patterns in the distribution and diversity of northern plant communities and the processes or historical events which have helped to shape these patterns. It reviews the current literature and identifies gaps in our current understanding of floristic history and compositional changes in plant communities during the last glacial-interglacial cycle in northern regions. The second part of this literature review provides a brief history of ancient DNA analysis and the development of sedimentary ancient DNA (*sed*aDNA) metabarcoding techniques. It reviews the value and representation of *sed*aDNA within lake sediments.

2.2 Present-Day Patterns in the Diversity and Distribution of Northern Plant Communities

The Arctic tundra biome (>60^{o}N latitude) is biogeographically young, but it has a complex evolutionary history as a result of the frequent reshuffling of populations through large-magnitude climate changes over its relatively short history (Abbott and Brochmann, 2003; Abbott, 2008; Birks, 2008; Eidesen *et al.*, 2013). It formed only 2 - 3 million years ago when the dense high latitude forests of the Tertiary period gave way to a continuous circumpolar tundra belt in response to planetary cooling at the transition to the Quaternary period *ca.* 2.6 million years ago (Matthews, 1979; Murray, 1995; McIver and Basinger, 1999; Abbott and Brochmann, 2003). Little is known of the origins of present-day arctic plants, although many are thought to have either derived from high latitude Tertiary forests, Quaternary migrants from high mountain regions in Asia and North America or result from *in situ* speciation during the Quaternary period (Hultén, 1937; Yurtsev, 1994; Murray, 1995; Abbott, 2008; Hoffmann *et al.*, 2010).

Although less rich in terms of species number than other biomes on Earth, the Arctic supports more than 21,000 species of often highly cold-adapted plants, mammals, birds, fish and fungi (CAFF, 2013). For vascular plants, several thermal and environmental limits exist which act to control the distribution of species and/or plant communities within the Arctic and Subarctic region (Billings, 1987; Walker, 1995b). Within the Arctic itself, vascular plants are categorised into 15 distinct vegetation-type categories (e.g. erect dwarf-shrub tundra, prostrate dwarf-shrub herb tundra, low-shrub tundra) according to dominant plant functional type (Figure 2.1; Walker *et al.*, 2005). More

than 90 % of high Arctic vascular plant species are circumpolar in their distribution, with their range extending the entire distance around the pole (Walker *et al*., 2005; Brochmann *et al*., 2013).

At its southern limit, the Arctic tundra biome forms an ecotone or transitional zone between tundra and boreal forest (taiga); it is the Earth's longest vegetation transition zone, stretching for more than 13,000 km and occupying around 5 % of the vegetated surface of the Northern Hemisphere (Harding *et al.*, 2002).

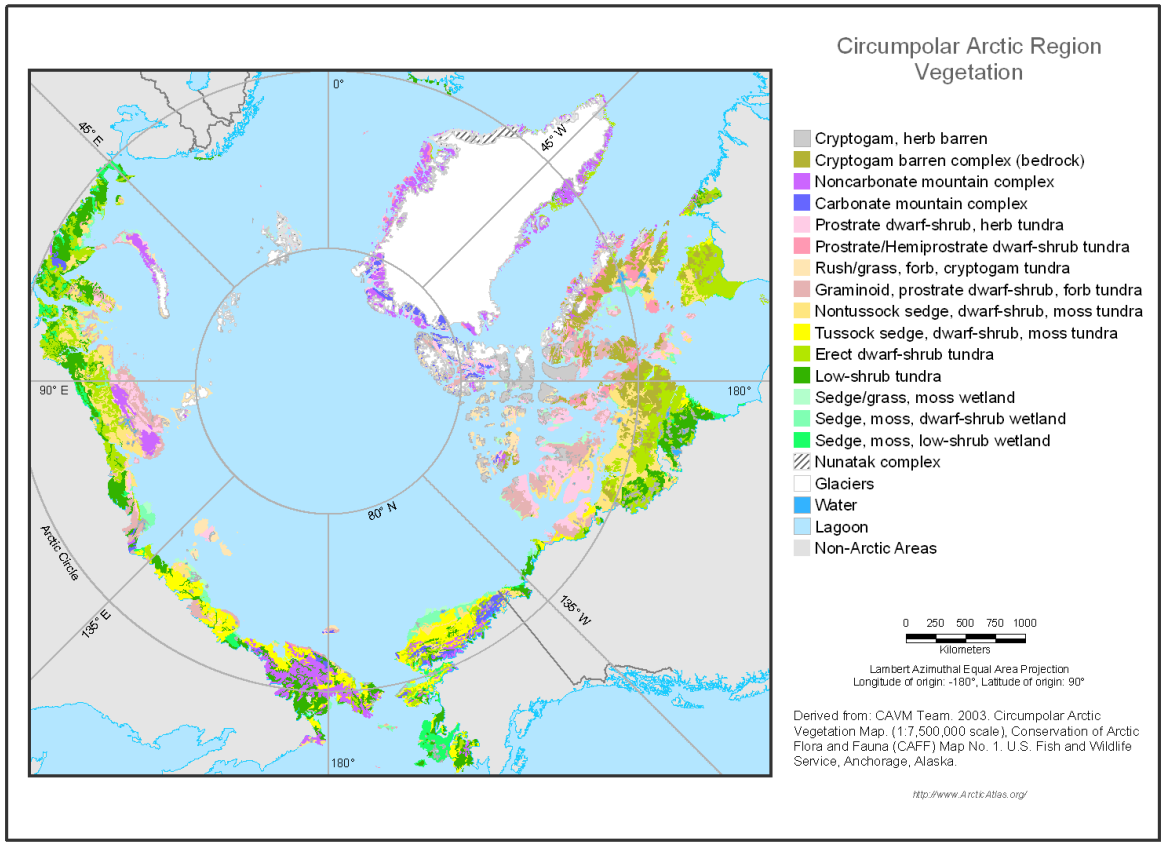

Figure 2.1 The Circumpolar Arctic Vegetation Map (from Walker *et al*., 2005).

In general, plant cover and species richness in northern regions shows a decreasing gradient from the lower latitudes towards higher latitudes, with less than 5 % plant cover and fewer than 50 species present within the polar desert or barren complexes in the north, compared with between 5 and 25 % plant cover and 50 - 100 species present within mountain complexes (Walker *et al.*, 2005). Species richness can also vary on a regional to local scale as a result of spatial heterogeneity in local growing conditions. Biotic factors such as competition, predation and age of the community, for example, can regulate species richness by determining the number and type of individuals which are present and able to persist in a given location and/or community (le Roux *et al.*, 2012). Moreover, several abiotic factors drive variability in species richness on the regional to local scale, including topography, microclimate, soil characteristics, sea-ice cover, presence of permafrost and even past glacial history (Grytnes *et al*., 1999, Gough *et al.*, 2000; Adler and Levine, 2007;

Stewart *et al.*, 2016). Mountain landscapes are spatially heterogeneous at a range of scales and microclimate and soil conditions often vary with topography and elevation to create a mosaic of different habitats and/or growing conditions (Scherrer and Körner, 2010; Graham *et al.*, 2012; Opedal *et al*., 2015; Graae *et al.*, 2018).

Imprints of past glaciations can still be observed in the regional patterns of plant diversity within northern regions; areas that remained ice-free (i.e. unglaciated) during the last glacial interval (*ca.* 30,000 - 11,800 cal. years BP) tend to support a higher species richness and genetic diversity than areas which were previously covered by ice (Stewart *et al.*, 2016). Present-day patterns in genetic diversity shows an increasing gradient from the previously glaciated Fennoscandia region eastward to unglaciated regions in northern Russia and Siberia, for example (Figure 2.2; Eidesen *et al.*, 2013; Stewart *et al.*, 2016). Recurrent glacial- interglacial cycles have also played a part in shaping the biogeographic distribution of Arctic plants. Following the end of a glacial interval, previously isolated populations began to merge. Some taxa were less successful than others at migrating back into their previously occupied distributions, instead retaining a fragmented distribution with large gaps occurring between geographically disjunct areas (Hultén, 1937, 1958; Abbott and Brochmann, 2003). Extreme examples of taxa which today have a geographically disjunct distribution are those which Hultén (1937, 1958) described as having an 'amphi-Atlantic' distribution, occurring on both sides of the Atlantic Ocean, but not elsewhere.

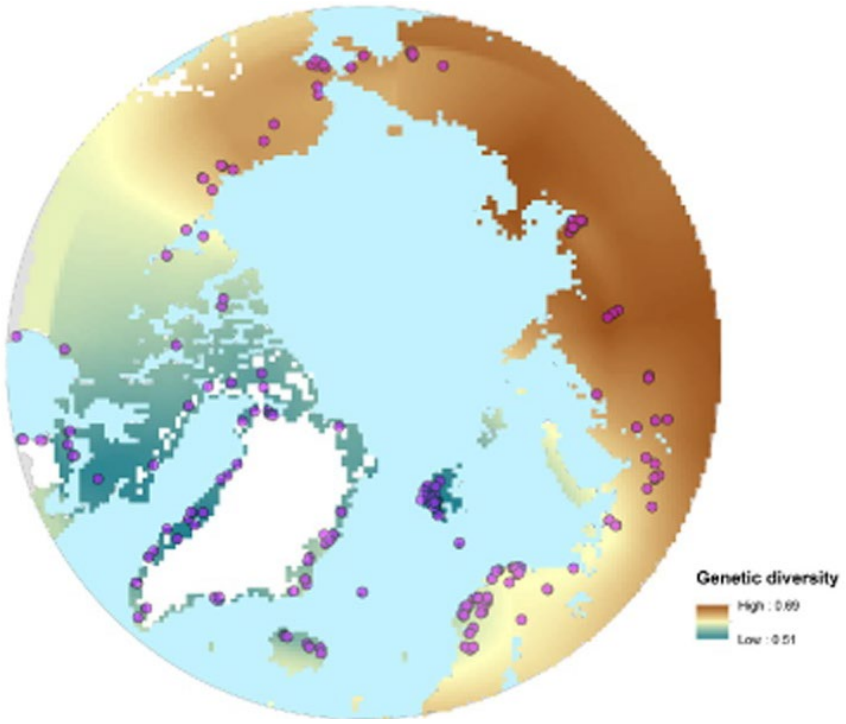

Figure 2.2 Map of interpolated genetic diversity patterns across northern high latitude areas based on Amplified Fragment Length Polymorphism (AFLP) variation in 23 common

arctic plant species (from Stewart *et al*., 2016). Filled purple circles show the locations of sampling sites for genetic data.

2.3 Floristic Changes Over the Last Glacial-Interglacial Cycle

2.3.1 The Last Glacial Maximum (LGM)

The climate, extent and configuration of ice sheets and the floristic composition of northern regions during the Last Glacial Maximum (LGM; 30,000 - 15,000 cal. years BP) has been the subject of long-standing interest within the fields of climate and ecosystem modelling, palaeoclimatology, palaeo- and modern ecology (Gray, 1884; Adams, 1905; Tarasov *et al.*, 2000; Binney *et al.*, 2017). During the LGM, orbital parameters were similar to today, but other climate forcings such as atmospheric CO_2 content, global ice volumes, sea level and mean annual temperatures were very different (Webb and Kutzbach, 1998; Jackson *et al.*, 2000). Knowledge of the composition of plant communities which persisted in ice-free northern regions during the LGM and how they changed following climate warming at the end of this interval is important for understanding the development of communities and ecosystems over long timescales (Willis, 1996; Bigelow *et al.*, 2003; Kaplan *et al.*, 2003; Edwards *et al.*, 2005), and distances and rates involved in postglacial migration and colonisation of previously glaciated regions (Cain *et al*., 1998; Pearson, 2006; Normand *et al.*, 2011)

At their maximum extent, continental ice sheets covered vast areas of North America, the British Isles, Scandinavia and northeast Europe during the LGM interval (Figure 1.1; Dyke *et al.*, 2002; Svendsen *et al.*, 2004; Clark *et al.*, 2009; Hughes *et al.*, 2016). The last Eurasian ice sheet complex (British-Irish, Scandinavian, and Svalbard-Barents-Kara Seas ice sheets) attained maximum extent (5.5 million km^2) and volume (~24 m sea-level equivalent) at *ca.* 21,000 cal. years BP, spanning >4,500 km from southwest Britain to northern Siberia (Hughes *et al.*, 2016; Patton *et al.*, 2017). The largest component of the Eurasian ice complex was the Scandinavian Ice Sheet (SIS), which was predominantly located on terrestrial lands, although the westernmost sector was grounded below present-day sea level on the continental shelf (Hughes *et al.*, 2016).

Presence of these extensive continental ice sheets during the LGM, combined with the prevailing cold and dry climate conditions, expelled plants and other biota from many areas where they occur today, such as previously glaciated areas in northern Fennoscandia (Figure 1.1). Thus, the present-day flora of previously glaciated regions must have spread into these regions sometime between deglaciation and the present-day. Knowledge of the ability of plants to shift their distribution and reform their previous range distributions following deglaciation and changes in

climate at the end of the last glacial period is crucial for understanding their present-day biogeographic distributions and may inform projections of migration rates with future climate changes (Bennett, 1986; Comes and Kadereit, 1998; Pearson, 2006; Cunze *et al*., 2013; Giesecke and Brewer, 2018).

2.3.2 Postglacial Colonisation and Development of Plant Communities Within Previously Glaciated Regions

Increasing summer insolation due to orbital forcing plus rising greenhouse gas concentrations from *ca.* 20,000 cal. years BP initiated the recession of the last Eurasian and North American ice sheet complexes (Clark *et al.*, 2009; Miller *et al.*, 2010; Hughes *et al.*, 2016). By around 18,000 cal. years BP, the SIS was fully separated from the British-Irish ice sheet (BIIS) and by *ca.* 15,000 cal. years BP, much of the Norwegian coastline and its outermost peninsulas (including Varanger Peninsula) had been deglaciated (Olsen *et al*., 1996; Stokes *et al.*, 2014; Hughes *et al.*, 2016; Stroeven *et al.*, 2016). By *ca.* 11,000 cal. years BP, only a very small ice sheet persisted over central Scandinavia.

Deglaciation of areas previously covered by ice resulted in the opening-up of land and increase in the availability of suitable habitat for northern plant taxa to recolonise and for previously isolated populations to reform. Traditionally, many arctic plants were considered to have a limited dispersal capability (Hultén, 1937, 1958; Dahl, 1963). However, more recent molecular evidence suggests that many arctic plants have adaptations for long-distance dispersal and/or colonisation ability (Alsos *et al.*, 2007, 2015), with dispersal via sea ice (e.g. Alsos *et al.*, 2016), driftwood (e.g. Johansen and Hytteborn, 2001), wind, ocean currents and birds (e.g. Bennike, 1999) all suggested as important mechanisms for long-distance dispersal of arctic plants at the end of the last glacial interval. For many arctic plants, it is likely that they did not have to migrate very far to reform their previous range distribution, dispersing instead from nearby northern regions which remained ice-free during the last glacial interval and are thus considered candidate glacial refugia, or safe-sites, for arctic plants (Tremblay and Schoen, 1999; Abbott *et al.*, 2000a; Allen *et al.*, 2015).

Large-scale surveys of pollen and plant macrofossil records have been used to infer rates of postglacial colonisation, patterns of postglacial succession and changes in floristic richness following climatic amelioration and/or deglaciation within previously glaciated regions (Bennett, 1985, 1988; Petit *et al.*, 2002; Giesecke and Bennett, 2004; Berglund *et al.*, 2008; Giesecke *et al.*, 2012). Intermittent absences (e.g. due to poor preservation and/or low concentrations, see Section 1.3) and false presences (e.g. due to long-distance dispersal of pollen; see Section 2.4.7) present a significant interpretational problem in pollen and plant macrofossil records, particularly for investigating the sequence of postglacial colonisation (Jackson, 2012; Gavin *et al*., 2014). Moreover,

biases resulting from non-linear relationships between pollen and/or plant macrofossils within sediments and their representation within the local vegetation can confound estimates of floristic richness (Prentice, 1985; Sugita, 1994; Meltsov *et al.*, 2011; Birks *et al.*, 2016).

Knowledge of the postglacial development and richness of plant communities which re-colonised previously glaciated northern areas at the end of the last glacial interval and how these plant communities have assembled through the current Holocene interglacial is poorly understood. The over-representation of pollen from dominant woody plant taxa, particularly in Holocene records, often occurs at the expense of low pollen-producing plant taxa (Lamb and Edwards, 1998; Bigelow, 2013; Gajewski, 2015). To address this gap in knowledge, *sed*aDNA will be analysed from a previously glaciated site of northeast Norway to investigate the postglacial colonisation of the region and development of plant communities over the Holocene (research question (1), Section 1.4). Analysis of sedimentary ancient DNA (*sed*aDNA) has the potential to identify key plant taxa that are poorly resolved by pollen and plant macrofossil analysis (e.g. Jørgensen *et al.*, 2012; Alsos *et al*., 2016; Sjögren *et al.*, 2017; Zimmermann *et al.*, 2017a) and, in contrast to pollen, it is less sensitive to 'swamping' by dominant woody taxa at the expense of entomophilous herbs (Alsos *et al.*, 2018).

2.3.3 Composition of the Full-Glacial Flora Within Ice-Free (i.e. Unglaciated) Regions

At the maximum LGM ice sheet extent, areas within northwest North America (Alaska and the Yukon Territory) and northern Russia remained free of extensive ice cover (Figure 1.1) and thus provided refuge for species that had been displaced by ice; they probably later acted as points of postglacial dispersal and/or expansion (Huntley and Webb, 1989; Abbott *et al.*, 2000a; Abbott and Brochmann, 2003; Birks, 2008; Binney *et al.*, 2009). Some ice-free regions adjacent to the last-glacial Northern Hemisphere ice sheets may have supported isolated, refugial populations of trees and shrubs during this interval (Kullman, 2002, 2005; Provan and Bennett, 2008; Parducci *et al.*, 2012; Westergaard *et al.*, 2019), although this remains debated (Brubaker *et al*., 2005; Birks *et al.*, 2012; Tzedakis *et al*., 2013; Edwards *et al.,* 2014).

Our understanding of the climate and composition of plant communities which prevailed within ice-free northern regions during the LGM has been hampered by the low density of sites with palaeoenvironmental records which span the interval, problems of data quality (e.g. dating of material) and limitations (e.g. biases in productivity, preservation and taxonomic resolution) of the palaeoecological techniques used (Jackson *et al.*, 2000). Knowledge of the floristic composition within ice-free northern regions could provide an environmental context for understanding these full-glacial landscapes which are known to have supported a diverse megafauna, including woolly

rhinoceros, woolly mammoth, wild horse and bison (Hubberten *et al.*, 2004; Sher *et al.*, 2005; Lorenzen *et al.*, 2011; Mann *et al.*, 2013), and early human populations (Pavlov *et al*., 2001; Kemp *et al.*, 2007; Potter *et al*., 2014; Hufthammer *et a*l., 2019). This thesis will contribute towards a better understanding of the full-glacial flora by developing *sed*aDNA records from two specific northern regions (the Polar Ural Mountains of northern Russia and interior Alaska) which remained ice-free (i.e. unglaciated) during the last glacial interval with the goal of reconstructing the community composition of the last glacial flora (research question (2), see Section 1.4).

One can argue that the last full-glacial plant communities are less well understood than the ecology, range distribution and population dynamics of the large-bodied mammals that consumed them (Murray, 1995; Anderson *et al*., 2004; Willerslev *et al.*, 2014). Due to the low taxonomic resolution and/or low pollen and plant macrofossil concentrations, the plant taxa which composed the full-glacial flora are poorly resolved and it still remains debated whether the prevailing flora was a form of shrub-tundra, herb-tundra or more like steppe which is characterised by dry, cryoxeric grassland communities (e.g. Cwynar and Ritchie, 1980; Guthrie, 2001; Yurtsev, 2001; Zimov *et al.*, 2012). Early authors coined the now widely used term 'Pleistocene mammoth steppe' (also called steppe-tundra or tundra-steppe) to describe a productive full-glacial ecosystem, comprised of cold- and drought-adapted plants which supported a large faunal biomass (Guthrie, 1968; Matthewes, 1982; Zimov *et al.*, 2012). It remains undetermined whether an analogue or remnants of this steppe-tundra ecosystem exist today, although small-scale modern analogues are thought to exist within Alaska, Yukon and eastern Siberia on steep, south-facing sites, rocky or fellfield habitats and within dry and/or disturbed sites (Cwynar and Ritchie, 1980; Edwards and Armbruster, 1989; Walker *et al.*, 2001; Chytrý *et al.*, 2019).

Low pollen influx rates at sites within Alaska and Yukon suggest that the full-glacial landscape was more sparsely vegetated and had a lower productivity than previously suggested; the pollen suggests it comprised arctic forb and graminoid communities and at least some parts of the landscape were more like fellfield habitats rather than dry, temperate steppe communities (Cwynar and Ritchie, 1980; Cwynar, 1982; Lozhkin *et al.*, 2019). Using *sed*aDNA, Willerslev *et al*. (2014) painted a rather different picture of Arctic vegetation during the last glacial interval, questioning the predominance of graminoids and suggesting that the importance of forbs had been underrepresented. They argued that the megafauna were likely opportunistic mixed-feeders rather than grass specialists (Willerslev *et al*., 2014). These results not only drew attention to the importance of forbs within the full-glacial vegetation mosaic, but also demonstrated the value of *sed*aDNA for documenting the past presence of insect-pollinated herbaceous plants, many of which are under-represented and/or poorly resolved by pollen analysis.

2.3.4 Traditional Palaeoecological Records

To date, most reconstructions of late-Quaternary vegetation dynamics have been derived from traditional palaeoecological approaches using pollen and/or plant macrofossils preserved within sediments. High-resolution, long and taxonomically detailed records have been developed from many northern localities and provide insights into the response of plant communities to climate changes over long timescales (e.g. Colinvaux, 1967; Matthews, 1974; Ager, 1982; Cwynar, 1982; Birks *et al*., 1996; Birks and Birks, 2008). However, many early studies suffer from poorly constrained age-depth models. Establishing a reliable chronology using Bayesian age-depth modelling and a high density of radiocarbon ages is essential for Quaternary studies investigating changes over time (Blaauw *et al*., 2018).

Due to issues with taxonomic resolution and long-distance wind dispersal of pollen grains, it has been argued that pollen analysis should be undertaken in conjunction with the analysis of plant macrofossil remains which tend to be local in origin (Birks and Birks, 2000). However, detailed reconstructions are highly time-consuming and require both an intensive sampling effort and an array of taxonomic expertise. Moreover, the success of pollen- and plant macrofossil-based reconstructions is highly dependent on the deposition and preservation of fossil material which tends to be highly variable between sites and/or species (Lamb and Edwards, 1988). It is here where ancient DNA extracted from sediments (*sed*aDNA) could make an important contribution towards resolving taxa poorly resolved and/or represented by pollen and plant macrofossils. This thesis aims to characterise long-term changes in the composition of northern plant communities over the late-Quaternary using *sed*aDNA and also aims to assess the value of *sed*aDNA as a palaeoecological tool for reconstructing past floras (Section 1.5).

2.4 Sedimentary Ancient DNA (*Sed*aDNA) Analysis

2.4.1 A Brief History of Ancient DNA (aDNA)

Ancient DNA (aDNA) refers to DNA that has been isolated from ancient specimens of organisms which have been deceased for hundreds to hundreds of thousands of years (Hofreiter *et al*., 2001; Willerslev and Cooper, 2005). When an organism is alive, damage to its DNA strands occurs quite frequently (e.g. during exposure to UV radiation) but this damage is repaired via an array of DNA repair pathways (e.g. Britt, 1999; Sancar *et al*., 2004; Bray and West, 2005; Iyama and Wilson, 2013). When an organism dies, this damage still occurs but the repair pathways no longer function and because these damages accumulate since the organism's death, most surviving DNA strands in ancient specimens are very short, often fewer than 100 base pairs (bp), and typically contain a

variety of chemical modifications (Pääbo, 1993; Sawyer *et al*., 2012; Fulton and Shapiro, 2019). The extent of DNA damage is sample-dependent and is linked to preservation conditions. Cold and temperature stable environments, such as permafrost regions in the Arctic and Subarctic and caves, tend to be the best sources of well-preserved specimens (Poinar and Stankiewicz, 1999; Willerslev *et al*., 2004; Schwarz *et al*., 2009).

The field of aDNA was born in the early 1980's when the first mitochondrial DNA (mtDNA) sequence was successfully recovered from a 140-year-old museum specimen of a quagga (*Equus quagga*), an extinct relative of the zebra (Higuchi *et al*., 1984). With the advent of PCR (polymerase chain reaction) in the late 1980's (Mullis *et al*., 1986; Saiki *et al*., 1988), it became possible to routinely amplify and produce millions of copies of surviving aDNA from only one or a few starting molecules (Pääbo, 1989). This enabled aDNA researchers to target specific genomic fragments of interest and significantly increased the breadth of potential applications and research topics that could be addressed (Pääbo, 1989; Pääbo and Wilson, 1988; Pääbo *et al*., 1989; Thomas *et al*., 1989). However, the high sensitivity and amplifying power of PCR increased the risk of contamination from modern DNA and simultaneously, the risk of contamination from previously amplified PCR products (Willerslev and Cooper, 2005). Since the surviving copies of endogenous DNA in most ancient specimens is degraded to some extent and in low concentration compared with the ubiquitous nature of non-degraded DNA in the modern environment, the potential for contamination is extremely high.

As techniques for minimizing contamination, recovering and authenticating ancient DNA improve, large-scale studies using an array of different sample types are becoming more attainable (Fulton and Shapiro, 2019). Over the past three decades, ancient DNA research has progressed from isolating small, partial fragments of mtDNA from ancient specimens (e.g. Higuchi *et al*., 1984; Pääbo, 1986; Thomas *et al*., 1989; DeSalle *et al*., 1992), to large-scale studies of ancient populations (e.g. Barnes *et al*., 2002; Haile *et al*., 2009; Naderi *et al*., 2009; Moren-Mayar *et al*., 2018), retrieval of whole genome sequences of extinct species (e.g. Gilbert *et al*., 2007; Rasmussen *et al*., 2010) to the analysis of DNA from a variety of remains, including coprolites, ice-cores, permafrost soil and lake sediments (reviewed in e.g. Rawlence *et al*., 2014; Parducci *et al*., 2017; Shapiro *et al*., 2019).

2.4.2 Environmental DNA (eDNA)

As organisms interact with their surroundings (Figure 2.3), they routinely expel DNA through shed leaves or plant rootlets (Willerslev *et al*., 2007), urine or faeces (Valiere and Taberlet, 2000; Andersen *et al*., 2012), hair (Lydolph *et al*., 2005; Gilbert *et al*., 2007), skin flakes (Swanson *et al*., 2006), feathers (Hogan *et al*., 2008), saliva (Nichols *et al*., 2012) or other secretive body fluids

(Ficetola *et al*., 2008). Environmental samples such as sediment, water, air or glacial ice, can thus contain DNA from a variety of different organisms currently or recently occupying the environment, including plants, mammals, fish, fungi and bacteria. This is termed environmental DNA (eDNA; Ogram *et al*., 1987; Taberlet *et al*., 2012).

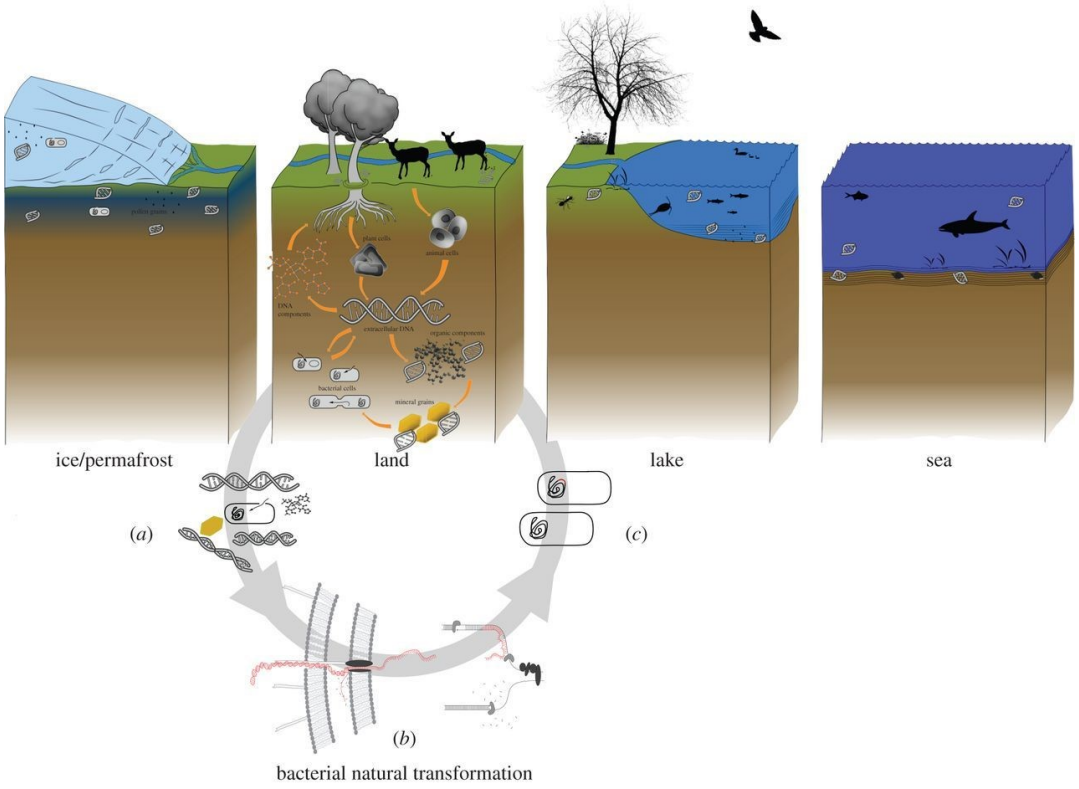

Figure 2.3 Typical depositional environments for environmental DNA (eDNA) (from Pedersen *et al*., 2015). eDNA can remain in the cells (cellular DNA) or be expelled following cell destruction (extracellular DNA) following the death of an organism. It may bind to inorganic sedimentary particles which protects the DNA from further degradation and allows it to persist in the environment over long timescales.

Research on eDNA originated in the field of microbiology, with the first reference to eDNA dating back to 1987 when microbial DNA was first successfully retrieved from marine sediments (Ogram *et al*., 1987). The emergence of eDNA methods was aided by the development of high-throughput next generation sequencing (NGS) technologies, which made it possible to amplify and sequence thousands of short (<120 base pairs; bp) DNA templates in a parallel fashion, thereby bypassing the laborious step of cloning PCR products using traditional Sanger sequencing (Binladen *et al*., 2007; Schuster, 2008; Shokralla *et al*., 2012). Today, analysis of eDNA has matured into an efficient, non-invasive and standardized approach. Diverse fields such as environmental pollution tracking (Caldwell *et al*., 2011), human forensics (van Oorschot *et al*., 2010), biodiversity monitoring and the study of invasive species (Thomsen *et al*., 2012; Mahon and Jerde, 2016; Valentini *et al*.,

2016) use eDNA for taxonomic identification, surveillance and genetic analysis in contexts where collection of intact organisms is impractical or even impossible.

DNA-based taxonomic identification and species detection have taken many different forms (e.g. traditional DNA barcoding, environmental barcoding, DNA metabarcoding, ecometagenetics) and the methodology employed is dependent upon the complexity and degradation of the DNA extract, in addition to the ecological question at hand (Ficetola *et al*., 2008; Taberlet *et al*., 2012a). Approaches differ in terms of the identification level possible and the type and length of marker used for amplification during PCR (Figure 2.4). For environmental samples, the complex mixture of DNA originating from multiple organisms calls for equilibrated co-amplification and taxonomic identification of all species; something which DNA metabarcoding excels at (Taberlet *et al*., 2018).

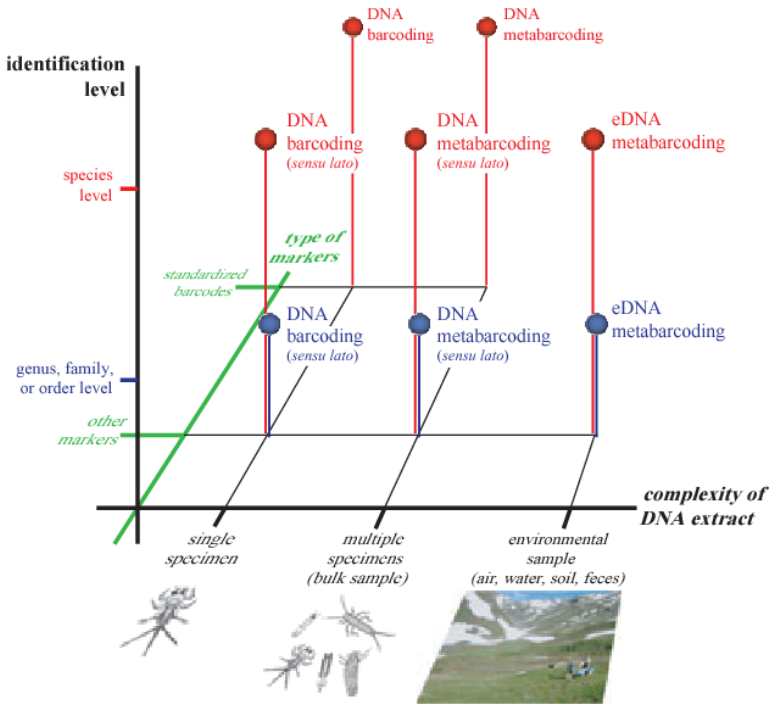

Figure 2.4 Outline of the different approaches used for DNA-based taxonomic identification (from Taberlet *et al*., 2012a).

2.4.3 Traditional DNA Barcoding Versus DNA Metabarcoding Methods

Coupling the principles of traditional DNA barcoding with NGS technology, DNA metabarcoding has relaxed the need for an intensive sampling effort in ecological studies and can be implemented for both modern and ancient environmental samples (Coissac *et al*., 2012). The principle of traditional DNA barcoding involves amplification of a selected region of the genome, referred to as a 'barcode', which is found across a range of target species and is used for taxonomic identification. The most widely used barcodes are located on the chloroplast genome for plants and usually the mitochondrial genome for animals. Traditional DNA barcoding has helped to simplify the

identification step of taxonomic and ecological studies enormously but has tended to fall short in reducing the sampling effort required for biodiversity assessments, with specimens still needed to be collected and sequenced individually (Meyer and Paulay, 2005; Will *et al*., 2005).

The DNA metabarcoding approach (Figure 2.5) diverges from these classical barcoding techniques in that it is capable of dealing with complex and degraded environmental samples and is able to provide automated, simultaneous taxonomic identification of all DNA templates present within the DNA extract (Coissac *et al*., 2012; Taberlet *et al*., 2012b). The method involves selective amplification of a short (often <100 bp), yet informative, portion of the genome found across a range of target species to tag and taxonomically identify sequences by matching them against a purposefully designed reference database.

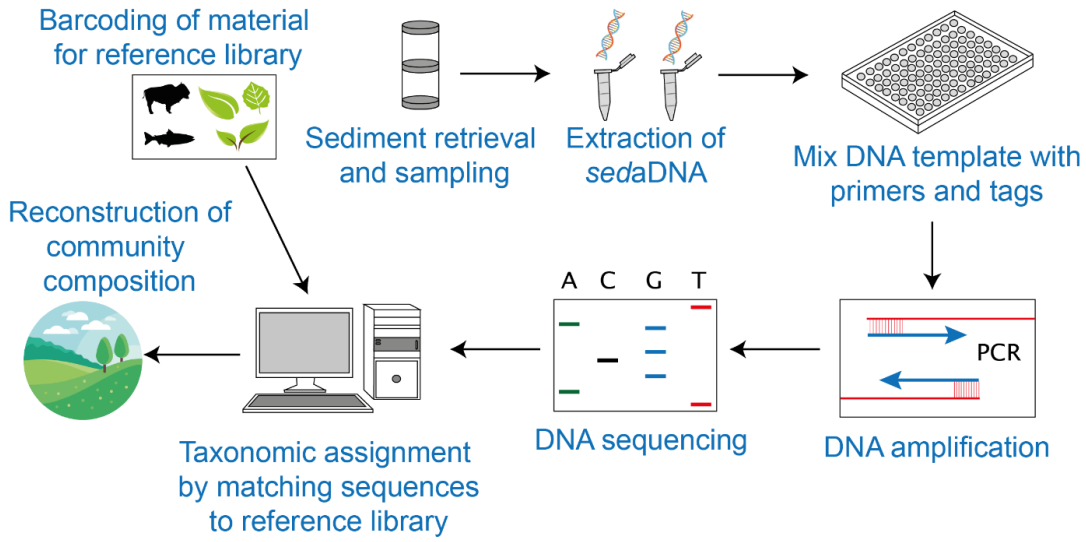

Figure 2.5 Schematic of the DNA metabarcoding approach for analysing ancient DNA extracted from sediments (*sed*aDNA)

The fundamental principle behind DNA barcoding and metabarcoding is based on the idea that a short region of the genome can be found which is consistent across individuals of a given species but differs among species of a target taxonomic group (Savolainen *et al*., 2005). The Consortium for the Barcode of Life (CBOL) first pioneered the use of barcode markers designed for specific target groups (e.g. vascular plants, fungi, birds, fish and other animals), later becoming the standardized method for rapid taxonomic identification (www.barcodeoflife.org; Ratnasingham and Herbert, 2007; Stockle and Hebert, 2008).

In theory, one nucleotide or molecule of DNA should be sufficient to detect a single species in an environmental sample using NGS technologies (Valentini *et al*., 2009). The challenge, however, stems from the degraded nature of eDNA and more so, ancient eDNA (Mitchell *et al*., 2005;

Willerslev and Cooper, 2005). As a result, the standard barcode markers proposed by the CBOL are not entirely adaptable to the analysis of eDNA or ancient eDNA due to the mismatch in length between the barcode markers (550-950 bp) and fragments (often <150 bp) typically recovered (Ficetola *et al*., 2010). An important consideration in eDNA metabarcoding applications is to ensure equal amplification of all target species present within an environmental sample. Use of longer barcode markers for eDNA applications is likely to result in few positive matches and over-amplification of certain sequences at the expense of others (Willerslev *et al*., 2003).

With the help of new software programs such as ecoPrimers (Riaz *et al*., 2011), new barcodes for eDNA metabarcoding applications have been developed, termed 'metabarcodes', and optimised to ensure minimal bias in co-amplification and high taxonomic resolution of the target group. Short metabarcodes for fungi, bryophytes, enchytraeids, beetles and birds (Epp *et al*., 2012) and mammals (Giguet-Covex *et al*., 2014) have been designed and used on environmental samples. For vascular plants, the most promising metabarcode to date is a fragment of the chloroplast *trn*L (UAA) intron, known as the P6 loop. The fragment varies between 10 to 143 bp in length and was developed by Taberlet *et al*. (2007). Universal primers, *g* and *h*, for this marker were designed over two decades ago (Taberlet *et al*., 1991) and are still used extensively. The locus appears to be universal among the plant kingdom and can be amplified in most plant taxa, often permitting closely related species to be distinguished and taxonomically identified (Särkinen *et al*., 2012).

2.4.4 Developments in DNA Reference Libraries

Use of species barcodes and/ or metabarcodes first requires the assembly of a comprehensive reference library to match DNA sequences to organisms. Reference libraries are commonly assembled by sequencing modern specimens collected in the field and/or from museum collections and therefore require sound taxonomy to ensure DNA sequences are not incorrectly assigned to species identifiers (Nilsson *et al.,* 2006). One of the most important contributions to the field of environmental (ancient) DNA analysis was the development of the NCBI GenBank® reference library (https://www.ncbi.nlm.nih.gov/genbank/), which contains publicly available DNA sequences for over 260,000 described plant and animal species (Benson *et al*., 2018). The detection and alignment success of retrieved DNA sequences against the reference library is heavily dependent on the barcode marker used and the complexity of the DNA template, however. GenBank®, stores DNA sequence data from a range of classic barcode markers (e.g. COI, matK, 16S) useful to traditional DNA barcoding applications, but these are less applicable to eDNA applications on degraded DNA templates.

More recently, a number of taxonomic reference libraries optimised for eDNA applications have become publicly available, facilitating the spread of eDNA methods into diverse research fields. A local taxonomic reference library based on the P6 loop of the *trn*L (UAA) intron is publicly available, currently comprising 815 arctic and 835 boreal vascular plant taxa (Sønstebø *et al*., 2010; Willerslev *et al*., 2014) and 455 bryophyte taxa (Soininen *et al*., 2015), which makes an enormous contribution for detailed eDNA studies of vegetation within northern high latitude regions. Matching DNA sequences to both a local and a global reference database, if available, is still encouraged to increase the detection success and minimise missing any taxa that are actually present (false negatives) but are yet to be added to the local reference library (Parducci *et al*., 2017).

2.4.5 Contamination and Authentication of Results

Contamination with exogenous DNA is a major challenge when working with degraded DNA templates in environmental samples and extreme caution must therefore be taken before accepting a taxonomic assignment and positive match to reference libraries. Human DNA is one of the most prevalent contaminants, alongside domestic or laboratory animals (Leonard *et al*., 2007), common food plants (Valentini *et al*., 2009) and PCR reagents (Willerslev and Cooper, 2005; Champlot *et al*., 2010). In addition to false-positives, amplification of contaminant DNA may also cause false-negatives as a result of competition or bias during PCR in favour of the often more abundant contaminants at the expense of the less abundant degraded environmental DNA (Gigli *et al*., 2009; Thomsen and Willerslev, 2015). Issues of contamination may arise in nearly all stages of the eDNA process; from sample retrieval, extraction, PCR and sequencing. No single strategy exists to remove all potential sources of contamination, although it is ultimately the responsibility of the researcher to ensure an appropriate, multi-strategy approach is followed to minimise and detect contamination issues (Champlot *et al*., 2010).

It is important that a strict clean protocol is followed for all field sampling and laboratory work for eDNA analyses, employing decontamination procedures (e.g. UV exposure, bleach baths and DNase treatments) to clean all equipment and work surfaces (Parducci *et al*., 2017; Shapiro *et al*., 2019). Extra precautions can be taken during sampling of eDNA samples by sterilizing all coring equipment (e.g. Feek *et al*., 2011) and applying an exotic tracer onto the piston and pipes to test the level of infiltration of DNA molecules from outer surface layers to the innermost sediments (Pedersen *et al*., 2016; Parducci *et al*., 2017). To minimise contamination from handling, sampling of sediments and other environmental samples should be undertaken wearing a full-body suit within a dedicated clean laboratory. For sediment cores, a set of sterile tools (e.g. knives or blades) should be used to discard the outermost 4-10mm and retrieve the innermost, undisturbed centre to minimise contamination from exposure to the air during the splitting of core sections (Parducci

et al., 2017). The inclusion of negative controls at all stages of the eDNA process (field, extraction and PCR blanks) is also essential in order to monitor issues of contamination.

2.4.6 Sedimentary Ancient DNA (*Sed*aDNA)

Following the death of an organism, extracellular DNA can be released into the environment as a result of cell membrane degradation and lysis (Nielsen *et al*., 2007; Pietramellara *et al*., 2009; Torti *et al*., 2015) and can absorb onto clay minerals, which greatly increases its resistance to microbial degradation (Goring and Barthohlomew, 1952; Pietramellara *et al*., 2009). This extracellular DNA can thus be preserved within sedimentary deposits for hundreds to potentially hundreds of thousand years and is termed sedimentary ancient DNA (*sed*aDNA).

In contrast to the now fairly long history of aDNA research, *sed*aDNA is a relatively young and rapidly developing research field. Frozen permafrost soils have proved excellent sources of well-preserved ancient DNA molecules and have thus been the most commonly used archive of *sed*aDNA to date (e.g. Willerslev *et al*., 2003, 2004; Lydolph *et al*., 2005; Jørgensen *et al*., 2012; Bellemain *et al*., 2013). However, the DNA signal from soils represents a highly local signal of plant communities, originating mainly from plant taxa growing within less than 1 m of the sampling point (Yoccoz *et al*., 2012; Edwards *et al*., 2018). For a more representative view of floristic composition, recent studies have demonstrated the potential of *sed*aDNA from lake sediments as they have much larger catchment areas than soil samples and they seem to represent the surrounding terrestrial environment well (e.g. Matisoo-Smith *et al*., 2008; Anderson-Carpenter *et al*., 2011; Pedersen *et al*., 2013; Alsos *et al*., 2016; Sjögren *et al*., 2017; Parducci *et al*., 2017; Alsos *et al*., 2018).

2.4.7 Plant *Sed*aDNA in Lake Sediments

Lakes are excellent sources of information on both aquatic and terrestrial communities and the continuous accumulation of sediment allows for the establishment of a well-defined chronological stratigraphy (Parducci *et al*., 2017). Unlike unfrozen soil columns (Poté *et al*., 2007) and cave sediments (Haile *et al*., 2007), vertical migration (leaching) of DNA does not seem to be a problem in lake sediments as they are permanently saturated, compacted and the anoxic conditions in the bottom water and below 1 - 2 cm sediment depth (Sobek *et al*., 2009) precludes the presence of burrowing animals and sediment reworking (Pansu *et al*., 2015; Parducci *et al*., 2017). Currently, the oldest plant *sed*aDNA sequences are from cold environments, dated between 450,000- and 800,000-years BP (Willerslev *et al*., 2007) but investigation of *sed*aDNA from temperate and tropical

environments is also proving possible (e.g. Epp *et al*., 2010; Boessenkool *et al*., 2014; Bremond *et al*., 2017).

A modern calibration study comparing the plant DNA signal in surficial lake sediments with detailed vegetation surveys revealed that the DNA records the dominant and common plant taxa present within the catchment with high accuracy and that 73 and 12 % of the taxa detected in the DNA were recorded in vegetation surveys within 2 and 50 m of the lake shore, respectively (Alsos *et al*., 2018). Analysis of plant *sed*aDNA from small lakes with limited inflowing streams may thus provide a more local signal of floristic composition and richness than records derived from pollen, as wind-dispersed pollen grains tend to be transported over long distances (Jacobson and Bradshaw, 1981; Rousseau *et al*. 2006).

To date, only a handful of studies have investigated the release, transport and deposition of DNA in the environment (Poté *et al*., 2007, 2009; Pietramellara *et al*., 2009; Barnes & Turner, 2016; Giguet-Covex *et al*., 2019) and thus, knowledge of the ecology and taphonomy of DNA within the environment is limited. Terrestrial plant DNA extracted from lake sediments is thought to derive from terrestrial soil inputs via processes such as inwash from streams, surface runoff and/or erosional events (Anderson-Carpenter, 2011; Giguet-Covex *et al*., 2019). If this is the case, there should be little evidence in the DNA signal of plant taxa that are not growing near the lake, but which deliver long-distance pollen to the site (Sjögren *et al*., 2017). Previous comparisons of the taxonomic overlap between pollen, plant macrofossil and plant *sed*aDNA analyses have shown a limited overlap between pollen and *sed*aDNA identifications, with *sed*aDNA showing a greater taxonomic overlap with plant macrofossils (Jørgensen *et al*., 2012; Parducci *et al*., 2013, 2015; Pedersen *et al*., 2013).

When rigorously applied, the analysis of *sed*aDNA from lake sediments may be able to detect more species per sample than other palaeoecological methods and permit the detection of plant taxa that are under-represented or poorly resolved taxonomically by pollen and/or plant macrofossil analysis (Jørgensen *et al*., 2012; Parducci *et al*., 2013, 2015, 2019; Pedersen *et al*., 2013; Alsos *et al*., 2016; Sjögren *et al*., 2017; Zimmerman *et al*., 2017a). In comparison to pollen, which is formed via sexual reproduction, *sed*aDNA largely exists in the sediment archive as extracellular DNA, originating from the plant tissues of above- and below-ground biomass (Yoccoz *et al*., 2012). The *sed*aDNA signal may therefore permit the detection of clonal plants with severely reduced sexual reproduction, a common trait at a species northern range limits (e.g. Dorken and Eckert, 2001).

Comparisons of the taxonomic overlap and pattern of occurrence of key plant taxa detected by *sed*aDNA and pollen to date have been rather conservative due to low pollen counts and/or poor

reference libraries (e.g. Pedersen *et al*., 2013). A more comprehensive comparison between *sed*aDNA and pollen analyses is necessary to assess the power and limitation of the two techniques for reconstructing plant community composition. To address this shortcoming, this thesis has the objective (see Section 1.5 and research question 4, Section 1.4) to compare a high-resolution *sed*aDNA record with a contemporaneous pollen record (counted at a high pollen count) derived from the same sediment core and which spans a long temporal interval.

Chapter 3 Holocene Floristic Diversity and Richness in Northeast Norway Revealed by Sedimentary Ancient DNA (*Sed*aDNA) and Pollen (Paper I)

This paper is published as:

Clarke, C.L., Edwards, M.E., Brown, A.G., Gielly, L., Lammers, Y., Heintzman, P.D., Ancin-Murguzur, F.X., Bråthen, K-A., Goslar, T. and Alsos, I.G. 2018: Holocene floristic diversity and richness in northeast Norway revealed by sedimentary ancient DNA (*sed*aDNA) and pollen. *Boreas,* 48(2), 299-316, doi: 10.1111/bor.12357.

This work has been presented as (selected examples):

Clarke, C. 2019: Arctic plant community dynamics and changes in floristic diversity: insights from sedimentary ancient DNA (*sed*aDNA). In: Biodiversity Network Seminar Series, University of Oxford. 1st May. Invited oral presentation.

Clarke, C., Alsos, I.G., Edwards, M.E., Bjune, A., Hughes, P., Gielly, L., Lammers, Y., Mangerud, J., Haflidason, H. and Svendsen, J.I. 2018: Ancient DNA from northern Eurasian lakes reveals community dynamics of vascular plants over the past 24,000 years. In: European Geosciences Union (EGU) 2018 Meeting, Vienna, 13th April. Poster Presentation.

3.1 Abstract

We present a Holocene record of floristic diversity and environmental change for the central Varanger Peninsula, Finnmark, based on ancient DNA extracted from the sediments of a small lake (*sed*aDNA). The record covers the period *ca.* 10,700 to 3,300 cal. years BP and is complemented by pollen data. Measures of species richness, sample evenness and beta diversity were calculated based on *sed*aDNA sampling intervals and 1,000-year time windows. We identified 101 vascular plant and 17 bryophyte taxa, a high proportion (86 %) of which are still growing within the region today. The high species richness (>60 taxa) observed in the early-Holocene, including representatives from all important plant functional groups, shows that modern shrub-tundra communities, and much of their species complement, were in place as early as *ca.* 10,700 cal. years BP. We infer that postglacial colonization of the area occurred prior to the full Holocene, during the Pleistocene-Holocene transition, Younger Dryas stadial or earlier. Abundant DNA of the extra-limital aquatic plant *Callitriche hermaphroditica* suggests it expanded its range northward between *ca.* 10,200 and 9,600 cal. years BP, when summers were warmer than present. High values of *Pinus* DNA occur throughout the record, but we cannot say with certainty if they represent prior local presence; however, pollen influx values >500 grains cm^{-2} $year^{-1}$ between *ca.* 8,000 and 7,300 cal.

years BP strongly suggest the presence of pine woodland during this period. As the site lies beyond the modern tree limit of pine, it is likely that this expansion also reflects a response to warmer early-Holocene summers.

3.2 Introduction

In the mid- to high-latitudes, the repeated waxing and waning of continental ice sheets throughout the Quaternary has regulated habitat availability for plants (Hultén, 1937; Abbott and Brochmann, 2003). By around 13,000 – 11,000 cal. years BP, the Fennoscandian Ice Sheet had retreated from the northeasternmost peninsulas of Finnmark, Norway (Sollid *et al*., 1973; Stokes *et al*., 2014; Hughes *et al*., 2016; Stroeven *et al*., 2016), and newly deglaciated land became accessible for plant colonization and vegetation development (Prentice, 1981; 1982). Today, these peninsulas harbour the ecotone from boreal forest to tundra. At and near their northern limit, tree populations are particularly sensitive to climate changes; climate variation may cause shifts in tree population ranges that may in turn generate changes in vegetation structure and habitat (Hyvärinen, 1975; Barnekow, 1999; Bjune *et al*., 2004; Jensen and Vorren, 2008). The deglacial history and proximity to the treeline suggest that records from the region can potentially address key ecological questions: how floristic richness and/or composition is affected by treeline dynamics and/or Holocene climate variation; whether taxa show discernible migration lags (and thus locally variable postglacial successional sequences); and how today's dominant plant communities assembled over the Holocene.

The understanding of treeline fluctuations has inspired palynological studies of vegetation history for many years (Hyvärinen, 1975; Seppä, 1996; Allen *et al*., 2007). Pollen percentage and influx values have been used to track treeline fluctuations across northeast Finnmark in response to Holocene climate changes (Hyvärinen, 1975; Hicks, 1994; Seppä, 1996; Hicks and Hyvärinen, 1999; Høeg, 2000; Huntley *et al*., 2013). During the regional Holocene Thermal Maximum (HTM; 8,000 – 6,000 cal. years BP; Seppä *et al*., 2009a; Huntley *et al*., 2013), the ecotonal boundaries between *Pinus* and *Betula* forests and *Betula* forest with low shrub-tundra had more northerly positions (Høeg, 2000). In contrast to treeline dynamics, less is known about the development and composition of the shrub and herb communities of northern Fennoscandia and their response to Holocene climate changes. Tundra pollen records are often interpreted in terms of broad-scale community dynamics rather than local compositional changes, due to features such as low accumulation rates and the over-representation of woody anemophilous taxa that mute the signal of entomophilous forbs in many records (Lamb and Edwards, 1998; Gajewski, 2015).

The Lateglacial and Holocene vegetation history of Finnmark has been documented in several pollen and plant macrofossil records since the 1970s (e.g. Hyvärinen, 1975; Prentice, 1981, 1982; Høeg, 2000; Allen *et al*., 2007; Birks *et al*., 2012; Sjögren and Damm, 2019), reflecting interest in understanding events linked to the early deglaciation of the region (Sollid *et al*., 1973; Stokes *et al*., 2014; Hughes *et al*., 2016; Stroeven *et al*., 2016). The initial postglacial landscape supported sparse, open, herbaceous vegetation with some shrubs (Hyvärinen, 1975; Prentice, 1981, 1982; Seppä, 1996). The oldest pollen record in northeast Finnmark is that of Østervatnet, southern Varanger Peninsula, which records the Older Dryas (*ca.* 13,900 – 13,600 cal. years BP) and Younger Dryas (*ca.* 13,500 – 11,500 cal. years BP) climate oscillations, with *Artemisia* replacing *Salix* and Poaceae in the cold stages (Prentice, 1981). The end of the Younger Dryas chronozone is distinguished by a rise in *Oxyria*/*Rumex*, *Salix*, Poaceae and Cyperaceae with species-rich meadows colonized by a succession of shrub and tree species in the Holocene (e.g. *Betula*, *Pinus*). More information on the rate of community assemblage and local variation in community development through time will help inform our understanding of the resilience and longevity of the current dominant communities in this area.

Pollen data have been used to reconstruct past changes in floristic diversity and richness, including studies in Scandinavia (Odgaard, 1999; Berglund *et al*., 2008a, b; Fredh *et al*., 2012; Reitlau *et al*., 2015). Records from Scandinavia indicate a rapid increase in species richness from the Lateglacial to the early-Holocene (*ca.* 12,000 – 8,000 cal. years BP), while spatially and temporally inconsistent trends characterize the middle- to late-Holocene (*ca.* 8,200 cal. years BP – present; Seppä, 1998; Berglund *et al*., 2008a, b; Birks and Birks, 2008; Felde *et al*., 2017). These later Holocene trends have been attributed to climate fluctuations, the first appearance of trees at a locality, and/or human impact. A significant ($p < 0.001$) negative relationship was identified between *Pinus* pollen influx and species richness at the boreal site Lake Rautuselkä, northern Finland, reflecting the importance of vegetation density on floristic richness (Seppä, 1998). A significant ($p < 0.05$) negative correlation between *Pinus* and *Betula* pollen influx and species richness was also identified at the tundra site Lake Hopseidet on the Nordkinn Peninsula, northeast Norway, which was not reached by northward expansion of the *Pinus* treeline during the Holocene (Seppä, 1998). High influx of wind-pollinated taxa in the tundra site probably reduced the statistical probability of other, less frequent insect-pollinated herbaceous types being counted (Birks and Line, 1992; Seppä, 1998). Records from central Scandinavia show that species richness has remained rather stable, with no long-term trends observed over the Holocene (Giesecke *et al*., 2012). Nevertheless, biases resulting from non-linear relationships between pollen and vegetation representation may confound richness estimates derived from pollen (Prentice, 1985; Sugita, 1994; Odgaard, 2001). Furthermore, the relationship between species

richness derived from pollen data (palynological richness) and the observed floristic richness in the landscape remains poorly understood (Meltsov *et al*., 2011; Goring *et al*., 2013).

Analysis of sedimentary ancient DNA (*sed*aDNA) has recently emerged as a promising proxy for reconstructing past floral diversity, augmenting information gained from pollen and macrofossil analyses (Jørgensen *et al*., 2012; Epp *et al*., 2015; Alsos *et al*., 2016; Pedersen *et al*., 2016; Parducci *et al*., 2017; Zimmerman *et al*., 2017a). When rigorously applied, the analysis of *sed*aDNA from lake sediments can detect more species per sample than other palaeoecological methods (Alsos *et al*., 2016). It also permits the detection of some key plant taxa that are poorly resolved taxonomically by pollen or plant macrofossil analysis alone (Parducci *et al*., 2013; Sjögren *et al*., 2017; Edwards *et al*., 2018). Recent investigation of the representation of contemporary vegetation in the DNA signal of superficial sediments in small lakes with limited inflowing streams from northern Norway revealed that 73 and 12 % of the taxa detected in the DNA were recorded in vegetation surveys within 2 and 50 m of the lake shore, respectively (Alsos *et al*., 2018). Thus, analysis of plant *sed*aDNA from small lakes with limited inflowing streams may give a more local signal of vegetation change and floristic richness than records derived from pollen, as wind-dispersed grains tend to be dispersed over long distances, particularly at the northern limit of trees (Rousseau *et al*., 2006).

This study represents the first palaeoecological exploration of Arctic vegetation dynamics in Finnmark using a *sed*aDNA record, in this case from sediments of a small lake on the Varanger Peninsula, northeast Finnmark. We use metabarcoding techniques (Taberlet *et al*., 2012a) to develop the *sed*aDNA record, together with X-ray fluorescence (XRF) to determine geochemical element concentrations over time, sedimentological data and pollen analysis. We then compare the results with published pollen records. Finally, we use the *sed*aDNA data to reconstruct Holocene trends in species richness using rarefaction and measures of beta diversity and evenness for all samples and for 1000-year windows.

3.2.1 Study Site

The lake (latitude 70°19’6.85348” N, longitude 30°1’43.83653” E; Figure 3.1) is unnamed on the 1:50 000 Norwegian Topographic Map (Norgeskart; https://www.norgeskart.no). We refer to it here informally as ‘Uhca Rohči’, or UR, the Sami name for an adjacent river feature. UR is a small lake (<1 ha) in a depression situated at 138 m above sea level (a.s.l.) within the river valley of Komagdalen on the Varanger Peninsula, northeast Finnmark, Norway. The peninsula is a plateau lying between 200 and 600 m a.s.l. with low relief: ridges are formed of Cambrian quartzites and sandstones, while valleys are eroded into shales and mudstones (Siedlecka and Roberts, 1992). After Pliocene uplift, the area was affected by sea-level change and subject to glacial erosion

(Fjellanger and Sørbel, 2007). UR Lake lies in the middle section of the glaciated southeast-draining Komagdalen valley. As shown in Figure 3.1D, the lake vicinity is associated with probable subglacial scour features on the former valley floor, which is now ~3 m above the present river. The valley lies between the Gaissattrinnet and Hovedtrinnet (Younger Dryas) moraines to the southwest and the older Ytre Porsangertrinnet and Korsnestrinnet moraines to the north and east (ice advance to NE, retreat to SW; Sollid *et al*., 1973). A reconstruction of the lower section of the Komagdalen valley by Olsen *et al*. (1996) suggests that the middle valley lies just north (outside) of an ice margin dated to 17,000 cal. years BP as well as a more southerly ice margin associated with the Vardø moraine stage (13,500 years BP) as mapped by Tolgensbakk and Sollid (1981). Cosmogenic dating at the head of Varangerfjorden and Tanafjorden suggests a local retreat age of *ca.* 15,400 – 14,200 cal. years BP, and it is certain that the peninsula was free of glacial ice by 13,000 – 12,000 cal. years BP (Stokes *et al*., 2014; Stroeven *et al*., 2016). UR Lake lies approximately 60 m above the main (Younger Dryas) postglacial shoreline (75 – 85 m a.s.l.), which is reflected by a markedly steeper valley floor reach at approximately 13 km downstream (Figure 3.1E; Fletcher *et al*. 1993). It is likely that the site received fluvial input from all of the upstream Komagdalen catchment, at least prior to downcutting associated with postglacial isostatic uplift in the later Holocene, and this is important in the interpretation of the *sed*aDNA data.

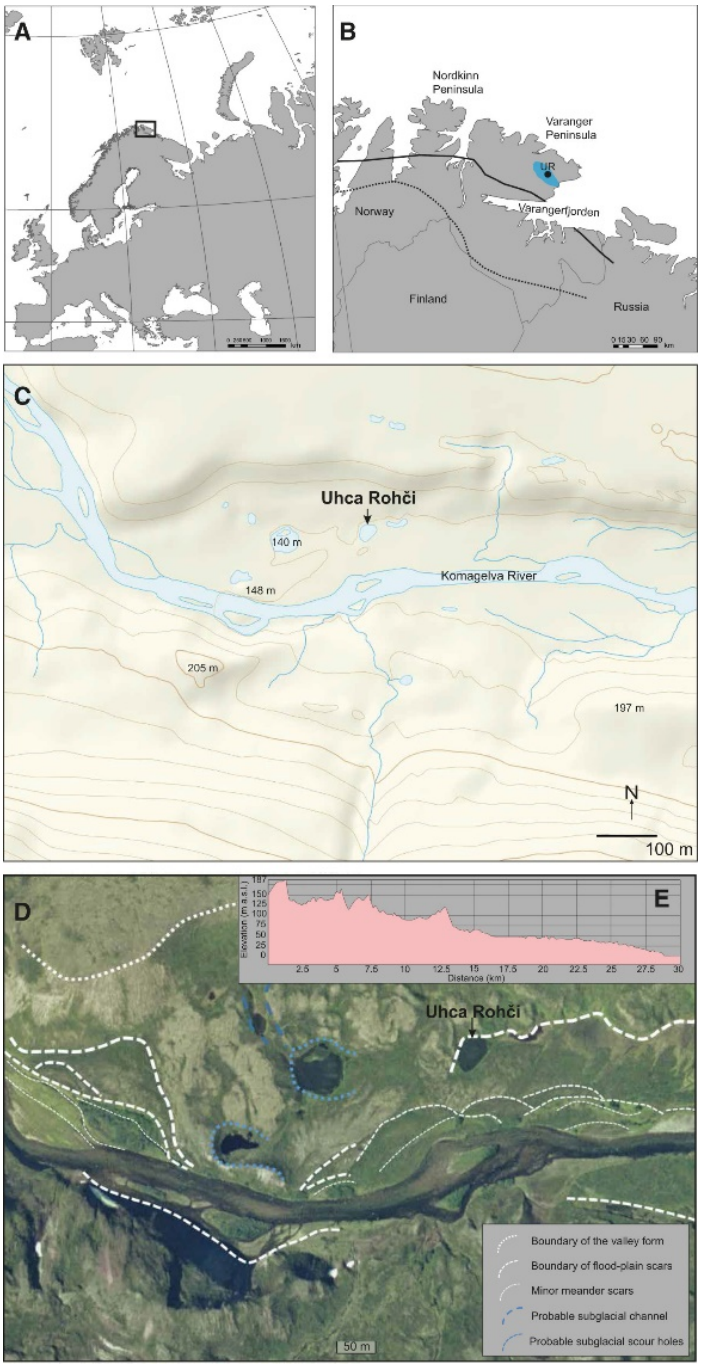

Figure 3.1 Location of Uhca Rohči Lake (unofficial name) on the Varanger Peninsula, northeast Finnmark, Norway. A. Map highlighting the location of Finnmark in northeastern-most Norway. B. Varanger Peninsula with watershed of Komagelva River indicated in blue. Dashed line indicates the *Pinus* forest limit according to Heikkinen (2005) and the solid black line indicates the tree limit of *Pinus* according to Hustich (1983). Location of Uhca Rohči Lake is indicated by a black circle. C. Map detailing the position of Uhca Rohči Lake within the Komagelva River (Komagdalen) valley (norgeskart.no). D. Geomorphological map created from aerial photography and inset E. valley floor height profile downstream of Uhca Rohči Lake created using Google Earth 2017.

The present-day climate of the Varanger Peninsula is characterized as sub-Arctic (<10 °C arctic isotherm in July), with annual precipitation between 500 and 800 mm. It is situated within the meeting zone of the westerlies and the sub-polar low-pressure system (with polar easterlies) and thus has highly variable weather (Hanssen-Bauer and Tveito, 2014). Large local heterogeneity exists due largely to the topography, and summer temperature may vary from 6 – 12 °C, with corresponding differences in local vegetation (Karlsen *et al*., 2005). Present-day tundra vegetation of the Varanger Peninsula is classified as erect shrub tundra (Virtanen *et al*., 1999; Walker *et al*., 2005), and is dominated by dwarf shrubs, such as *Empetrum nigrum* subsp. *hermaphroditum* and *Betula nana*. Species-rich meadows occur along the wide riparian plains of the Komagelva River where tall shrubs such as *Salix lanata*, *S. hastata* and *S. glauca* form a spatially and temporally diverse vegetation mosaic with mesic forbs such as *Bistorta vivipara*, *Thalictrum alpinum* and *Viola biflora*, and graminoids such as *Avenella flexuosa*, *Deschampsia cespitosa* and *Eriophorum angustifolium* (Ravolainen *et al*., 2013; Bråthen *et al*., 2017). The headwaters of the Komagelva River originate from the north to northwest of the main Komagelva channel, which flows eastwards to Varangerfjord (Figure 3.1B). The entire watershed lies outside of the present-day *Pinus* limit (Figure 3.1B). UR Lake is one of several small lakes surrounded by the species-rich riparian meadows of the Komagdalen valley, which is one of the principal sites for the Climate Ecological Observatory for Arctic Tundra (Henden *et al*., 2011; Ravolainen *et al*., 2011, 2013; COAT, 2018).

Early-Holocene climate in northern Fennoscandia was affected by summer insolation that was higher than present (Berger, 1978; Berger and Loutre, 1991). Quantitative summer temperature estimates based on aquatic plant macrofossils suggest an early onset of the Holocene Thermal Maximum (HTM), with temperatures ~2 °C warmer than pollen-based estimates between 11,700 – 7,500 cal. years BP (Väliranta *et al*., 2015). However, pollen-based summer temperature reconstructions indicate July temperatures of +1.5 ± 0.5 °C above modern (1961–1990) values in northern Fennoscandia during the regional HTM identified at *ca.* 8,000 – 6,000 cal. years BP (Møller and Holmeslet, 2002; Jensen and Vorren, 2008; Seppä *et al*., 2009b; Sejrup *et al*., 2016). Northeast Finnmark was characterized by warmer summer temperatures and higher precipitation (Allen *et al*., 2007). Warm conditions were interrupted by a short cold spell at *ca.* 8,200 cal. years BP (Seppä *et al*., 2009a).

3.3 Material and Methods

3.3.1 Core Retrieval and Sampling

A 10-cm-diameter and 2.5-m-long lake sediment core (UR-1) was retrieved in February 2016 from the winter ice surface using a modified Nesje piston-corer (Nesje, 1992) with a 4-m-long continuous section of acrylonitrile-butadiene-styrene (ABS) pipe. Total lake depth including winter ice thickness was 2 m, as measured by a single-beam echo-sounder (Echotest II Plastimo) and tape measure. Coring and retrieval of sediments started at 2 m, with the assumption, based on the echo-sounder data, that the top of the core sequence would include surface or near-surface sediments. After retrieval of the sediments, the pipe was cut into 1-m sections and sealed immediately to minimize the risk of contamination by airborne or other modern environmental DNA. The core sections were stored in a shed (2–6 °C) to prevent freezing before transport and stored at 4 °C in the cold room at the Tromsø University Museum (TMU), Norway. Core sections were opened by longitudinal splitting. One half was used for subsampling, and the other half kept for archival purposes. Core UR-1 was subsampled at 1-cm resolution within a dedicated ancient DNA clean-room facility at TMU using sterile tools, a full bodysuit, facemask, and gloves. Following the protocol described by Parducci *et al*. (2017), the outer 10 mm of sediment was avoided or discarded and an ~20-g subsample was retrieved from inside the freshly exposed centre only.

An additional short 50-cm-long and 7-cm-diameter core (UR-2), which included a clear sediment-water interface, was retrieved using a UWITEC gravity corer (UWITEC Corp., Austria) lowered from the surface of the lake ice. Subsampling of core UR-2 was not performed in the clean-room at TMU, as this core was only used for pollen analysis and age-depth model determination (described below).

3.3.2 Lithological and Elemental Analyses

Subsamples (2 cm^3) were taken for bulk density and loss-on-ignition (LOI) analyses at 3-cm intervals using a volumetric sampler. Samples were weighed in crucibles and dried overnight at 100 °C before dry weight and bulk density were determined (Chambers *et al*., 2011). Samples were then ignited at 550 °C for 2 h, placed in a desiccator to cool to room temperature and reweighed. Total LOI was calculated as the percentage loss of dry weight after ignition (Heiri *et al*., 2001). Magnetic susceptibility and XRF analyses were performed on the archival core halves at the Department of Geosciences, UiT - Arctic University of Norway. Magnetic susceptibility was measured at 1-cm intervals using a Bartington point sensor on the Geotek Ltd. Multi-Sensor Core Logger using a 10-s exposure time. Quantitative element geochemical measurements were performed with an

Avaatech XRF core scanner. XRF scanning was performed at 1-cm resolution with the following settings: 10 kV, 1000 µA, 10 s exposure time and no filter. To minimize closed sum effects, we normalized the raw peak area data against Ti, as this element is considered a reliable indicator of allochthonous catchment inputs (Croudace and Rothwell, 2015).

3.3.3 Radiocarbon Dating and Age-Depth Model Construction

Seven samples of terrestrial plant macrofossils from Nesje core UR-1 and an additional two samples from UWITEC core UR-2 were radiocarbon (^{14}C) dated with accelerator mass spectrometry (AMS) at the Poznań Radiocarbon Laboratory (Goslar *et al*., 2004). All radiocarbon ages were calibrated according to the terrestrial IntCal13 curve (Reimer *et al*., 2013), and an age-depth relationship was established using the Bayesian framework calibration software 'Bacon' (v. 2.2; Blaauw and Christen, 2011), which was implemented in R v. 3.2.4 (R Core Team, 2017).

3.3.4 Pollen Analysis

In total, 16 pollen samples were analysed from core UR-1 and an additional two samples from UR-2. Subsamples of 1.5 cm^3 were prepared using standard methods (acid-base-acid-acetolysis; Fægri and Iversen, 1989) and were mounted in glycerol. Two *Lycopodium* spore tablets (Batch no. 3862; n = 9666) were added to each sample to calibrate pollen concentration estimation. At least 300 pollen grains of terrestrial taxa were identified per sample using taxonomic keys (Fægri and Iversen, 1989) and type material held in the Palaeoecology Laboratory at the University of Southampton. Results are presented as pollen percentages, with trees, shrubs, herbs and graminoids based on the sum of total terrestrial pollen (∑P), and percentages for spores and aquatics based on ∑P + ∑spores and ∑P + ∑aquatics, respectively.

3.3.5 DNA Extraction, Amplification, Library Preparation, and Sequencing

DNA extraction, PCR amplification, PCR product pooling and purification, and sequencing follow the protocols of Alsos *et al*. (2016) unless otherwise stated. Within the TMU clean-room facility, DNA was extracted from 80 sediment subsamples and nine negative extraction controls, which consisted of no sediment and were used to monitor for contamination. Aliquots of DNA extracts were then shipped to the Laboratoire d'ÉCologie Alpine (LECA, University Grenoble Alpes, France) for metabarcoding. Each DNA extract and negative extraction control was independently amplified using uniquely tagged generic primers that amplify the *trn*L P6 loop of the plant chloroplast genome (Taberlet *et al*., 2007), a widely applied marker for the identification of vascular plants in environmental samples. Each sample and negative control underwent eight PCR replicates to

increase confidence in the results and improve the chance of detecting taxa with small quantities of template in the DNA extracts. We also ran 13 negative PCR controls, consisting of no DNA template. Pooled and cleaned PCR products were then converted to two Illumina-compatible amplicon libraries using the single-indexed, PCR-free MetaFast method (FASTERIS SA, Switzerland). These libraries were then sequenced on the Illumina HiSeq-2500 platform for 2× 125 cycles at FASTERIS.

3.3.6 Sequence Analysis and Taxonomic Assignments

Next-generation sequence data were filtered using the OBITools software package (Boyer *et al*., 2016; http://metabarcoding.org/obitools/doc/index.html) following the protocol and criteria defined by Alsos *et al*. (2016). Taxonomic assignments were performed using the *ecotag* program (Boyer *et al*., 2016) by matching sequences against a local taxonomic reference library comprised of 815 arctic and 835 boreal vascular plant taxa, and 455 bryophytes (Sønstebo *et al*., 2010; Willerslev *et al*., 2014; Soininen *et al*., 2015). In order to minimize any erroneous taxonomic assignments, only taxa with a 100 % match to a reference sequence were retained. We further considered a taxon to be undetected in a PCR replicate if it was represented by fewer than 10 reads. Moreover, sequences that displayed higher average reads in negative extraction or PCR controls than lake sediment samples were also removed. Identified taxa were compared with the local flora from Komagdalen (Ravolainen *et al*., 2013), Species Map Service 1.6 (https://artskart1.artsdatabanken.no/Default.aspx), the Norwegian Flora (Elven, 2005) and the circumpolar flora (Hultén and Fries, 1986). Sequences assigned to taxa not present in northern Scandinavia today were checked against the NCBI BLAST database for multiple or alternative taxonomic assignments (http://www.ncbi.nlm.nih.gov/blast/).

3.3.7 Indices of Richness, Diversity and Evenness

Species diversity was measured by three parameters – beta diversity (β-diversity), richness, and evenness (Magurran, 2004; Soininen *et al*., 2012) – and analysed for both the DNA sampling intervals (3-cm resolution) and 1000-year time windows. On average, each 1000-year time window encompassed nine *sed*aDNA samples, whereas time windows of 500 years or less contained too few samples (>4), on average, for estimating long-term changes in diversity. Following Koleff *et al*. (2003), β-diversity was measured using Whittaker's (b_w) index computed using the PAST v. 3.19 software package (Hammer *et al*., 2001). A comparison of richness between samples with different count sizes can be biased, as the chance of detecting rare taxa increases simultaneously with count size (Birks and Line, 1992; Brown, 1999). We, therefore, rarefied the *sed*aDNA data based on the minimum count of 5,306 DNA reads to estimate the number of vascular plant taxa that would have

been detected if the DNA read count had been standardized amongst samples. Rarefaction analysis was performed using the minimum count size in the Vegan (Oksanen *et al*., 2017) package for R (R Core Team, 2017). We chose the Simpson evenness index ($E_{1/D}$) to measure DNA sample evenness across the 1000-year time windows, following Meltsov *et al*. (2011). This index of evenness is independent of the number of taxa detected.

3.4 Results

3.4.1 Chronology and Lithostratigraphy

In total, seven AMS radiocarbon dates were obtained from the UR-1 core (Nesje). Ages span 3,330±30 to 9,480±50 ^{14}C years BP, which corresponds to a calibrated weighted-mean range of 3,606 – 10,705 cal. years BP (Table 3.1). The resulting age-depth model (Figure 3.2) suggests a fairly linear sedimentation rate, with the exception of a period of faster accumulation of sediment between 125 and 150 cm depth (as measured from the top of core UR-1; *ca.* 7,600 – 8,100 cal. years BP). We consider these radiocarbon ages to be reliable for age-depth modelling as they are in the correct stratigraphical sequence, have small errors and are all derived from terrestrial plant macrofossils. It can, therefore, be assumed that the uppermost sediments that correspond to the last *ca.* 3000 years are missing from core UR-1. This was most likely caused by a lake-depth measurement error, potentially from the ice layer affecting the echo-sounder, which resulted in the non-retrieval of upper sediments. To test this assumption, two additional AMS radiocarbon dates were obtained from the surface core UR-2 (UWITEC), which displayed a clear sediment–water interface. Radiocarbon ages from these two additional samples (275±30 and 1,485±30 ^{14}C years BP; a calibrated weighted-mean range of 15 and 1,433 cal. years BP) confirm that UR-1 is missing the uppermost sediment.

Table 3.1 Radiocarbon ages of plant macrofossil remains from Uhca Rohči Lake shown with their 1σ error, calibrated weighted mean, calibrated median and calibrated 95 % confidence age ranges. All radiocarbon ages were calibrated using the IntCal13 curve (Reimer *et al*., 2013).

Core no.	**Lab. ID**	**Depth below sediment surface (cm)**	**Age±1σ (^{14}C years BP)**	**Cal. weighted mean age (cal. years BP)**	**Cal. median age (cal. years BP)**	**Cal age, 2σ (cal. years BP)**	**Material**
UR-2	Poz-98146	1–2	275±30	15	6	−2 to 150	*Salix* leaves
UR-2	Poz-98147	42–43	1485±30	1433	1417	1305–1598	*Salix* leaves
UR-1	Poz-93338	9–10	3330±30	3606	3592	3479–3819	*Salix* leaves
UR-1	Poz-87278	22–23	3850±40	4246	4244	4059–4408	*Salix* leaves
UR-1	Poz-87277	74–75	5490±50	6280	6287	6040–6432	Charcoal
UR-1	Poz-87276	120–121	6890±50	7715	7717	7572–7842	*Empetrum* wood
UR-1	Poz-93339	140–141	7150±40	8038	8024	7920–8195	*Empetrum* wood
UR-1	Poz-87275	178–179	8240±35	9208	9205	9027–9402	Charcoal
UR-1	Poz-87274	231–232	9480±50	10 706	10 702	10 417–11 030	*Salix* leaves

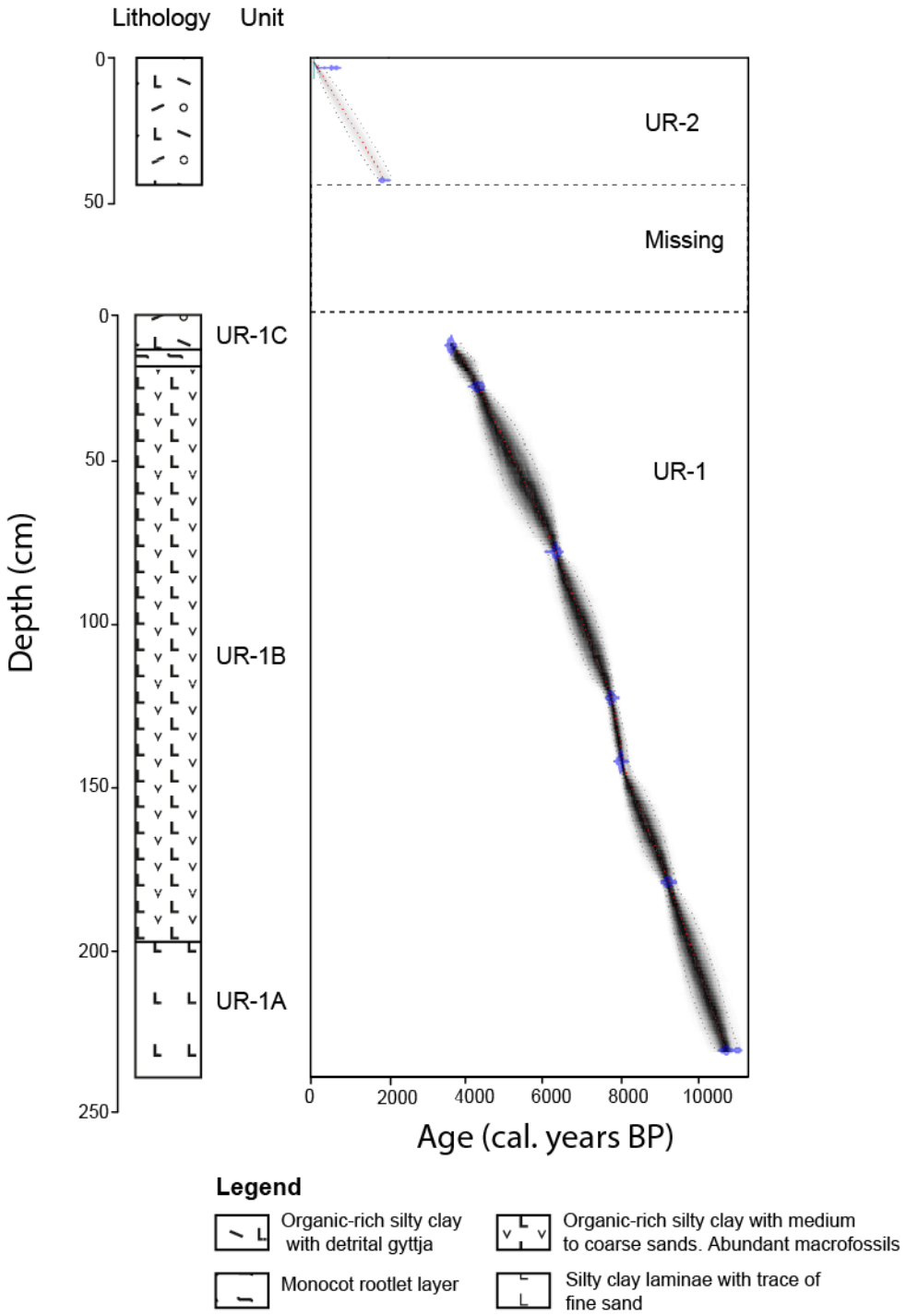

Figure 3.2 The age-depth relationship and lithostratigraphical units (labelled UR-1A to C) for Uhca Rohči Lake, Varanger Peninsula. Age-depth relationships for core UR-1 (Nesje) and UR-2 (UWITEC) were analysed independently due to the hiatus remaining unknown. Radiocarbon ages were calibrated following IntCal13 (Reimer *et al.*, 2013) and the age-depth model produced using the software Bacon (Blaauw and Christen, 2011). Note that only UR-1 was analysed further in this study.

LOI values vary around 20 % in the lower part of the core and reach a maximum of 47 % in the upper part, indicating a generally increasing organic component with time (Figure 3.3). Core UR-1 is divided into three main lithostratigraphical units, labelled A–C from the core base (Figures 3.2, 3.3):

Unit UR-1A (234–204 cm; ca. 10,700 – 9,900 cal. years BP). Silty-clay with traces of fine sand characterize this unit. Coarse (˜2–3-cm thick bands) greenish-brown banding is evident. LOI values range from 15 to 22 % (mean 18 %) and mean bulk density is 0.4 g cm^{-3}. The unit is characterized by high Ti, K and Fe, suggesting that sedimentation is driven by input of terrigenous minerogenic sediment.

Unit UR-1B (204–15 cm; ca. 9,900 – 3,800 cal. years BP). A sharp transition into more organic-rich silty clay with small monocot rootlets and wood fragments occurs at 204 cm, marking the base of Unit B. Thin (1–3 mm) laminae of medium-coarse sand alternating with olive-brown organic silty clay comprise this second unit, with silt content decreasing upwards. Higher mean LOI values of 27 % and fluctuations in Ti, K and Si characterize Unit B, with peaks in Ca probably related to the presence of inclusions, such as shells, within this unit. Bulk density values range from 0.2 to 0.4 g cm^{-3} and display a general decline through this unit, with the exception of a short-lived interval of higher values between 165 and 138 cm, which may result from sediment compaction after cutting the core sections.

Unit UR-1C (15–0 cm; ca. 3,800–3,300 cal. years BP). This unit comprises mid- to dark-brown silty-clay gyttja with some detritus, including abundant monocot rootlets. A small decline in LOI values from 37 to 33 % in this unit suggests a slight rise in terrigenous minerogenic input. The lithology of this unit is similar to that of core UR-2.

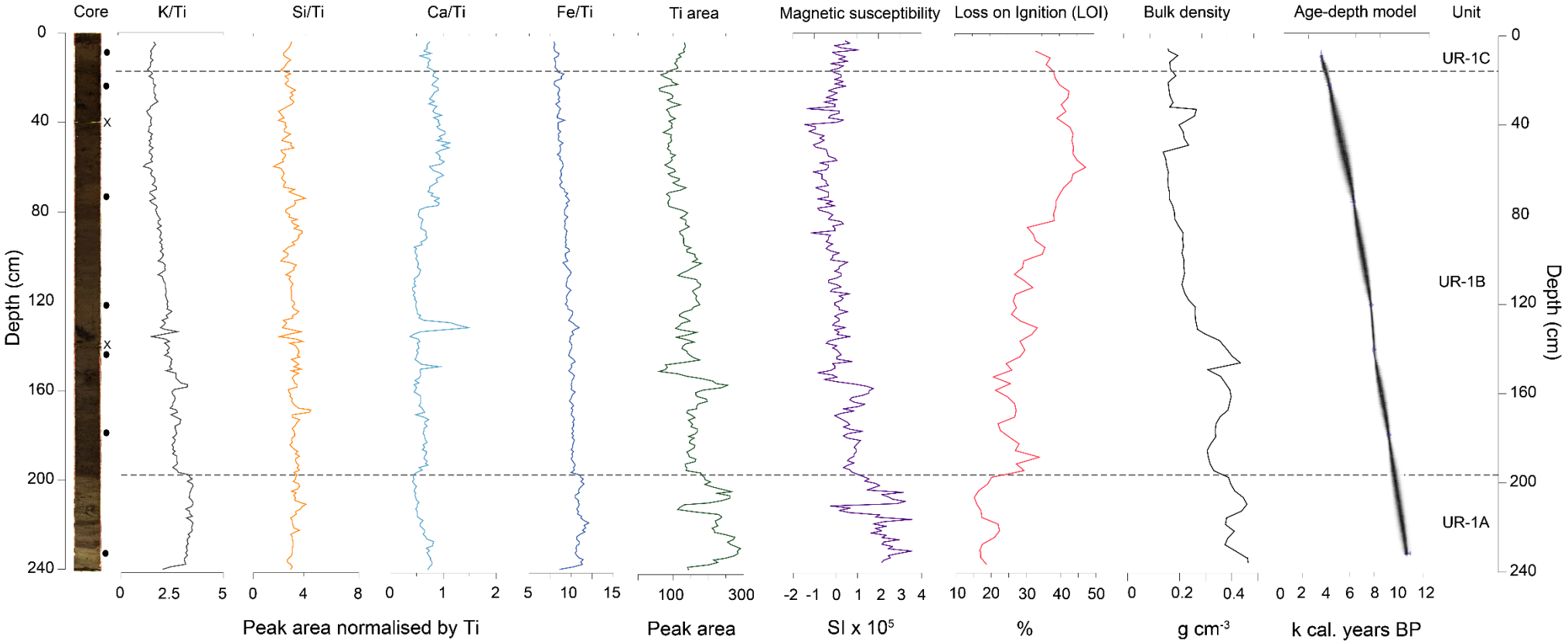

Figure 3.3 Sediment properties and element profiles for Uhca Rohči Lake, Varanger Peninsula. Lithostratigraphical units (UR-1A to C) described in the text are indicated. Black circles indicate the depth of ^{14}C samples analysed and black crosses indicate the position of core breaks after splitting the continuous Nesje core (UR-1) sequence. Selected elements measured by XRF are given as a ratio to Ti (see Material and methods). Loss-on-ignition (LOI) is given as a percentage of dry sediment weight.

3.4.2 DNA Analysis

In total, we obtained around 72 000 000 raw reads for the two UR libraries (Appendix A Table A.1). Following post-identification filtering, 118 taxa remained, of which 41 % were identified to species level, 47 % to the genus and 12 % to the family (Appendix A Figures A.1–A.3). Of the taxa detected in the *sed*aDNA, 44 % are found growing in the Komagdalen valley today and a large proportion (86 %) within Finnmark and the Kola Peninsula today (Appendix A Table A.2). Salicaceae and *Pinus* were present in nearly all samples but display variation in the number of PCR replicates (out of eight) in which they were detected; this was unrelated to sample depth/age. The steep drop in *Pinus sed*aDNA in the uppermost three samples (*ca.* 3,600 – 3,300 cal. years BP) appears to be an artefact of the rapid increase in *Salix sed*aDNA during this time.

Although not present in the study region today, stands of *Pinus* forest occur ~50 km south of the Komagdalen valley (Elven, 2005) and scattered trees are observed at the nearby site Østervatnet on the southern Varanger Peninsula (Prentice, 1981). In addition, the *sed*aDNA results indicate that Holocene vegetation was dominated by woody taxa such as *Betula*, *Empetrum*, *Vaccinium* spp. and the *Rhododendron tomentosum* complex, the latter of which is also not found in the catchment today. The most common terrestrial forb was *Bistorta vivipara* followed by *Cakile* and Apiaceae, all common in the area today, although *Cakile* occurs mainly at the coast. The most dominant aquatic taxon, *Limosella aquatica*, is restricted to four inner fjord sites in Finnmark today (Alta, Lakselv, Neiden and Pasvik) whereas the second most common aquatic taxon, *Callitriche hermaphroditica*, has a slightly wider distribution in the inner fjord zone and along the main valleys. However, it does not occur within Varangerhalvøya National Park today (Appendix A Table A.2).

Trees and tall shrubs (e.g. *Pinus*, *Betula*, *Empetrum*, Salicaceae) dominate the *sed*aDNA record, accounting for 50 % of total DNA reads on average (Figure 3.4), followed by total terrestrial forbs (23 %) and graminoids (12 %; Figure 3.5). The percentage dominance of functional groups remains relatively constant across samples, except for a distinct peak in the *sed*aDNA of aquatics between 10,200 – 9,600 cal. years BP. This short period is described by the appearance of *Callitriche hermaphroditica*, *C. palustris*, and *Potamogeton* (Figure 3.5). *Callitriche hermaphroditica* is a northern species (<53 °N) typically found in shallow lakes and slow-moving rivers; it is on the IUCN Red List (http://www.iucnredlist.org/details/167828/0), and based on current distribution in Finland, it is inferred to indicate a minimum July temperature of 13 – 14 °C (Väliranta *et al*., 2015). *Callitriche palustris* and *Potamogeton* (not identified to species level) are more common and found in a wide range of aquatic habitats.

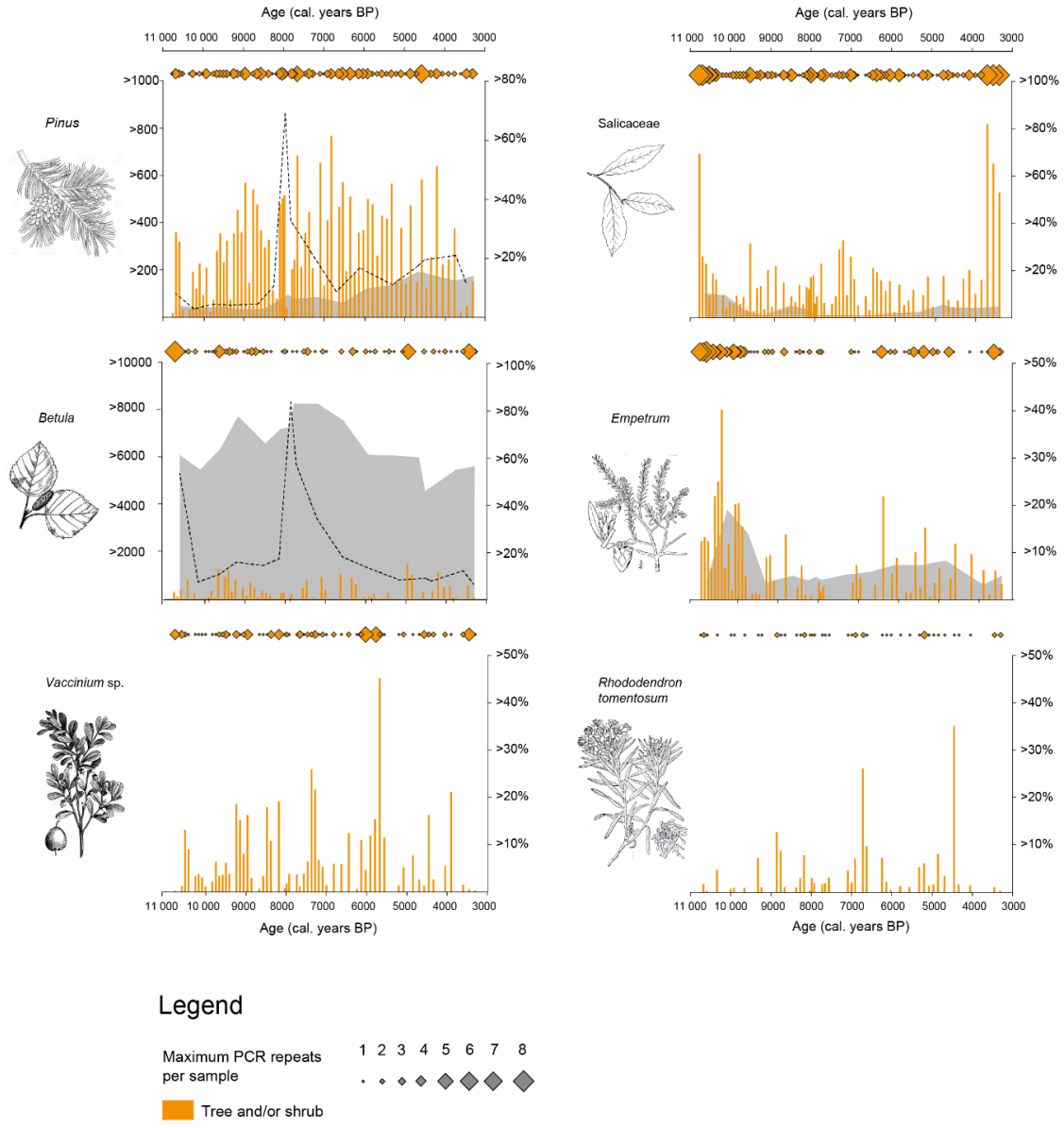

Figure 3.4 Selected woody taxa as a percentage of total DNA reads per sample (histogram; right-hand y-axis) and maximum number of PCR replicates (diamond symbols) for the Uhca Rohči Lake record. Grey shaded area depicts pollen percentages based on sum of total terrestrial pollen (∑P; right-hand y-axis) with pollen influx for *Pinus* and *Betula* indicated by a dashed line (left-hand y-axis). Note that the height of the y-axis varies amongst panels.

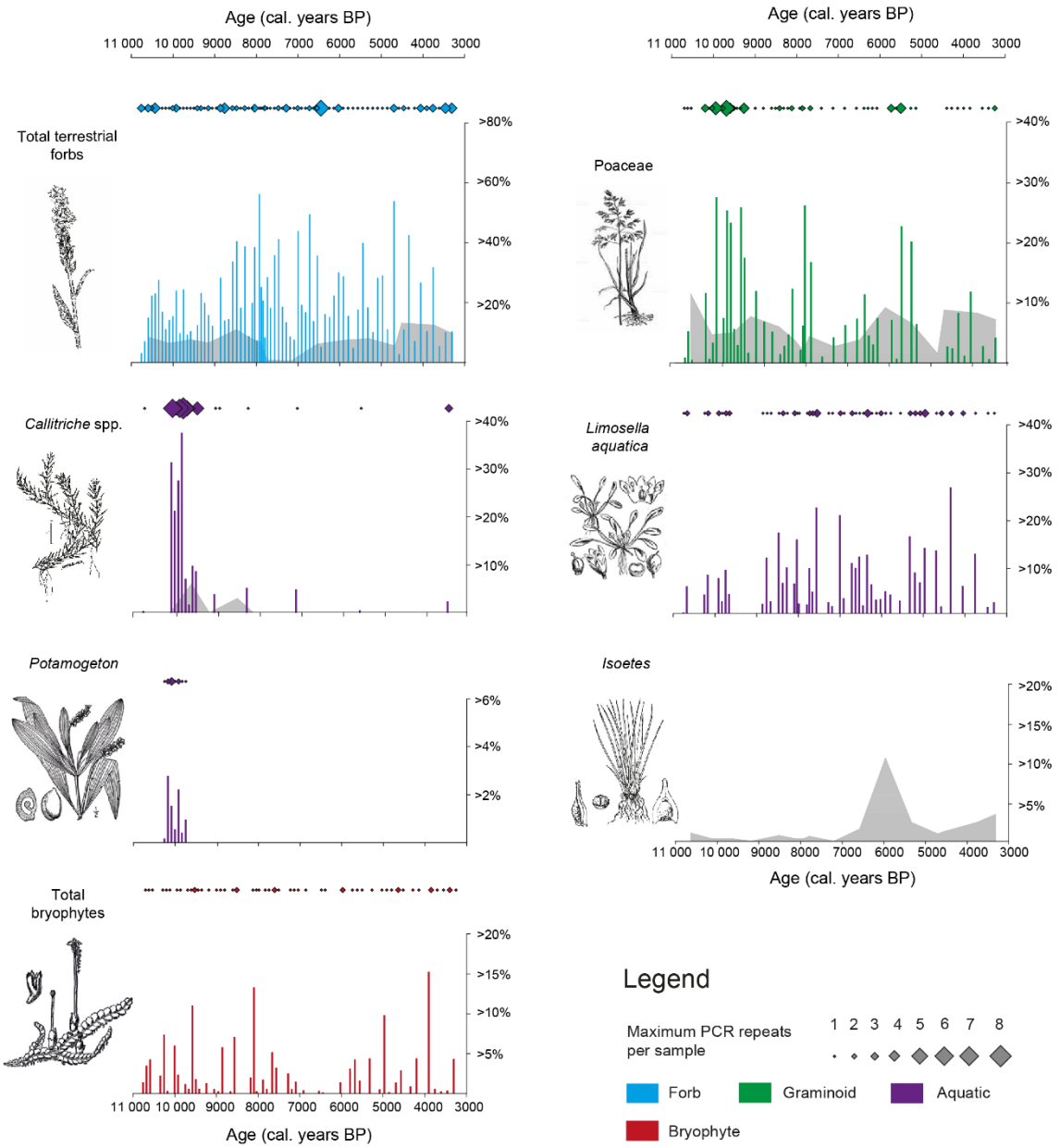

Figure 3.5 Selected herbaceous taxa as a percentage of total DNA reads per sample (histogram) and maximum number of PCR replicates (diamond symbols) for the Uhca Rohči Lake record. Grey shaded area depicts pollen percentages based on sum of total terrestrial pollen (∑P). Proportion of aquatics and spores are calculated based on the sum of total terrestrial pollen plus aquatics (∑P + ∑aquatics) or spores (∑P + ∑spores). Pollen percentages for *Callitriche* spp. are presented with a 10× exaggeration. Note that the height of the right-hand *y*-axis varies amongst panels.

3.4.3 Pollen Analysis

Pollen analysis detected 39 taxa across the 16 samples analysed from UR Lake, with *Betula* dominating the pollen percentages and accounting for 65 % of total land pollen (TLP) and influx of 2263 grains cm^{-2} $year^{-}1$, on average (Figure 3.6). Of the 39 taxa, 72 % could be identified to genus

level, 21 % to family level and 7 % to species level. Four taxa (Ericales, *Myrica gale*, *Picea* and *Ulmus*) are assumed to be long-distance dispersed based on their current native ranges (Appendix A Table A.2). Three distinct zones are identified based on the pollen (Figure 3.6), numbered from the base as follows:

Ur-Ia (234–190 cm; ca. 10,700 – 9,500 cal. years BP) – Betula-Empetrum-Salix zone. This basal pollen assemblage is characterized by high and rising percentages of *Betula* (up to 70 %) and *Empetrum* (up to 20 %) with high *Salix* (5 – 15%). Poaceae percentages are up to 17 % at the beginning of the zone, decreasing to around 5 % near the end of the zone boundary. *Pinus* values remain low at around 5 % and influx of 70 grains cm^{-2} $year^{-1}$. *Filipendula* and Cyperaceae are present at percentages of 5 and 3 %, respectively.

Ur-Ib (190–155 cm; ca. 9,500 – 8,400 cal. years BP) – Betula-Salix-Empetrum zone. This is a short-lived subzone characterized by the rapid decline in values for *Empetrum* (up to 7 %) from the previous (Ur-Ia) subzone. *Pinus* values remain consistently low at around 5 % and influx of 50 grains cm^{-2} $year^{-1}$. The Ur-I/Ur/II boundary is defined by a decrease in *Empetrum* and *Salix* to low values.

Ur-II (155–80 cm; ca. 8,400 – 6,400 cal. years BP) – Betula-Pinus-Empetrum zone. Rising *Pinus* values from 5 to 10 % (110–860 grains cm^{-2} $year^{-1}$) accompany an increase in *Betula* values up to a peak of 80 % (˜1600–8300 grains cm^{-2} $year^{-1}$) in this zone. *Salix* values are low or zero whilst *Empetrum* values gradually increase.

Ur-III (80–0 cm; ca. 6,400–3,300 cal. years BP) – Betula-Pinus-Empetrum-Poaceae zone. The Ur-II/Ur-III boundary is defined by rising values of Poaceae and *Pinus* and a decline in *Betula*. *Pinus* reaches maximum values in this zone, increasing from around 10 % at the start of the zone up to a peak of 18 % (200 grains cm^{-2} $year^{-1}$). *Empetrum* continues to rise through the transition between Ur-II and Ur-III zones coincident with increasing percentages of herbaceous taxa such as Chenopodiaceae and *Rumex*. Poaceae and *Salix* rise slightly. *Isoetes* reaches a maximum value of 10 % before declining towards the end of the zone.

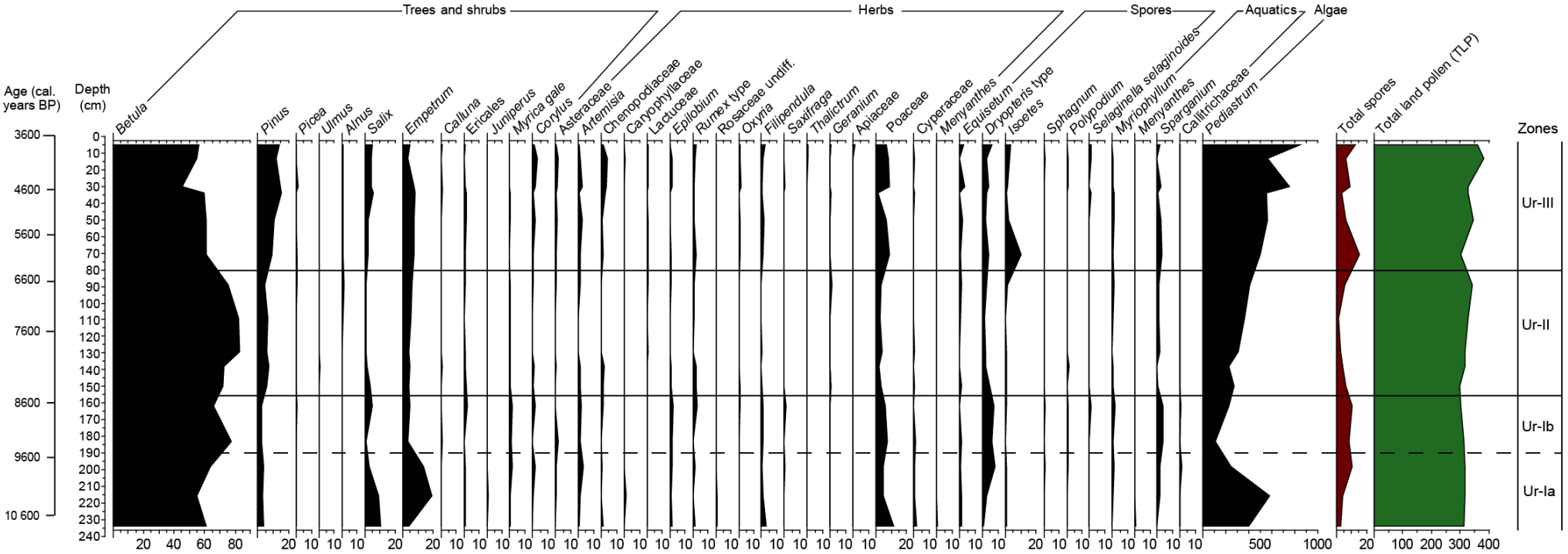

Figure 3.6 Percentage pollen diagram for Uhca Rohči Lake with local pollen assemblage zones (LPAZs) Ur-I to Ur-III indicated. Pollen percentages are based on the sum of total terrestrial pollen (∑P). Proportion of aquatics and spores are calculated based on the sum of total terrestrial pollen plus aquatics (∑P + ∑aquatics) or spores (∑P + ∑spores).

3.4.4 Comparison Between Pollen and *sed*aDNA

The combined approach of *sed*aDNA and pollen analysis resulted in 137 taxa of 64 families identified to varying taxonomic levels (Appendix A Table A.2). In total, 20 families were shared between pollen and *sed*aDNA, with poor taxonomic resolution seen for families such as Poaceae, Cyperaceae and Caryophyllaceae based on pollen whilst identification to genus or even species level was possible with *sed*aDNA. Of the 39 taxa detected by pollen analysis, 12 were also identified in the *sed*aDNA to the same taxonomic level. Selected taxa found as pollen are presented in Figures 3.4 and 3.5 as a percentage of total terrestrial pollen and compared with the results of *sed*aDNA analysis. No algal taxa were detected in the *sed*aDNA record based on the vascular plant *trn*L P6 loop marker, whilst a high abundance of *Pediastrum* was identified throughout the pollen record at UR Lake (Appendix A Table A.2). Both the pollen and *sed*aDNA record are dominated by trees and shrubs, but they differ in terms of the percentage dominance of key taxa. For example, *Betula* accounts for 65 % of total terrestrial pollen on average, followed by *Pinus* (8 %) and *Empetrum* (7 %) whilst *Pinus* and Salicaceae are found to be dominant in the *sed*aDNA, accounting for 21 and 20 % of total DNA reads, respectively. The greater dominance of *Empetrum* in the early-Holocene (*ca.* 10,700 – 9,500 cal. years BP) revealed by *sed*aDNA is mirrored in the pollen record (Figure 3.4). Moreover, the absence of *sed*aDNA belonging to the family Poaceae between *ca.* 5,500 and 4,500 cal. years BP is simultaneous with a rapid and short-lived decline in Poaceae pollen (Figure 3.5). Furthermore, two pollen grains of *Callitriche* sp. were found within samples at 198 and 162 cm depth, coincident with the interval of high *sed*aDNA values for *Callitriche hermaphroditica* and *C. palustris* (Figure 3.5).

3.4.5 Floristic Richness and Diversity Results

No long-term trends in floristic richness were observed over the time period investigated (Figure 3.7) although species richness reconstructed from individual samples shows high-frequency variation. *Sed*aDNA detected an anomalously high number of taxa (48 taxa pre-rarefaction) within a single sample at *ca.* 6,020 cal. years BP (68 cm depth; 39 taxa remaining following rarefaction). Differences in the composition of this sample compared to adjacent samples largely result from the presence of many forb taxa such as *Viola biflora*, *Stellaria borealis*, *Rumex*, *Oxyria digyna*, *Geranium* and *Dryas*. Some bryophyte (e.g. *Andreaea rupestris*, *Dicranum*, *Sphagnum russowii*) and woody taxa (e.g. *Alnus*, *Kalmia procumbens*, *Vaccinium* spp.) are also present but are not found in adjacent samples. There is no clear explanation from the lithological (Figure 3.2) or geochemical (Figure 3.3) data for the anomalously high floristic richness observed in this sample.

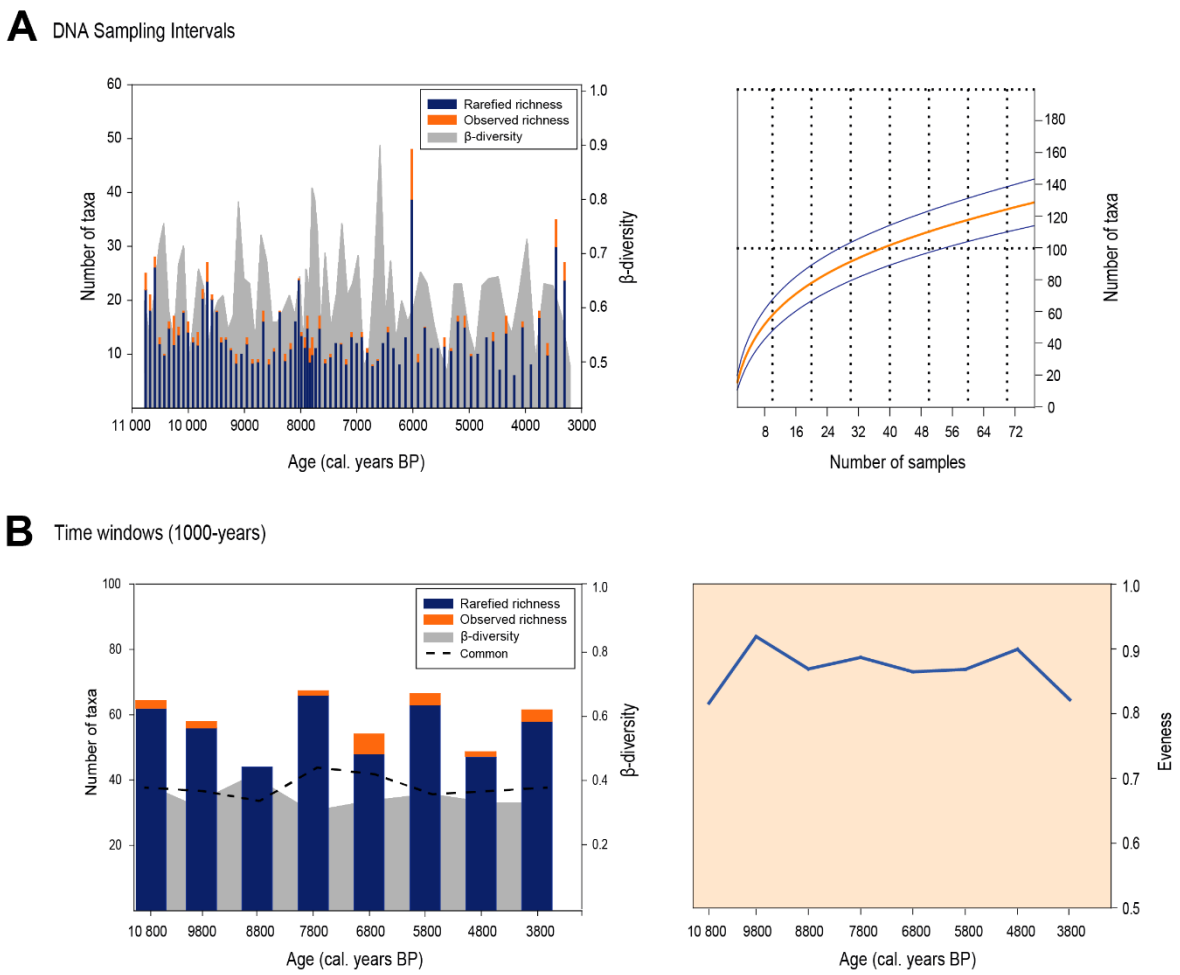

Figure 3.7 Measures of species richness, beta diversity and sample evenness for (A) DNA sampling intervals (3-cm resolution) with a sample rarefaction curve presented in the right-hand graph outlining the relationship between sample size and the number of taxa identified (orange line) with 95 % confidence intervals (blue lines) and (B) the same data amalgamated into 1000-year time windows for the Uhca Rohči Lake *sed*aDNA record. Dashed line indicates the number of taxa common between 1000-year time windows.

β-diversity calculated based on individual samples displayed variation (SD = 0.09), despite species richness remaining fairly constant amongst samples (Figure 3.7A). Whilst the number of taxa detected by *sed*aDNA remains similar amongst samples, the taxonomic composition differed between adjacent samples. Typically, the woody taxa remain a common component of adjacent samples but the herb (forb and graminoid) taxa show sporadic occurrences throughout the record. Merging samples into 1000-year time windows largely removes the effect of these sporadic occurrences, with β-diversity displaying little variation between time windows (Figure 3.7B). The number of taxa identified as common between adjacent 1000-year time windows remained consistently high throughout the record, accounting for, on average, 70 % of all taxa detected. Six

taxa belonging to trees and shrubs (Salicaceae, *Pinus*, *Empetrum*, *Betula*, *Rhododendron tomentosum*, *Vaccinium uligonosum*) seven to forbs (Anthemideae, Asteraceae, Apiaceae, *Bistorta vivipara*, *Comarum palustre*, *Dryas*, *Limosella aquatica*) and three to graminoids (Agrostidinae, *Festuca*, Poaceae) were consistently detected across all time windows. The small variation observed in β-diversity (SD = 0.04) and sample evenness (SD = 0.06) across 1000-year time windows therefore results from the typically sporadic occurrences of the remaining 30 % of taxa detected (Figure 3.7B).

3.4.6 Floristic Diversity and Treeline Changes

Rarefied species richness based on pollen (palynological richness) from UR Lake varies between 10 and 24 taxa. Compilation of palynological richness patterns from Lake Rautuselkä and Hopseidet (Seppä, 1998) with estimates obtained at UR Lake indicates long-term trends in richness reconstructed from pollen data (Figure 3.8).

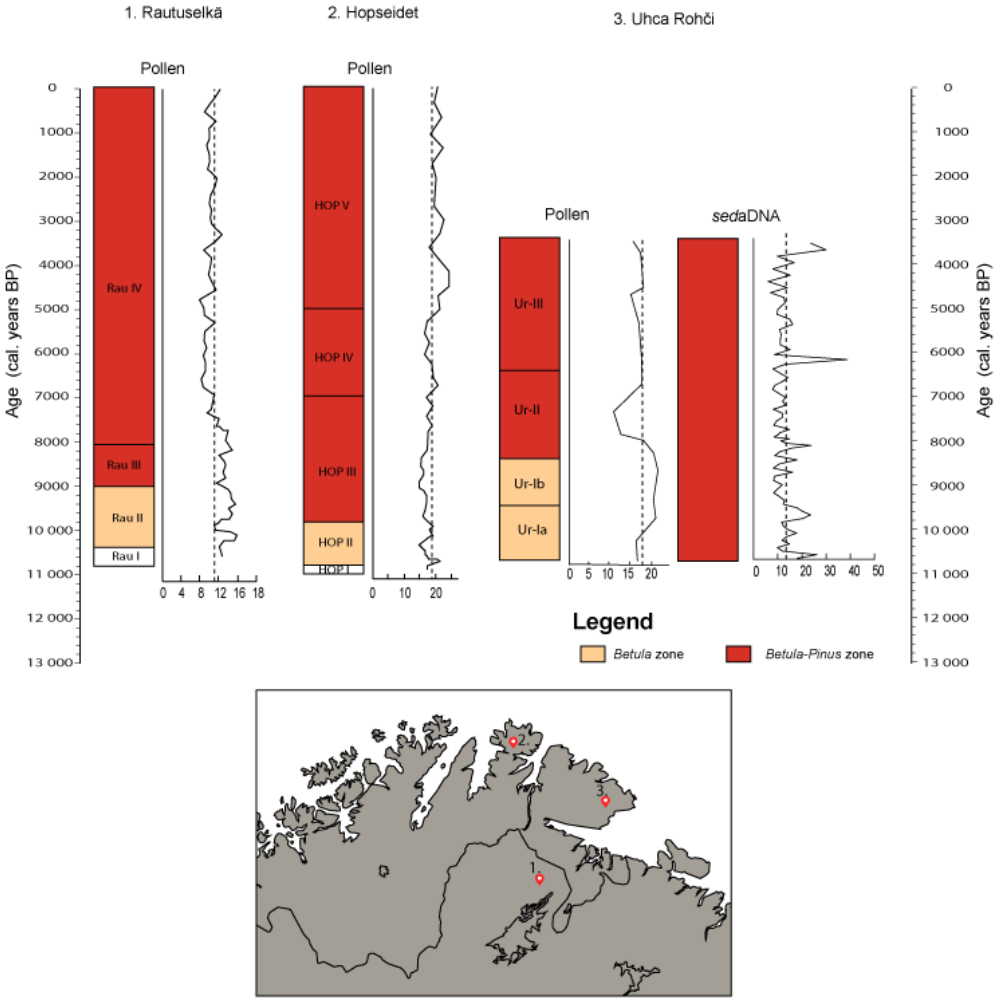

Figure 3.8 Compilation of palynological richness for two published records (Rautuselkä and Hopseidet; Seppä, 1998) and the Uhca Rohči pollen record (this paper). Floristic richness derived from *sed*aDNA record from Uhca Rohči Lake also indicated (this paper). Dashed line indicates mean species richness. *Betula* and *Betula-Pinus* pollen

zones are indicated. Map (inset) details location of sites with 1 = Lake Rautuselkä; 2 = Lake Hopseidet and 3 = Uhca Rohči Lake.

A sharp decline in species richness from 24 to 10 taxa is observed in UR Lake between *ca.* 8,000 – 7,300 cal. years BP, coincident with maximum values for *Pinus* pollen influx (~110 to 860 grains cm^{-2} $year^{-1}$), which occur in zone UR-II (Figures 3.4 and 3.6). No significant relationship (r^2 = 0.08, p > 0.01) between *Pinus* pollen influx and palynological richness was identified at UR Lake, however. Floristic richness reconstructed from *sed*aDNA from UR Lake displays no long-term trends (Figure 3.8), indicating richness remains relatively stable with the exception of the anomalously high richness observed at 68 cm depth (*ca.* 6,000 cal. years BP).

3.5 Discussion

3.5.1 Holocene Development of Uhca Rohči Lake and the Surrounding Landscape

The lithostratigraphical record (Figures 3.2 and 3.3) indicates a continuous input of fine sediment into UR Lake throughout the Holocene. Our results suggest an increase in lake production at *ca.* 9,900 cal. years BP, with a gradual transition from minerogenic sediments, composed of silty-clay laminae with low LOI and high Ti and magnetic susceptibility, to silty-clay gyttja with gradually increasing values for LOI (Figure 3.3). Following this transition, lithostratigraphical properties remain relatively stable for the remainder of the record with only small fluctuations observed in LOI, magnetic susceptibility, and geochemical elements in the uppermost zone. The position of the lake on the early- to middle-Holocene floodplain (Figure 3.1) and laminated nature of the minerogenic sediments in lithostratigraphical unit A and to a lesser extent, unit B (Figure 3.3), probably reflect periodic flood events of the Komagelva River prior to river incision forced by continued isostatic uplift (Fletcher *et al*., 1993; Fjellanger and Sørbel, 2007). Very local slope wash may also have played a role at this time. Furthermore, the high sand content of these laminae suggests the influence of a relatively high-energy system in these early lithostratigraphical units.

Our results suggest UR Lake was isolated from riverine influence at *ca.* 3,800 cal. years BP, at the transition from silty-clay with thin laminae (unit B) to silty-clay gyttja with detritus (unit C), when the Komagelva River subsequently downcut to an elevation below the lake. Thus, the source of the *sed*aDNA has probably changed over time, with the Komagelva River delivering some of the DNA to the lake from a larger source area of the upstream catchment during periodic flood events prior to downcutting in the later Holocene. Nevertheless, no distinct change in the taxonomic composition of *sed*aDNA samples is observed between units A, B and C, nor was any clear pattern

observed between the presence of banding and/or laminae and the taxonomic composition of samples.

The prominence of aquatic taxa such as *Callitriche hermaphroditica*, *C. palustris* and *Potamogeton* in the *sed*aDNA between *ca.* 10,200 – 9,600 cal. years BP (Figure 3.5) may reflect particularly good growing conditions in the lake, for example clear water conditions, warmer temperatures and/or more nutrients. In addition, aquatic plants are efficiently dispersed by birds and are therefore likely to show a rapid geographical response to climate change (Birks, 2000; Väliranta *et al*., 2015). *Callitriche hermaphroditica*, which is not found in Varangerhalvøya National Park today, occurs in more continental sites in Finnmark and appears to require a minimum July temperature of 13 – 14 °C (Väliranta *et al*., 2015). The pattern of occurrence of the aquatic taxa suggests a response to warmer-than-present early-Holocene summers.

A handful of other taxa (*Rhododendron tomentosum*, *Limosella aquatica*) that are dominant in the *sed*aDNA signal (Figures 3.4 and 3.5) do not occur in the region today, with current native ranges more than 50 km south or southeast of the Varanger Peninsula (see above). Thus, the continued presence and dominance of these taxa in the *sed*aDNA signal suggest a warmer climate between, at least, *ca.* 10,000 – 4,000 cal. years BP. This is in accordance with the general interpretation from pollen-based temperature reconstructions in northern Fennoscandia, which indicate July temperatures of +1.5±0.5 °C during the HTM (e.g. Ritchie *et al*., 1983; Møller and Holmeslet, 2002; Jensen and Vorren, 2008; Seppä *et al*., 2009a; Sejrup *et al*., 2016).

3.5.2 Interpreting the Major Vegetation Patterns

Little change is observed in the relative dominance of functional groups between *ca.* 10,700 and 3,300 cal. years BP, with trees and tall shrubs such as *Pinus*, Salicaceae, *Betula* and *Empetrum* accounting for a high percentage of the terrestrial pollen (mean 87 %) and total DNA reads (mean 50 %; Figure 3.4). Wind-pollinated woody taxa (e.g. *Betula*, *Pinus*) are generally over-represented in pollen studies (Prentice, 1985; Sugita, 1994). Likewise, we note that the abundance of these plant growth forms may be over-represented in our *sed*aDNA dataset due to polymerase-related biases that generally occur during metabarcoding PCR (Alsos *et al*., 2018; Nichols *et al*., 2018), although calibration against modern vegetation suggests a bias in the opposite direction (Yoccoz *et al*., 2012).

Whilst a large majority (>85 %) of the taxa detected in the *sed*aDNA are also found growing in Finnmark and the Kola Peninsula region today, the occurrence of *Pinus* in the *sed*aDNA signal from UR Lake raises questions. *Pinus* is found in nearly every sample, usually in high abundance, yet it is not a major component of the present-day flora of the Varanger Peninsula. The nearest forest

stands occur around 50 km south of Varangerfjorden, although some scattered trees are present in the southern Varanger Peninsula (see above; Figure 3.1B). The watershed of the Komagelva River, a likely source of *sed*aDNA to UR Lake during the time represented by lithostratigraphical units UR-1A and 1B, is situated outside of the present-day *Pinus* limit (Figure 3.1B). The sustained high abundance of *Pinus* throughout the record, including lithostratigraphical unit UR-1C when the lake is presumed to have been isolated from riverine influence, suggests a source of *Pinus sed*aDNA to UR Lake other than the Komagelva River.

It is possible that *Pinus sed*aDNA in UR Lake originates from pollen and thus may indicate long-distance dispersal rather than local growth. Unlike angiosperms, pollen grains derived from gymnosperms contain some chloroplast DNA (cpDNA) within their reproductive cells (Suyama *et al*., 1996; Parducci *et al*., 2005) that, theoretically, could be introduced into the sediment matrix, either naturally or during DNA extraction. However, current thinking from *sed*aDNA studies suggests that DNA extracted from sediments does not derive from pollen grains (Jørgensen *et al*., 2012; Pedersen *et al*., 2016; Sjögren *et al*. 2017; Wang *et al*., 2017), but instead from other components embedded in the sediment matrix (Parducci *et al*., 2017). This is probably due to the generally lower biomass of pollen compared to stems, roots and leaves, and to the resilience of their sporopollenin coats, which requires a separate lysis step in the extraction of DNA (Kraaijeveld *et al*., 2015). The extraction of cpDNA from fossil pollen grains has proven difficult (Parducci *et al*., 2005; Bennett and Parducci, 2006), which suggests that consistent detection of pollen-derived cpDNA from the sediment matrix itself is unlikely. Another possibility is contamination. *Pinus* passed the filtering stage, but there was a high number of *Pinus* reads in the negative controls (Appendix A Table A.2). Therefore, our *Pinus* record may support the inference of local presence, but there is enough doubt that other proxy data are required to establish whether pine was locally present during the Holocene.

Pinus was found in all of the pollen samples analysed from UR Lake (Figure 3.6; mean 8 % TLP; mean concentration 6100 grains g^{-1}). Maximum *Pinus* pollen influx rates (~200 – 870 grains cm^{-2} $year^{-1}$) are observed between *ca.* 8,000 – 7,300 cal. years BP (Appendix A Table A.4). These are comparable to influx values reported from nearby Østervatnet (400 – 650 grains cm^{-2} $year^{-1}$) *ca.* 8,000 cal. years BP (Appendix A Table A.3) and Mortensnes (2000 grains cm^{-2} $year^{-1}$), which is only 46 km to the SW (Høeg 2000). A threshold value of 500 grains cm^{-2} $year^{-1}$ given by Hyvärinen (1985) and Hicks (1994) for indicating pine presence means that from our pollen data, *Pinus* may have been present at the site *ca.* 8,000 – 7,300 cal. years BP (Appendix A Table A.3). This accords with the northward range expansion across northern Fennoscandia beginning *ca.* 8,500 cal. years BP (Hyvärinen, 1975; Seppä, 1996; Huntley *et al*., 2013) that probably reflected early- and middle-Holocene summer warmth, but

demonstrates a probable lag in response, compared to aquatic taxa. Maximum *Betula* influx rates (~1300 – 8300 grains cm^{-2} $year^{-1}$; Appendix A Table A.4) are also observed during this interval *ca*. 8,000 – 7,300 cal. years BP (Figure 3.4), suggesting a mixed birch-pine forest.

3.5.3 Continuity and Stability in the Holocene Flora

The record obtained from UR Lake reveals stability in the Holocene flora, with over 85 % of the total taxa detected by *sed*aDNA still growing within the catchment and/or in the broader region today (Appendix A Table A.2). Our *sed*aDNA results indicate that the erect shrub-tundra and the riparian grassland communities became established early: dominant taxa of the shrub communities (e.g. Salicaceae, *Betula*, *Empetrum*, *Vaccinium uligonosum*) and riparian meadows (*Bistorta vivipara*, *Caltha*, *Viola biflora*, *Rumex*, *Stellaria longifolia* and *S. borealis*) had appeared by *ca.* 10,700 cal. years BP (Figures 3.4 and 3.5), in accordance with pollen records from nearby Østervatnet and Bergebyvatnet showing a *Salix*-Poaceae assembly from *ca.* 12,500 cal. years BP (Prentice, 1981, 1982). Presence of these taxa continued to *ca.* 3300 cal. years BP and they are common in the catchment today.

Overall, *sed*aDNA permitted identifications at a higher taxonomic resolution than was possible with pollen; the main exception is Salicaceae, which, based on the *trn*L marker, can only be identified to family level due to hybridization and similarity in cpDNA sequences amongst individuals. The *sed*aDNA signal detected taxa from a range of different ecological habitats including shrub-tundra, riparian meadows and/or open grassland rich with herbs and bryophytes. It also reveals the dynamics of aquatic taxa. In contrast, the pollen record from UR Lake is largely confined to northern boreal and low arctic taxa (e.g. *Betula*, *Pinus*, *Salix*, *Empetrum*), with a minor signal deriving from herbaceous (e.g. Asteraceae, Chenopodiaceae, *Rumex*, Poaceae) and aquatic taxa (e.g. *Sparganium*, Callitrichaceae). In contrast to the *sed*aDNA record, entomophilous forbs typical of low tundra settings, such as *Bistorta vivipara*, are largely absent or in low percentages in the pollen record from UR Lake (Figure 3.6, Appendix A Table A.2). The limited number of pollen samples and dominance of taxa such as *Betula* and *Pinus* probably restrict or mask the presence of low pollen-producing entomophilous taxa in the pollen record from UR Lake.

Direct comparison of the number of taxa contributed by each proxy to overall species richness is not appropriate due to disparity in sampling effort. Although previous comparisons between pollen and *sed*aDNA have shown only partial overlap of taxa (Pedersen *et al*., 2013; Parducci *et al*., 2015), our data demonstrate high similarity in the records for taxa such as *Empetrum*, *Callitriche* and Poaceae, as well as a dominance of *Betula* and *Pinus* (Figures 3.4 and 3.5). Thus, in contrast to lakes that only receive inflow from catchments/small streams

(Alsos *et al*., 2018), the DNA signal in UR Lake, which may have been affected by sediment inputs from the large, upstream catchment, more closely resembles the regional vegetation signal typical of many pollen records (Jacobson and Bradshaw, 1981; Rousseau *et al*., 2006).

3.5.4 Postglacial Patterns in Floristic Richness and Diversity

Floristic richness reconstructed from *sed*aDNA displays only minor variations over the Holocene interval investigated, particularly when the effect of sporadic occurrences of herb taxa is minimized by merging samples into 1000-year time windows (Figure 3.7). High floristic richness (61 taxa) characterizes the earliest time window between 10,700 – 9,800 cal. years BP, and richness remains consistently high throughout the record; small fluctuations may be due to flood-related inputs from the extensive, up-river catchment. No long-term trends in floristic richness are evident, based on either individual samples or 1000-year time windows (Figure 3.7). High sample evenness across time windows indicates the persistence of dominant taxa over the Holocene, with a large percentage (>70 %) of taxa detected as common amongst 1000-year time windows (Figure 3.7B). Our findings derived from *sed*aDNA support the conclusions of Normand *et al*. (2011) and Giesecke *et al*. (2012), which are derived from pollen, that the current distribution of plants in previously glaciated regions established quickly after the onset of the Holocene. For the Holocene at least, there is no evidence for increased floristic richness over time that might be due to the delayed immigration of species, but it should be noted that as the area was deglaciated prior to the end of the Younger Dryas (see above), the earliest part of the record is missing.

Palynological richness from UR Lake and two published records from Lake Rautuselkä and Hopseidet (Seppä, 1998) show more variation than floristic richness reconstructed from *sed*aDNA (Figure 3.8). Palynological richness displays long-term trends in response to variation in the pollen abundance of anemophilous woody taxa (e.g. *Pinus* and *Betula*). Dominance of taxa producing high amounts of pollen (e.g. *Pinus*, *Picea* and *Betula*) occurs at the expense of entomophilous forbs and leads to a likely underestimation of floristic richness. On the other hand, where low palynological richness coincides with high *Pinus* pollen influx, this may reflect an ecological effect whereby dense forest reduces niche availability for herbaceous taxa (Seppä, 1998). Our *sed*aDNA data do not show such patterns, whereas the UR pollen data do, albeit at a coarse sampling resolution. The limitations on the effectiveness of pollen spectra for estimating species richness (Meltsov *et al*., 2011; Goring *et al*., 2013) mean that *sed*aDNA can provide improved estimates; less sensitive to 'swamping' by dominant taxa, as total numbers are not limited by counting time, and all valid reads contribute to the floristic list.

3.6 Conclusions

The pollen and *sed*aDNA records from this site on the Varanger Peninsula show a largely consistent pattern. Erect shrub-tundra vegetation was established early in the Holocene. This was soon followed by the establishment of *Betula* forest probably mixed with *Pinus* as indicated by high pollen influx rates of both taxa. This, together with the persistence of *Limosella aquatica* and *Rhododendron tomentosum* through the *sed*aDNA record, indicates a climate warmer than present throughout most of the early- and middle-Holocene.

As species richness reconstructed from pollen data is limited by differential pollen productivity, dispersal and often low taxonomic resolution, alignment with contemporary data often proves difficult if not impossible. Reconstructions of past changes in floristic diversity and richness can be improved by using *sed*aDNA, as it better reflects local floristic composition and diversity. The *sed*aDNA data on floristic richness between *ca.* 10,700 and 3,300 cal. years BP show that high diversity, richness and sample evenness prevailed across the record, despite known climatic variations in the Holocene. Nevertheless, when considering past and future responses to climate change, important questions remain as to how the crossing of critical climatic thresholds may interact with sub-regional heterogeneity and drivers of vegetation composition and change, including herbivory. Here, as this paper shows, s*ed*aDNA studies can make an important contribution.

3.7 Acknowledgements

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3.8 Author Contributions

K.A. Bråthen surveyed potential study sites and C. L. Clarke, A. G. Brown, I. G. Alsos and F. J. Ancin-Murguzur cored Uhca Rohči Lake. C. L. Clarke extracted the DNA and L. Gielly performed the amplifications and ran ObiTools. C. L. Clarke analysed the DNA data with contributions from Y.

Lammers, P. D. Heintzman and I. G. Alsos. A. G. Brown described the sediment properties, provided the geomorphological background and compiled data on *Pinus* pollen influx rates. Radiocarbon dating was performed by T. Goslar. C. L. Clarke constructed the age-depth models and performed XRF, LOI, magnetic susceptibility and pollen analysis. M. E. Edwards contributed to pollen analysis. I. G. Alsos, K. A. Bråthen and M. E. Edwards provided ecological background. C. L. Clarke drafted the first version of the manuscript of which all co-authors commented on.

Chapter 4 Persistence of Arctic-Alpine Flora During 24,000 Years of Environmental Change in the Polar Urals (Paper II)

This paper has been submitted to Scientific Reports and is currently under peer-review:

Clarke, C.L., Edwards, M.E., Gielly, L., Ehrich, D., Hughes, P.D.M., Morozova, L.M., Haflidason, H., Mangerud, J., Svendsen, J-I. and Alsos, I.G. 2019: Persistence of arctic-alpine flora during 24,000 years of environmental change in the Polar Urals. Manuscript in review at *Scientific Reports.*

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Clarke, C.L., Edwards, M.E., Hughes, P.D.M., Haflidason, H., Mangerud, J. Svendsen, J-I., and Alsos, I.G, 2017: Polar Ural Mountains: A surprisingly rich flora for the past 24,000 years. *PAGES Open Science Meeting (OSM)*. Zaragoza, 12th May. Oral Presentation.

4.1 Abstract

Plants adapted to extreme conditions can be at high risk from climate change; arctic-alpine plants, in particular, could “run out of space” as they are out-competed by expansion of woody vegetation. Mountain regions could potentially provide safe sites for arctic-alpine plants in a warmer climate, but empirical evidence is scattered. Here we present a 24,000-year record of species persistence based on sedimentary ancient DNA (*sed*aDNA) from Lake Bolshoye Shchuchye (Polar Urals). We provide robust evidence of long-term persistence of arctic-alpine plants through large-magnitude climate changes but document a decline in their diversity during the expansion of woody vegetation for a period in past. Nevertheless, most of the plants that were present during the last glacial interval, including all of the arctic-alpines, are still found in the region today. This underlines the conservation significance of mountain landscapes via their provision of a range of habitats that confer resilience to climate change, particularly for arctic-alpine taxa.

4.2 Introduction

Arctic-alpine plants are at greater risk of habitat loss and local extinction under future climate change than plants of lower elevations (Dirnböck *et al*., 2003; Engler *et al*., 2011; Niskanen *et al*., 2019). Yet observed extinction rates on mountain tops have been low, despite climate warming over the past century (Matteodo *et al*., 2013; Wipf *et al*., 2013). As modelling and large-scale predictions often fail to account for the importance of local-scale factors that control plant distributions (Kulonen *et al*., 2018; Niskanen *et al*., 2017), it may be that extinction probabilities have been overestimated. This uncertainty underlines the importance of identifying specific geographical locations and/or attributes of habitats that have helped to sustain communities over long periods of varying climate and documenting their long-term history. Recent advances in sedimentary ancient DNA (*sed*aDNA) analysis combined with a high-quality sediment record may provide new insights into the diversity of the arctic-alpine flora and how it has fared through large-magnitude climate changes in the past. We collected data on species persistence using *sed*aDNA analysis on a high-resolution and well-studied lake sediment core spanning the last 24,000 years from the Polar Ural Mountains of the Russian Arctic.

Areas that facilitated long-term species persistence in the past can be considered a priority for conserving biodiversity and genetic diversity under a changing climate (Reside *et al*., 2019). Spatially heterogeneous mountain landscapes should provide viable future habitat for taxa with a range of environmental requirements. In such landscapes, microclimate and soil vary with topography and elevation to create a mosaic of different conditions at the mesoscale (Scherrer and Körner, 2010), thus providing a buffer against regional climate changes (Dobrowski, 2011; Scherrer and Körner, 2011; Suggitt *et al*., 2018). At the same time, compressed vertical and horizontal gradients enable species to track their bioclimatic niches effectively as climate changes (Graae *et al.,* 2018; Opedal *et al*., 2015). Most evidence that mountain regions function as long-term refugia or safe sites is, however, indirect, being based on contemporary observation and/or modelling (Niskanen *et al*., 2017, 2019; Patsiou *et al*., 2014).

With future warming exacerbated by arctic amplification (Serreze and Barry, 2011; Walsh, 2014), much terrain in northern lowlands may be taken over by woody plant communities (Brodie *et al*., 2019; Hagedorn *et al*., 2014; Pearson *et al*., 2013), making northern mountain areas important as localities in which competition-sensitive arctic-alpines can persist. While we have some knowledge about long-term refugia for warm-adapted species during cold, glacial periods (Stewart *et al*., 2010), identifying locations for arctic-alpine species in warm, interglacial periods has received much less attention. Identifying such locations requires a challenging integration of long temporal timescales and fine spatial scales (Gavin *et al*., 2014). Fossils (i.e. pollen/plant

macrofossils) provide the best evidence for presence of a plant species at a given time in the past, but records can be constrained by several factors: levels of preservation and taxonomic resolution, specific nature of the site, etc. (Gajewski, 2015; Prentice, 1985; Sugita, 1994). Previous studies have often had to rely on extrapolation to argue for long-term species persistence, based on sporadic occurrences of a taxon in the fossil record and its presence within the present-day vegetation mosaic (Cwynar, 1982; Napier *et al*., 2019; Taberlet and Cheddadi, 2002).

Analysis of *sed*aDNA has the potential to provide more detail on past community composition, particularly with regard to arctic-alpine species, as the method is well suited to cold climates (Clarke *et al*., 2018; Willerslev *et al*., 2014; Zimmermann *et al*., 2017b). It can significantly augment information on plant community composition and species persistence derived from traditional fossil records (Alsos *et al*., 2016; Giguet-Covex *et al*., 2014; Parducci *et al*., 2017). In contrast to pollen, the *sed*aDNA signal is less sensitive to "swamping" by woody anemophilous taxa (e.g. many north-temperate and boreal trees and shrubs) at the expense of low pollen-producing and insect-pollinated arctic-alpine herbs (Jørgensen *et al*., 2012; Sjögren *et al*., 2017; Zimmermann *et al*., 2017a).

Here we present a continuous 24,000-year record of plant community composition and species persistence based on ancient DNA extracted from lake sediments (*sed*aDNA). We use the well-described sediments of Lake Bolshoye Shchuchye (Haflidason *et al*., 2019; Regnéll *et al*., 2019; Svendsen *et al*., 2019), the largest and deepest lake in the Polar Ural Mountains (Figure 4.1, Appendix B Supplementary Figures B.1 – B.2). The sediment record is unique for western Eurasia as it represents 24,000 years without any breaks and/or disturbances (Figure 4.2). The site has remained ice free for at least the last 60,000 years, thus providing insight into a well-established and diverse flora compared to adjacent regions which were deglaciated after the Last Glacial Maximum (LGM). Moreover, the lake catchment supported the establishment of forest at lower elevations for a period in the Holocene between *ca.* 9000 and 4000 cal. years BP.

Compared with the smaller lakes typically used for reconstructions of vegetation histories based on *sed*aDNA (Alsos *et al*., 2016, 2018; Epp *et al*., 2015; Pansu *et al*., 2015), Lake Bolshoye Shchuchye's sediments may capture a signal of plant communities over an elevational range of 187 - 1,100 m a.s.l. within its large (215 km^2) hydrologic catchment. The lake is surrounded by steeply sloping hillsides and is fed by the River Pyriatanyu, which drains much of the catchment; this means that the lake probably receives direct local slope-wash plus fluvially transported material from a much wider area. The lake catchment is physiographically diverse and thus holds many of the criteria suggested by modelling studies to facilitate long-term species persistence. We use metabarcoding techniques (Taberlet *et al*., 2012a) to establish the *sed*aDNA record and combine

the results with information on the present-day occurrence of identified taxa within the catchment vegetation mosaic.

Figure 4.1 Location of the study area. Digital elevation model of the Polar Urals with the location of Lake Bolshoye Shchuchye marked. Map inset shows the location (rectangle) of main map. The ice sheet limit during the Last Glacial Maximum (white line) from Svendsen *et al*. (2004) and the present-day boreal treeline (black dashed line) are also indicated.

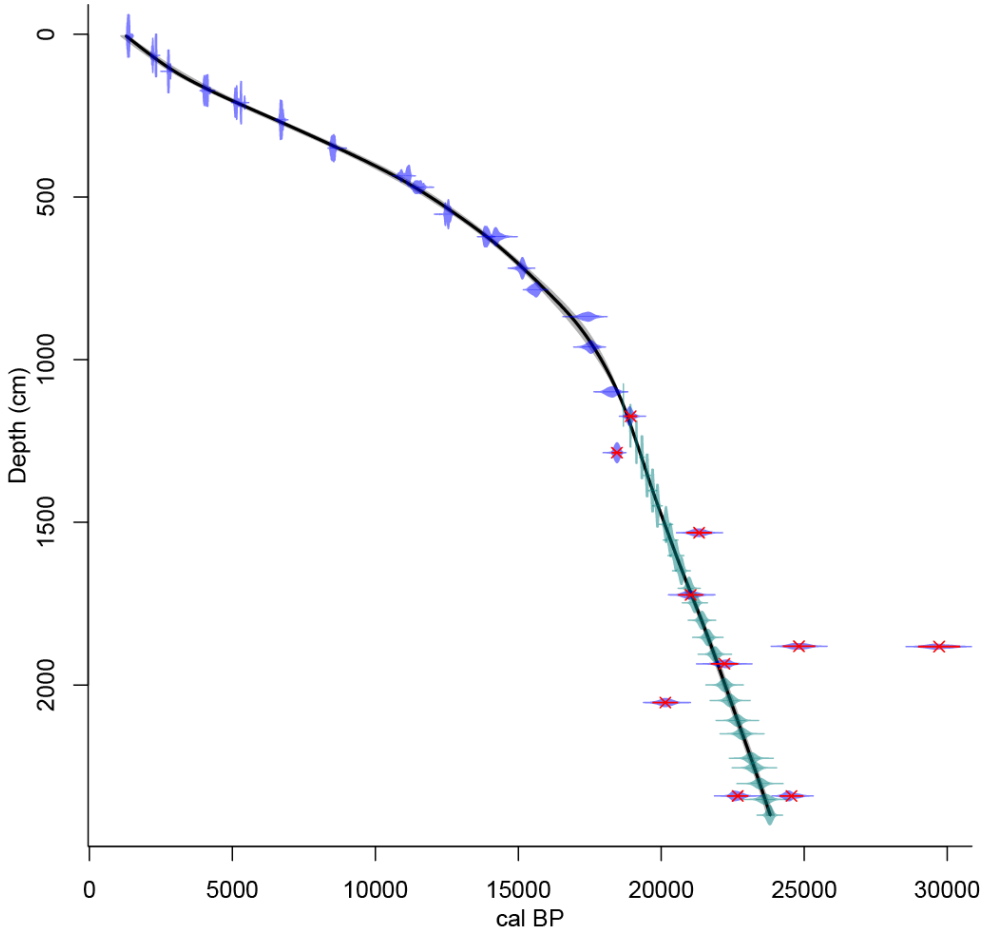

Figure 4.2 The age-depth relationship for core no. 506-48 from Lake Bolshoye Shchuchye, Polar Urals (from Svendsen *et al.*, 2019). Small grey-shaded area shows the 95 % probability interval. The green marks show chronology retrieved from counting of annual laminations (varves) from 11.4 m depth to the base of the core. The varve chronology is anchored to 18,679 ± 128 cal. years BP (95 % confidence interval) and gives a basal age of 23,813 ± 306 cal. years BP (see Regnéll *et al.*, 2019). The blue marks show the probability distribution of AMS radiocarbon ages; outliers are marked with red crosses.

Our main aim is to test the degree of persistence of Lake Bolshoye Shchuchye over the past 24,000 years, with a particular focus on the fate of competition-sensitive, arctic-alpine plants during the past expansion of woody taxa. Our results provide robust evidence that this spatially heterogeneous mountain landscape has functioned effectively as a refugium for cold-adapted plants in a warm climate. We find that the catchment has supported plant communities that have diversified through time, including those typical of an arctic-alpine community but also taxa of shrub-tundra and boreal forest. The *sed*aDNA record provides insight into long-term preservation of floristic diversity at the catchment scale in a mountain landscape and also documents the response of individual arctic-alpine plants to expanding woody vegetation. We conclude that in the

Arctic, as elsewhere, conservation priorities should go beyond protecting areas that harbour high biodiversity today and instead move towards monitoring and protecting areas that have also shown to be resilient to past changes.

4.3 Results

4.3.1 Plant *Sed*aDNA Record

We obtained around 100 million raw DNA reads for the 153 sediment samples from Lake Bolshoye Shchuchye (Appendix B Supplementary Table B.1). After filtering out sequencing artefacts and sequences with <98 % match to the DNA reference library (see Material and Methods), we retained 19 million reads, corresponding to 134 vascular plant and 28 bryophyte taxa. Of these, 40 % are identified to species level, 45 % to genus and 15 % to a higher taxonomical level (Appendix B Supplementary Table B.2). Where possible, genera which were not identified to species level were assigned to a likely inferred species (marked with an asterisk) based on their biogeographic distribution (Appendix B Supplementary Table B.2).

The identified taxa represent a wide range of different modern ecological habitats, including forest (e.g. *Larix sibirica*, *Picea* sp.) and its understory (e.g. *Dryopteris fragrans*, *Gymnocarpium dryopteris*), tall shrubs (e.g. *Alnus*, *Betula*, Saliceae), dwarf shrub-tundra (e.g. *Vaccinium uligonosum*, *V. vitis-idaea*/*myrtillus*, *Arctostaphylos uva-ursi*, *Empetrum nigrum**, *Dryas octopetala**), herb-tundra (e.g. *Puccinellia*, *Festuca*, *Draba*, *Lagotis glauca*), mesic sedge (e.g. *Carex*, *Eriophorum*) and bryophyte (e.g. *Andreaea, Aulacomnium turgidum*, Dicranaceae) communities, and (in all probability) mixed plant communities in between these categories (Appendix B Supplementary Figures B.3 – B.5).

A sustained, long-term increase in floristic diversity is observed in the *sed*aDNA record over time (Figure 4.3, Appendix B Supplementary Figures B.3 – B.5). Of the total 162 plant taxa detected using *sed*aDNA, 70 % (114 taxa) were detected within the full-glacial period (*ca.* 24,000 - 15,000 cal. years BP), 75 % (85 taxa) of which persist into the Holocene period (*ca.* 11,700 - 1,300 cal. years BP) along with the addition of 47 new plant taxa not present in the full-glacial period. The majority (87 %) of the vascular plant taxa detected within the full-glacial period and 89 % of all the vascular plant taxa detected by *sed*aDNA are still found in the vegetation today (Figure 4.3, Appendix B Supplementary Table B.2). Thus, much of the present-day flora at Lake Bolshoye Shchuchye was already in place in the full-glacial period; subsequently, there has been continuity in taxonomic composition but also a gradual addition of new plant taxa over time to form the present-day vegetation mosaic.

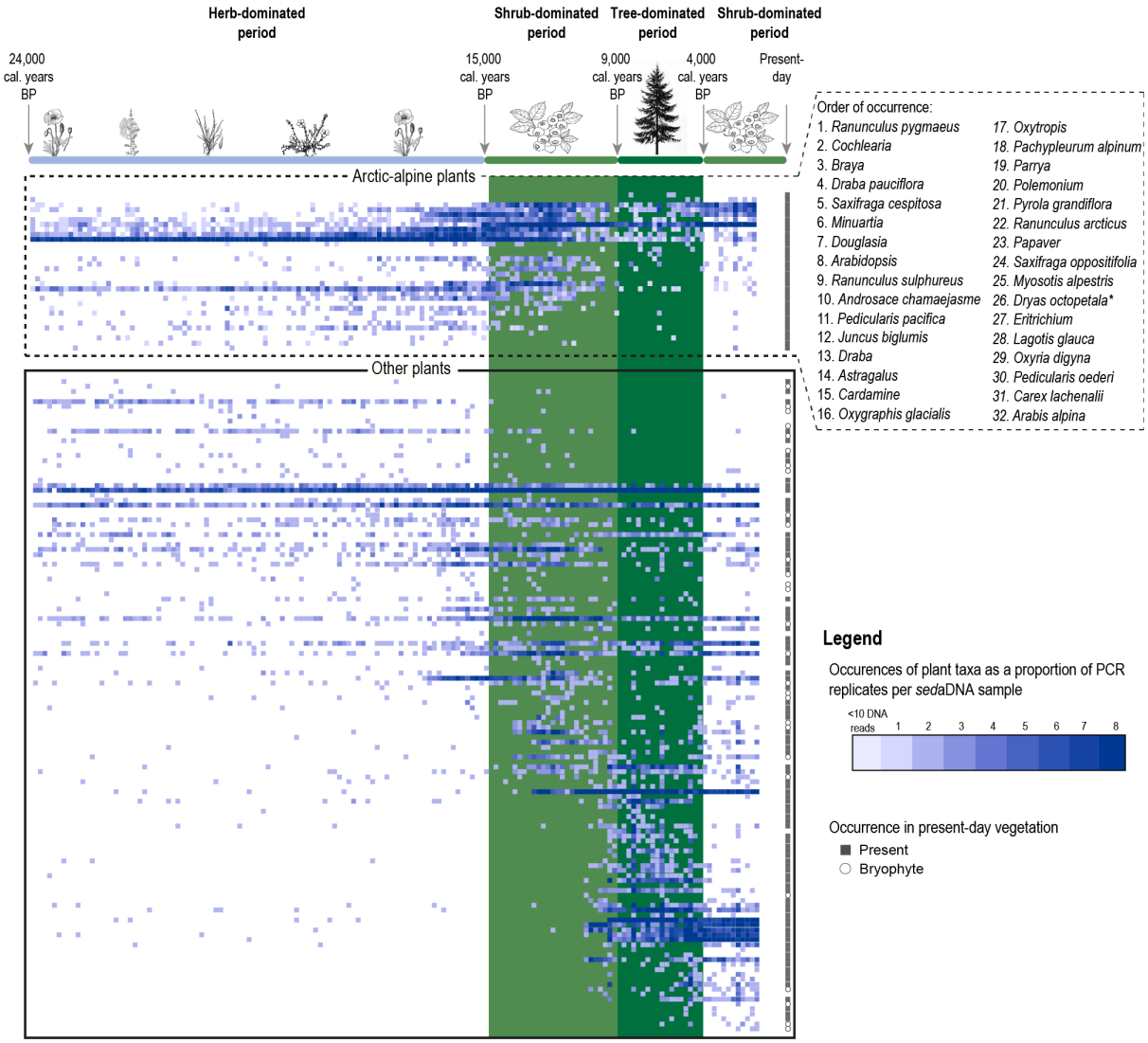

Figure 4.3 Incidence matrix of plant taxa detected in Lake Bolshoye Shchuchye's sediments using *sed*aDNA analysis. All identified plant taxa are presented as a proportion of PCR replicates (out of eight) per *sed*aDNA sample. Plant taxa classified as 'arctic-alpine' are located within the grey dashed area and taxon names are given. Occurrences of arctic-alpine plants with <10 *sed*aDNA reads in a single PCR replicate are also indicated. Plant taxa inferred to species level based on their biogeographic distribution are marked with an asterisk (*). The x-axis of the incidence matrix refers to the *sed*aDNA sample number, with a decreasing age (towards the present-day) from left to right. The y-axis presents each plant taxa detected using *sed*aDNA sorted according to their median distribution within the *sed*aDNA samples. Plant taxa which are still present within the vegetation of the Polar Urals today are marked with a grey square on the far right-hand side of the incidence matrix; bryophytes are excluded as the present-day biogeographic distribution of many of the identified taxa are poorly resolved. Green shaded boxes indicate the timing of past shrub and forest tree establishment in the vicinity of the lake.

Saliceae, a tribe of the willow (Salicaceae) family, is common throughout the record, occurring in nearly all PCR repeats of all samples. It most likely represents dwarf shrubs (e.g. *Salix polaris*, *S. reptans*, *S. nummularia*) in the early full-glacial, with the addition of shrub forms (e.g. *Salix lanata*, *S. glauca*, *S. phylicifolia*) in the Lateglacial period and potentially tree forms in the Holocene (e.g. *Salix caprea*, *S. cinerea*). The full-glacial and early Lateglacial zone (*ca.* 24,000 - 15,000 cal. years BP) is characterized by herb-tundra vegetation with a rich diversity of forbs, such as *Papaver*, *Draba*, *Bistorta vivipara* and *Saxifraga oppositifolia*, and graminoids, such as *Puccinellia*, *Festuca* and *Juncus biglumis* (Figure 4.4). The mat-forming dwarf shrub *Dryas octopetala** becomes more common from 15,000 cal. years BP, followed by a sequential arrival of additional dwarf shrubs and tall shrubs/deciduous trees (e.g. *Betula*, *Empetrum*, *Vaccinium* sp.).

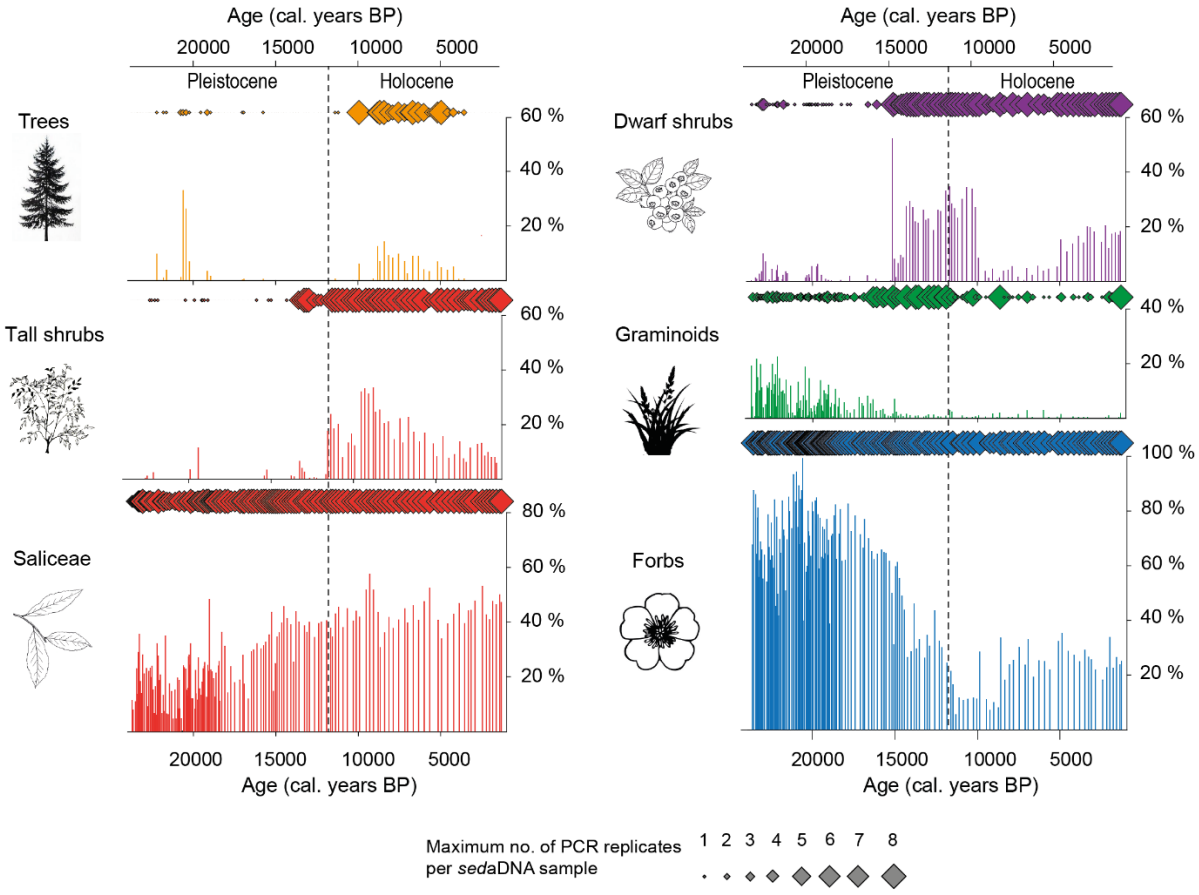

Figure 4.4 Turnover in plant functional groups over the past 24,000 years. Selected functional groups are presented as a percentage of total *sed*aDNA reads per sample (histogram) and maximum number of PCR replicates (out of eight) per sample (diamond symbols) for the Lake Bolshoye Shchuchye record. Note that the height of the y-axis varies amongst panels. The grey dashed line indicates the divide between the last glacial interval in the Pleistocene and the current Holocene interglacial period.

The coniferous trees *Picea* sp. and *Larix sibirica* became common elements of the vegetation *ca.* 9,000 - 4,000 cal. years BP, alongside many boreal herbs (Figure 4.4, Appendix B Supplementary Figures B.3 – B.5). Coniferous forest withdrew around 4,000 cal. years BP; the vegetation subsequently reverted to dwarf-shrub tundra with a diverse herb flora, similar to the vegetation mosaic seen in the Lateglacial and early Holocene interval, as well as the present-day vegetation mosaic of the Polar Urals.

4.3.2 Species Persistence and Floristic Richness

We assigned a classification, where possible, to vascular plant taxa detected by *sed*aDNA based on their present-day native distribution (see Methods). In total, 31 taxa were classified as "arctic-alpine", 49 as "boreal" and 22 as occupying a dual "arctic-boreal" distribution (full details of the classifications are presented in Appendix B Supplementary Table B.2). A steady increase in the diversity of arctic-alpine plants is observed throughout the full-glacial and Lateglacial period, peaking around 12,700 cal. years BP before a distinct decline to low values (Figure 4.5a). The arctic-boreal taxa, which include widespread genera such as *Empetrum* and *Vaccinium*, show low diversity in the full-glacial period but then increase, with fluctuations similar to the arctic-alpine pattern from around 17,000 cal. years BP. The initial decline in arctic-alpine plants occurs slightly before the main increase in the diversity of boreal taxa, at the time when floristic richness of arctic-boreal taxa is high and Saliceae is abundant. A further decline is seen *ca.* 10,000 cal. years BP, when the boreal taxa expand and maintain a high diversity until around 4,500 cal. years BP, with four prominent peaks visible in the Holocene period (Figure 4.5a). Overall, the proportion of DNA reads of the three distribution categories (Figure 4.5b) show similar trends to floristic diversity, with the exception that arctic-alpines reached their maximum proportion (>98 % of total DNA reads) around 20,000 cal. years BP, considerably earlier than their maximum diversity was reached.

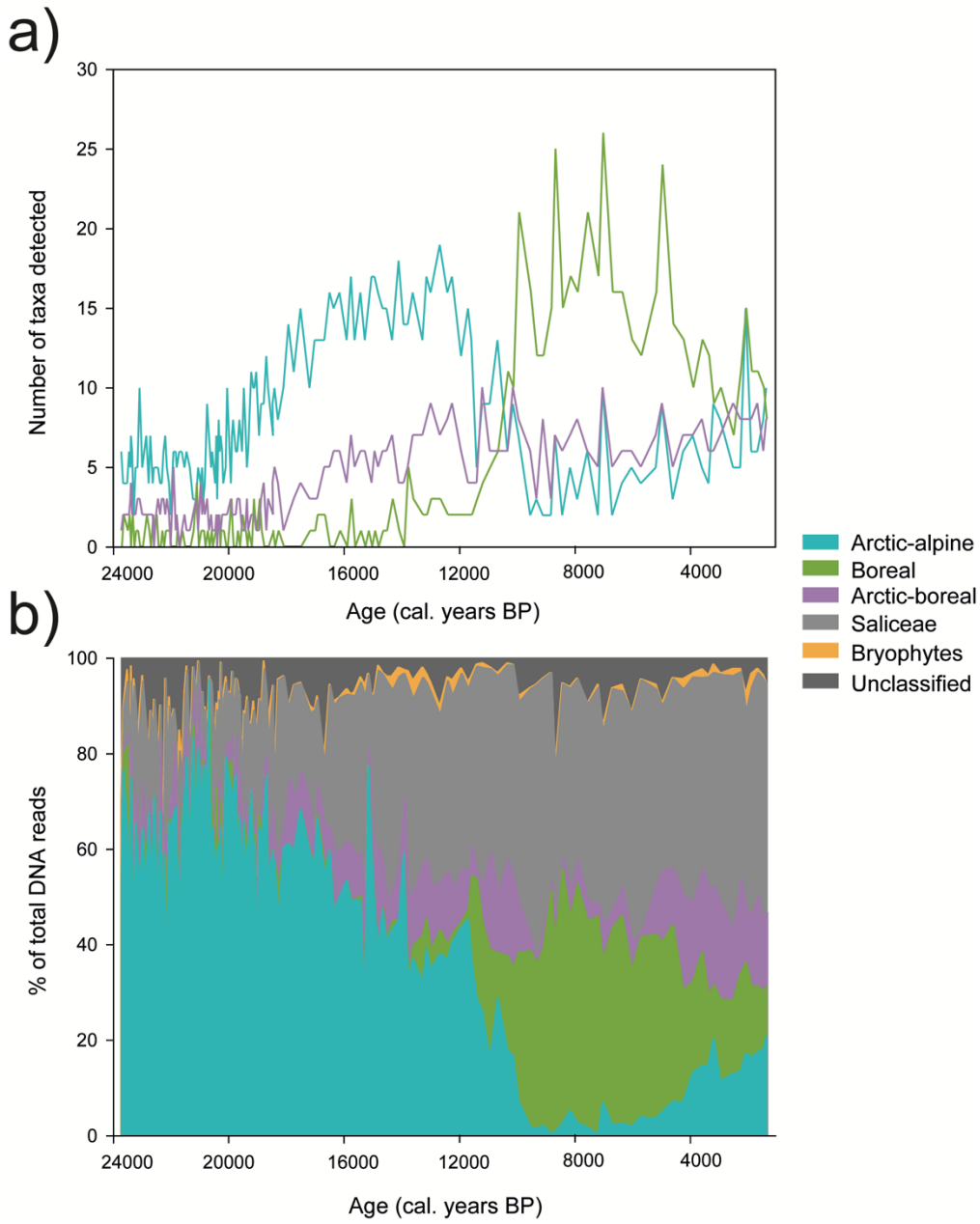

Figure 4.5 Response of arctic-alpine plants to the past establishment of woody, boreal plants at Lake Bolshoye Shchuchye. (a) Floristic diversity over time and (b) the proportional abundance of plants detected by *sed*aDNA within each distribution category. For full details of the distribution classifications, see Appendix B Supplementary Table B.2.

We investigated the response of individual arctic-alpine plant taxa to the Holocene establishment of dwarf shrub and tall shrub/ trees (e.g. *Dryas octopetala**, *Vaccinium* sp., *Empetrum nigrum**, *Betula*, *Alnus*) and later forest trees (e.g. *Larix sibirica*, *Picea* sp.) in the lake's vicinity between *ca.* 15,000 - 4,000 cal. years BP (Figure 4.3). In total, 32 arctic-alpine plant taxa were detected by *sed*aDNA, of which 31 are detected within the full-glacial interval; *Arabis alpina* was recorded during the Holocene interval only (see Appendix B Supplementary Table B.2). Of the 31 arctic-alpines that are detected within the full-glacial interval, 27 (28 including occurrences with

<10 DNA reads) are recorded after dwarf shrubs and tall shrubs/trees began to establish from 15,000 cal. years BP and 15 (21) are recorded in the later period when forest tree taxa established between *ca.* 9,000 - 4,000 cal. years BP. Of the 17 arctic-alpines that are not detected in the *sed*aDNA during the period of forest tree establishment, all disappear prior to the establishment of forest tree taxa during the preceding shrub period (Figure 4.3). Nevertheless, three (four) of these taxa reappear when coniferous forest withdrew in the more recent period (*ca.* 4,000 - 1,300 cal. years BP), and all of the 32 arctic-alpine plant taxa detected in the *sed*aDNA record are found in the local vegetation today.

4.4 Discussion

Several studies have emphasised the advantage of *sed*aDNA for higher taxonomic resolution and richness compared to traditional methods of pollen and plant macrofossils (Alsos *et al*., 2016; Paus *et al*., 2015; Zimmermann *et al*., 2017a). The *sed*aDNA record from Lake Bolshoye Shchuchye is taxonomically diverse, even compared with most other *sed*aDNA studies (Birks and Birks, 2016; Zimmermann *et al*., 2017b); this may be explained by its long temporal record, optimised methods and use of a more complete local reference library. Moreover, the sediment lithology is predominantly fine-grained clays and silts; these tend to be highly suitable for the preservation of DNA since extracellular DNA can bind to the relatively large and charged surface areas of clay colloids (Cai *et al*., 2006; Huang, 2014; Pietramellara *et al*., 2001). The lake has a large (215 km^2) and topographically complex hydrological catchment with steep slopes, high rates of erosion and runoff, with considerable riverine input to the lake (Svendsen *et al*., 2019), all of which probably contribute to the floristically rich *sed*aDNA record obtained. The *sed*aDNA signal in Lake Bolshoye Shchuchye likely reflects sediment inputs from a much larger source area, capturing a range of plant communities occupying the lake's hydrologic catchment. Since *sed*aDNA was able to identify many taxa that are often poorly represented in traditional palaeorecords, the Lake Bolshoye Shchuchye record provides a unique insight into the response of the artic-alpine flora to environmental change, including the millennial-scale, phased expansion of woody growth forms in the Lateglacial and Holocene.

There was both continuity and change in plant community composition at Lake Bolshoye Shchuchye over the past 24,000 years; the lake's catchment supported a growing set of plant communities, and species diversity increased over millennia with few taxon losses to the present day. More than two thirds of all recorded plant taxa identified were already in place in the full-glacial period (*ca.* 24,000 - 15,000 cal. years), when the study region remained free of extensive ice cover (Hughes *et al*., 2016; Svendsen *et al*., 2004, 2019), and the data provide a rich floristic record of full-glacial vegetation close to local glaciers but outside of the Eurasian Ice Sheet margin, which

was situated further to the north (Hughes *et al*., 2016). Subsequently, new taxa appeared over time, and there was a shift in the dominance of plant functional groups during a long period of changing and (predominantly) warming climate. While the timing and nature of the general vegetation changes observed since the last glacial period at Lake Bolshoye Shchuchye are comparable to those documented in nearby pollen records from the region (Andreev *et al*., 2005; Panova *et al*., 2003; Svendsen *et al*., 2014), our record is longer, well-dated and more complete. Furthermore, the *sed*aDNA permits the identification of a sequential arrival of different dwarf-shrub taxa from 15,000 cal. years BP and the clear identification of larch (*Larix sibirica*) forest in the vicinity of the lake between *ca.* 9,000 and 4,000 cal. years BP (*Larix* is notorious for its low pollen productivity and underrepresentation in pollen records).

It is well accepted that arctic-alpine and boreal-steppe taxa are primarily sensitive to competition for light (rather than sensitive to heat *per se*), and that they can be shaded out by larger, shrubby growth forms (Bjorkman *et al*., 2019; Martin *et al*., 2017; Wesser and Armbruster, 1991). From the *sed*aDNA data, it appears that the Lateglacial, stepwise addition of shrub and sub-shrub taxa may well have led to competition and the reduction of populations of many arctic-alpine taxa, resulting in decreasing diversity of arctic-alpines. The reduction occurred prior to the arrival of trees; changes in plant dominance were well underway by the time of forest establishment. Thus, the ongoing expansion of shrub-tundra (Martin *et al*., 2017; Vowles and Björk, 2019) may pose a threat to the abundance and diversity of arctic-alpines, especially in homogenous tundra habitats where much of this expansion has been observed.

The timing of forest tree establishment in the vicinity of Lake Bolshoye Shchuchye (~9,000 cal. years BP and continuing until ~4,000 cal. years BP) is concurrent with the known northward treeline expansion into the lowlands of the Polar Urals and surrounding areas, which many authors consider representing warmer summer temperatures (Binney *et al*., 2009; Kaakinen and Eronen, 2000; Kremenetski *et al*., 1998; Salonen *et al*., 2011). During this period, when arctic-alpine taxa would have been most disadvantaged, nearly half of the 31 arctic-alpine taxa were detected, at least in small quantities, which strongly suggests their continuous presence in the catchment from full-glacial times; all 31 are found in the region today.

Pollen and plant macrofossil records seldom show such clear evidence of continuity, because of the swamping effect of Holocene anemophilous pollen at virtually all northern sites and differential preservation of identifiable plant remains (Birks, 2008; Väliranta *et al*., 2015). A survey of European late-Quaternary pollen data showed no increase in diversity into the Holocene in northern sites, in part probably due to this feature (Giesecke *et al*., 2012). However, exceptional records with high pollen counts in regions never colonized by trees in the Holocene do demonstrate

the local persistence of arctic-alpines from the Lateglacial through the Holocene, for example, that from Hanging Lake in mountains of the northern Yukon (Cwynar, 1982; Kurek *et al*., 2009). Although *sed*aDNA cannot fully resolve all aspects of the arctic-alpine plant community, it can greatly enhance the documentation of species persistence in the face of changing environmental conditions.

Intermittent absences inevitably present an interpretational problem in palaeorecords (Gavin *et al*., 2014; Jackson, 2012). For the subset of taxa which do show absences during the periods of shrub and/or tree expansion, the possibilities are either that they became locally extinct, or that they were present but their DNA input to the sediment dropped below the threshold for detection, probably as a result of their decreased biomass on the landscape at a time when the biomass of woody taxa expanded. Furthermore, taxa that may have been growing near the lake in full-glacial time most likely shifted their range up slope with increasing warmth and associated competition, removing them from the lake edge and valley floor, which is a prime locality for DNA recruitment to sediment (Alsos *et al*., 2018). Thus, as with pollen of entomophilous taxa, but probably not to the same extent, there is likely a limitation in detectability for rare taxa in the *sed*aDNA signal.

A complementary line of evidence used to evaluate the likelihood of species persistence is that of modern genetic diversity patterns, where, generally, regions that have supported a flora over a relatively long period show higher levels of genetic diversity than regions recently recolonised. Genetic diversity shows an increasing gradient from Fennoscandia eastward to unglaciated areas of northern Russia and Siberia. Within this gradient, the Polar Urals are classified as intermediate (Eidesen *et al*., 2013; Stewart *et al*., 2016). This suggests a degree of long-term persistence (compared with Fennoscandia) and could reflect the presence of plant communities since the last major deglaciation of the northern segment of the Ural Mountains at ~60,000 cal. years BP (Hughes *et al*., 2016; Svendsen *et al*., 2004, 2019).

The arctic-alpine flora has been identified as one that is exceptionally threatened by projected future climate change (Niskanen *et al*., 2019); it is also one that is often difficult to track via traditional palaeorecords. The Lake Bolshoye Shchuchye record provides unusually robust empirical evidence of the persistence of an arctic-alpine flora through a long period of environmental change, demonstrating the buffering capacity of a spatially heterogeneous landscape. However, a distinct decline in abundance, which is likely related to biomass, began as soon as woody taxa started to expand, suggesting that in a future warming scenario, local species loss may occur long before tree establishment occurs. These empirical results strongly support the

need for conservation awareness of potential biodiversity loss in arctic environments, particularly those with less topographic buffering capacity.

4.5 Material and Methods

4.5.1 Study Site

Lake Bolshoye Shchuchye (67°53'24"N, 66°18'36"E) is located at 187 m a.s.l. in the central part of Polar Urals mountain chain, ~105 km north-east of the mining town of Vorkuta (Figure 4.1). The lake itself is 13 km long and 1 km wide and has a maximum water depth of 140 m in the centre of the basin. Seismic reflection profiling revealed that the basin fill contains more than 160 m of acoustically laminated sediments with minimal disturbances from mass movements (Haflidason *et al*., 2019; Svendsen *et al*., 2019). The lake has a catchment area of 215 km^2, with a deltaic inlet at its northern shore caused by inflow of the river Pyriatanyu and an outlet along its southern shore draining into the Bolshoye Shchuchya River, a tributary of the Ob River. The lake is surrounded by steep-sided valley slopes and exposed rock faces with high mountain peaks reaching 500 - 1,100 m a.s.l. at its north-western shore, intersected by valleys.

Present-day climate conditions are characterised as cold and continental, with a mean summer temperature (June-July-August) of 7 °C at the Bolshaya Khadata station (Solomina *et al*., 2010), located 25 km to the south of Lake Bolshoye Shchuchye at 260 m a.s.l. The lake lies within arctic bioclimatic sub-zone E (Walker *et al*., 2005). The catchment vegetation mosaic comprises dwarf shrub-tundra with patchy thickets of *Alnus viridis* growing on south-facing slopes up to an elevation of 300 m a.s.l. At the higher elevations, the vegetation is discontinuous with exposed rocky surfaces supporting alpine grass and forb communities. The lake is situated close to the northern distributional limit of *Larix sibirica*, with isolated trees observed a few kilometres to the southeast of the lake.

Lake Bolshoye Shchuchye is considered to have been formed by glacial erosion during repeated past glaciations, following weaknesses along ancient NW-SE striking faults (Svendsen *et al*., 2019). The lake and its catchment are located well outside the maximum extent of the Barents-Kara Ice Sheet during the LGM *ca.* 25,000 - 17,000 cal. years BP and the region is thought to have remained ice-free for at least the last 50,000 - 60,000 cal. years BP (Hughes *et al*., 2016; Svendsen *et al*., 2004, 2019). During time periods around 90,000 cal. years BP (MIS 5b) and 70,000 - 60,000 cal. years BP (MIS 4), large ice sheets formed over the Barents-Kara Sea region, creating major ice-dammed lakes that flooded the adjacent lowlands on both sides of the Ural Mountains chain (Mangerud *et al*., 2004; Svendsen *et al*., 2014). It remains controversial whether large ice caps

formed over the Polar Urals, the northernmost part of the mountain chain, during this time (Astakhov, 2018) but it is clear that sizeable outlet glaciers from mountain valleys reached the foothills of the Polar Urals *ca.* 60,000 cal. years BP. In contrast to previous glaciations, the Barents-Kara Ice Sheet did not reach the northern rim of the mainland during the LGM and almost the entire Arctic seaboard of Russia to the east of the Arkhangelsk region remained ice-free during this glaciation (Mangerud *et al*., 2004). Cosmogenic (^{10}Be) exposure dating revealed that the small cirque glaciers that exist today within the Polar Urals were only slightly larger during the LGM than today (Mangerud *et al*., 2008; Svendsen *et al*., 2019).

4.5.2 Sediment Core Retrieval

Six separate cores were retrieved from Lake Bolshoye Shchuchye, full details of which are provided by an earlier accompanying paper (Svendsen *et al*., 2019). A 24-m long core (number 506-48) retrieved in July 2009 from the southern end of the lake (67°51'22.248"N, 66°21'30.096"E) is the focus for this study. The core was retrieved with a UWITEC piston corer using a combination of 2-m long by 10-cm diameter PVC sample tubes for most sections and 2-m long by 9-cm diameter steel tubes for the deepest sections. A detailed sedimentological description and the core chronology, which is based on a series of AMS radiocarbon dates supported by a sequence of annual laminations (varves), is presented in two earlier publications (Regnéll *et al*., 2019; Svendsen *et al*., 2019).

4.5.3 Radiocarbon Dating and Sampling of the Sediment Core

In total, 26 samples of terrestrial macrofossils were radiocarbon (^{14}C) dated with Accelerator Mass Spectrometry (AMS) at the Poznań Radiocarbon Laboratory of the Adam Mickiewicz University, Poland. All radiocarbon ages were calibrated according to the terrestrial IntCal13 curve (Reimer *et al*., 2013) using the online Calib program (Stuiver *et al*., 2017). Subsampling of the core was undertaken at *ca.* 15-cm resolution in a laminar flow cabinet within a clean laboratory within the Centre for Geobiology and Microbiology at the Department of Earth Science, University of Bergen, Norway, using sterile tools, full bodysuit, facemask, and gloves. Subsampling was undertaken in the presence of subsampling controls (open water samples) in order to detect potential lab aerial contamination. Following the protocol described by Parducci *et al*. (2017), the outer 10 mm of sediment was avoided and an ~20 g subsample was retrieved from inside the freshly exposed centre only.

4.5.4 DNA Extraction, Amplification, Library Preparation and Sequencing

DNA extraction, PCR amplification, PCR product pooling and purification, and sequencing followed the protocols of Alsos *et al*. (2016) unless otherwise stated. DNA was extracted from 153 sediment subsamples within the dedicated ancient DNA facility at Tromsø University Museum (TMU), Norway. In addition, DNA was extracted from 17 negative extraction, nine negative PCR and nine negative water (subsampling) controls, which contained no sediment and were used to monitor for contamination. Aliquots of DNA extracts were then shipped to the Laboratoire d'ÉCologie Alpine (LECA, Université Grenoble Alpes, France) for metabarcoding. Each DNA extract and negative extraction control was independently amplified using uniquely-tagged generic primers that amplify the *trn*L P6 loop of the plant chloroplast genome (Taberlet *et al*., 2007) in eight PCR replicates to increase confidence in the results and improve the chance of detecting taxa with small quantities of template in the DNA extracts (Ficetola *et al*., 2015). Pooled and cleaned PCR products were then converted to four Illumina-compatible amplicon libraries using the single-indexed, PCR-free MetaFast method (FASTERIS SA, Switzerland). These libraries were then sequenced on the Illumina HiSeq-2500 platform for 2× 125 cycles at FASTERIS.

4.5.5 DNA Sequence Analysis and Taxonomic Assignments

Next-generation sequence data were filtered using the OBITools software package (Boyer *et al*., 2016; http://metabarcoding.org/obitools/doc/index.html) following the protocol and criteria defined by Alsos *et al*. (2016). Taxonomic assignments were performed by first matching sequences against a reference library of 2445 sequences comprising 815 artic (Sønstebø *et al*., 2010) and 835 boreal vascular plants (Willerslev *et al*., 2014), in addition to 455 arctic/boreal bryophytes (Soininen *et al*., 2015). Sequences were then matched to a second reference library generated after running *ecopcr* on the global EMBL database (release r117).

In order to minimise any erroneous taxonomic assignments, only taxa with a 98 % match, or greater, to a reference sequence were retained. We further removed sequences which displayed higher average frequency in the negative extraction or PCR controls than in the lake sediment samples. Identified taxa were compared to the circumpolar flora (Hultén and Fries, 1986), Pan Arctic Flora Checklist (PAF; Elven *et al*., 2011) and flora of the Polar Urals (Gorchakovskiy *et al*., 1994; Morozova *et al*., 2006; Rebristaya, 1977). Sequences assigned to taxa that are not present in northern Russia today were checked against the NCBI BLAST database for multiple or alternative taxonomic assignments (http://www.ncbi.nlm.nih.gov/blast/). Taxonomy follows the Pan Arctic Flora Checklist (Elven *et al*., 2011), if present.

4.5.6 Distributional Classification of Taxa

Where possible, we assigned a classification of either “arctic-alpine”, “boreal” or “arctic-boreal” to each vascular plant taxon identified by *sed*aDNA based on their present-day native distribution (Hultén, 1971; Hultén and Fries, 1986). Plant taxa classified as “arctic-alpine” had two-thirds or more of their main distributional area located north of the forest limit for boreal tree taxa *Pinus*, *Picea*, *Larix* and *Betula*. Those classified as “boreal” had two-thirds or more of their distributional area located within the boreal forest limits. Finally, those assigned an “intermediate” classification occupied an area where approximately half of their main distributional area was located within each biome. For those taxa which were identified to genus or family level only by *sed*aDNA, a classification was given based on the distribution of all likely species within the identified genus or family which are present within northern Russia today. Full details of the classifications, including likely species of which the classification is based upon, are presented in Appendix B Supplementary Table B.2. Bryophytes were excluded from the analysis due to the lack of sufficient information on their present-day distribution.

To determine the degree of species persistence over time and long-term continuity of individual plant taxa identified by *sed*aDNA, an assessment of their present-day occurrence within the local vegetation mosaic of Lake Bolshoye Shchuchye’s hydrologic catchment was performed based on data collected by L. Morozova and colleagues during the years 2000, 2001 and 2006 for a period of 46 days in total. Botanical data were collected systematically along topographical/ecological transects from rivers/lakes up to the top of a ridge or mountain. In addition, botanical information was retrieved from several sources (Gorchakovskiy *et al*., 1994; Morozova *et al*., 2006; Rebristaya, 1977).

4.5.7 Acknowledgements

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4.5.8 Author Contributions

C.L.C., I.G.A., M.E., J.I.S., H.H. and J.M. contributed to the concept and designed the study. J.I.S., H.H. and J.M. surveyed and cored Lake Bolshoye Shchuchye and constructed the age-depth model.

C.L.C. subsampled the sediment core and extracted the DNA and L.G. amplified the DNA and performed the initial sequence analysis and taxa assignment. C.L.C. further analysed the DNA data and performed the post-identification filtering with contributions from I.G.A and M.E. Ecological expertise was contributed by I.G.A, M.E., D.E. and P.D.M.H. for the distributional classification of taxa. L.M. provided botanical expertise on the flora of the Polar Urals and the present-day occurrence of taxa identified by DNA within the local vegetation. J.I.S., H.H. and J.M. contributed their expertise on the geomorphological background and glacial history of the site. C.L.C. wrote the manuscript with the contributions of all co-authors.

Chapter 5 Arctic Plant Community Dynamics Over the Past 24,000 Years in the Polar Urals: New Insights from Sedimentary Ancient DNA (*Sed*aDNA) and Pollen (Paper III)

This paper is currently in the pre-submission stage of the publication process:

Clarke, C.L., Bjune, A.E., Edwards, M.E., Gielly, L., Paus, A., Hughes, P.D.M., Haflidason, H., Mangerud, J., Svendsen, J.I. and Alsos, I.G. 2019: Arctic plant community dynamics over the past 24,000 years in the Polar Urals: New insights from sedimentary ancient DNA (*sed*aDNA) and pollen. *Manuscript in preparation*.

This work has been presented as (selected examples):

Clarke, C.L. 2018: A 24,000-year combined ancient DNA and pollen record from the Polar Urals. In: *Climate and Chronology Seminar Series*. Research Lab for Archaeology, University of Oxford. 20th February. Invited Oral Presentation.

Clarke, C.L. and Edwards, M.E. 2018: An ancient DNA record of 24,000 years of arctic plant community dynamics. In: *International Quaternary Webinar Series*. University of Massachusetts. 6th January. Online Webinar Presentation.

5.1 Abstract

Knowledge of the Last Glacial Maximum (LGM) flora in the Russian Arctic and changes in its composition over the late Quaternary is to a large extent based on short and fragmented stratigraphic sequences found in exposed sedimentary sections. Here, we present a new 24,000-year record of plant community dynamics based on pollen and ancient DNA extracted from the sediments (*sed*aDNA) of Lake Bolshoye Shchuchye, the largest and deepest lake in the Polar Ural Mountains. Our results suggest several shifts in the dominance between and within plant functional groups over time but, nevertheless, survival of most taxa *in situ*. A diverse arctic-alpine herb flora characterises the LGM interval (*ca.* 24,000 - 15,000 cal. years BP) and persists into the Holocene. Sedges (e.g. *Carex*) and bryophyte taxa (e.g. *Bryum*, *Aulacomnium*) increase around 17,000 cal. years BP, followed by the establishment of shrub-tundra communities of *Betula*, *Dryas*, *Vaccinium* sp. a little later from around 15,000 cal. years BP. Forest trees, such as *Picea* and *Larix*, and ferns (e.g. *Dryopteris fragrans*, *Gymnocarpium dryopteris*) establish in the vicinity of the lake between *ca.* 9000 - 4000 cal. years BP before withdrawing and allowing expansion of a dwarf-shrub tundra and diverse herb community, similar to the prevailing present-day vegetation mosaic. The *sed*aDNA and pollen records show a largely consistent pattern in the occurrence of key plant taxa, with

*sed*aDNA revealing several important features that the pollen stratigraphy failed to detect; a turnover in grass and forb genera over the Pleistocene-Holocene transition, a diverse and variable bryophyte flora through time and a sustained increase in floristic richness since the last glacial interval.

5.2 Introduction

Palaeoecological records have helped to elucidate a broad array of vegetation responses to past climate changes (Jackson and Overpeck, 2000; Nolan *et al*., 2018), yet interpretations of long-term dynamics have often been constrained by limits of pollen and plant macrofossil data (e.g. productivity, preservation, taxonomic resolution) or specific nature of the site (Prentice, 1985; Sugita, 1994; Gajewski, 2015). Ancient DNA from sediments (*sed*aDNA) has the potential to provide more detail on plant community composition and its response to past climate changes, augmenting information gained from pollen and plant macrofossil analyses (Alsos *et al*., 2016; Jørgensen *et al*., 2012; Epp *et al*., 2015; Pedersen *et al*., 2016; Parducci *et al*., 2017; Zimmerman *et al*., 2017a,b; Clarke *et al*., 2018).

Here we present a combined pollen and *sed*aDNA record of plant community composition over the last 24,000 years from a lake in the Polar Ural Mountains of the Russian Arctic. The 24-m long sediment sequence retrieved from this site can potentially address several key ecological questions: whether there was complete compositional turnover or a minor reshuffling of taxa following the Pleistocene-Holocene transition, whether there was a difference in the response of different plant functional groups and/or communities to late Quaternary environmental changes and whether the *sed*aDNA and pollen records show similar changes in plant community composition over the past 24,000 years.

The Polar Urals, the northernmost part of the Ural Mountain chain, is a promising region for investigating the range of responses of individual plant taxa or communities to past climate changes (Shiyatov *et al.,* 2005) because the mountain chain itself marks an important biogeographic boundary between the floras of the western Siberian and east European lowlands (Eidesen *et al*., 2013; Elven *et al*., 2011) and climate conditions vary across small spatial scales due to the spatially heterogeneous nature of the mountain region. The Polar Urals lie within the forest-tundra ecotone (MacDonald *et al*., 2007; Shiyatov and Mazepa, 2011). A northward shift in the ecotone is expected in response to future climate changes (ACIA, 2004; CAFF, 2013), which will potentially have far-reaching consequences for northern ecosystems and for climate feedbacks due to changes in albedo (Bonan, 2008). The present treeline has a relatively simple composition, primarily composed of Siberian larch (*Larix sibirica*), enabling an investigation of climate-induced

treeline changes. The region has remained ice-free for at least the last 50,000 - 60,000 cal. years BP (Astakhov *et al*., 1999; Svendsen *et al*., 2004, 2014; Hughes *et al*., 2016) and only small, mostly cirque glaciers existed during the Last Glacial Maximum (LGM; 30,000 - 15,000 cal. years BP; Mangerud *et al.,* 2008; Clark *et al.,* 2009). More information on plant community composition will help inform understanding of the full-glacial ecosystem which hosted a rich herbivore fauna (Astakhov, 2004; Hubberten *et al*., 2004; Sher *et al*., 2005) and an early human population (Pavlov *et al*., 2001; Svendsen *et al*., 2010; Slimak *et al*., 2011; Pitulko *et al*., 2017; Hufthammer *et al*., 2019).

The last Eurasian ice sheet complex (British-Irish, Scandinavian, and Svalbard-Barents-Kara Seas ice sheets) attained maximum extent and volume at *ca.* 21,000 cal years. BP (Hughes *et al.,* 2016), when mean annual temperatures over parts of the Arctic were as much as 20 ^{o}C lower than at present (Dahl-Jensen *et al.,* 1998; Elias *et al.,* 1996; Miller *et al.,* 2010). At their LGM maxima, the ice-sheet complexes covered vast areas of the British Isles, Scandinavia and the Barents-Kara Sea and adjacent regions, yet a large land mass that spanned from Spain eastwards across Eurasia and the Ural Mountains to far easternmost Siberia remained ice-free (Guthrie, 1990; Svendsen *et al.,* 2004; Sejrup *et al.,* 2005; Hughes *et al.,* 2016). This large ice-free area provided refuge for northern species that were displaced by ice and was later the source of subsequent population expansions (e.g. Huntley and Webb, 1989; Abbott and Brochmann, 2003; Sommer and Nadachowski, 2006; Binney *et al*., 2017). Palaeoecological records from the region suggest that a predominantly treeless ecosystem known as 'steppe-tundra' (also called 'tundra-steppe' in Russian literature or 'mammoth steppe' by Guthrie, 1982, 1990; Zimov *et al*., 2012 and others) persisted throughout these ice-free regions, characterised by a predominance of cold- and drought-adapted taxa (e.g. Kaplan *et al*., 2003; Sher *et al*., 2005; Bezrukova *et al*., 2010; Kuzmina, 2015; Chytrý *et al*., 2019).

Increasing summer insolation due to orbital forcing from *ca.* 20,000 cal. years BP, plus rising greenhouse gas concentrations, initiated the recession of the Eurasian ice sheet complex, with a noticeable increase in the rate of recession seen by *ca.* 16,000 cal. yrs BP (Clark *et al.,* 2009; Miller *et al*., 2010; Hughes *et al*., 2016). By *ca.* 11,000 cal. years BP, the Barents-Kara ice sheet had retreated from mainland Russia and only a small ice sheet persisted over parts of Scandinavia (Hughes *et al*., 2016). From *ca.* 9,000 cal. years BP and lasting until *ca.* 4000 cal. years BP, pollen-based estimates in northern Russia suggest mean July temperatures were at least 1-3 ^{o}C higher than present (Andreev *et al.,* 2005; Salonen *et al.,* 2011) and forest trees such as *Picea*, *Pinus*, *Larix* and *Alnus* reached their northernmost extent (Panova *et al*., 2003; Andreev *et al*., 2005; Jankovska *et al*., 2006; Salonen *et al.,* 2011; Svendsen *et al.,* 2014).

Current knowledge of the long-term environmental history of the Polar Urals is hampered by the lack of well-dated continuous records, with most palaeorecords in the region being based

on exposed coastal and river sections (e.g. Andreev *et al*., 1998; 2003; Jankovska *et al*., 2006). Although useful, such sedimentary archives are often fragmentary and cover only short time windows, making interpretation of long-term dynamics challenging. While a handful of records based on lake and peat sediments have appeared more recently, most cover the Holocene interval only (Panova *et al*., 2003; Andreev *et al*., 2005; Panova *et al*., 2010).

Here we present a continuous 24,000-year record of plant community composition based on *sed*aDNA and pollen from Lake Bolshoye Shchuchye, the largest and deepest lake in the Polar Urals. We use metabarcoding techniques (Taberlet *et al.,* 2012a) to develop the *sed*aDNA record, in this case from the sediments of a well-dated 24-m sediment core retrieved from the southern end of the lake that spans the last 24,000 cal. years BP. The *sed*aDNA record from this core detected a diversity of arctic-alpine plants which are seen to persist over the past 24,000 years, despite the past expansion of woody vegetation (see Clarke *et al*., 2019). We combine the *sed*aDNA record with a detailed pollen stratigraphy from the same sediment core to determine the nature and rate of responses of different plant functional groups and/or plant communities to late Quaternary environmental changes. Moreover, we compare the taxonomic overlap and resolution of these two proxies as a means of reconstructing past vegetation changes. The chronology and sediment characteristics of the sediment core from Lake Bolshoye Shchuchye are described in a series of publications elsewhere (Haflidason *et al*., 2019; Regnéll *et al*., 2019; Svendsen *et al*., 2019).

5.2.1 Study Site

Lake Bolshoye Shchuchye (67.89°N, 66.31°E, 187 m a.s.l.) is located in the central part of Polar Urals mountain chain, *ca.* 105 km north-east of the mining town of Vorkuta (Figure 5.1). The lake has a catchment area of 215 km^2 (Figure 5.1), with a deltaic inlet at its northern shore caused by inflow of the river Pyriatanyu and an outlet along its southern shore draining into the Bolshoye Shchuchya River, a tributary of the Ob River. The lake is surrounded by steep-sided valley slopes and exposed rock faces with high mountain peaks reaching 500 - 1,100 m a.s.l. at its north-western shore. Compared with smaller lakes often used for reconstruction of past vegetation communities (e.g. Jacobson and Bradshaw, 1981; Sugita, 2007; Alsos *et al.,* 2016, 2018), the sediments in Lake Bolshoye Shchuchye may capture a signal of plant communities over an elevational range of 187 - 1100 m a.s.l. which, when using a lapse rate of 0.7 °C $(100\ m)^{-1}$ (Rolland, 2003), represents a gradient of ±6.4 °C in July air temperature.

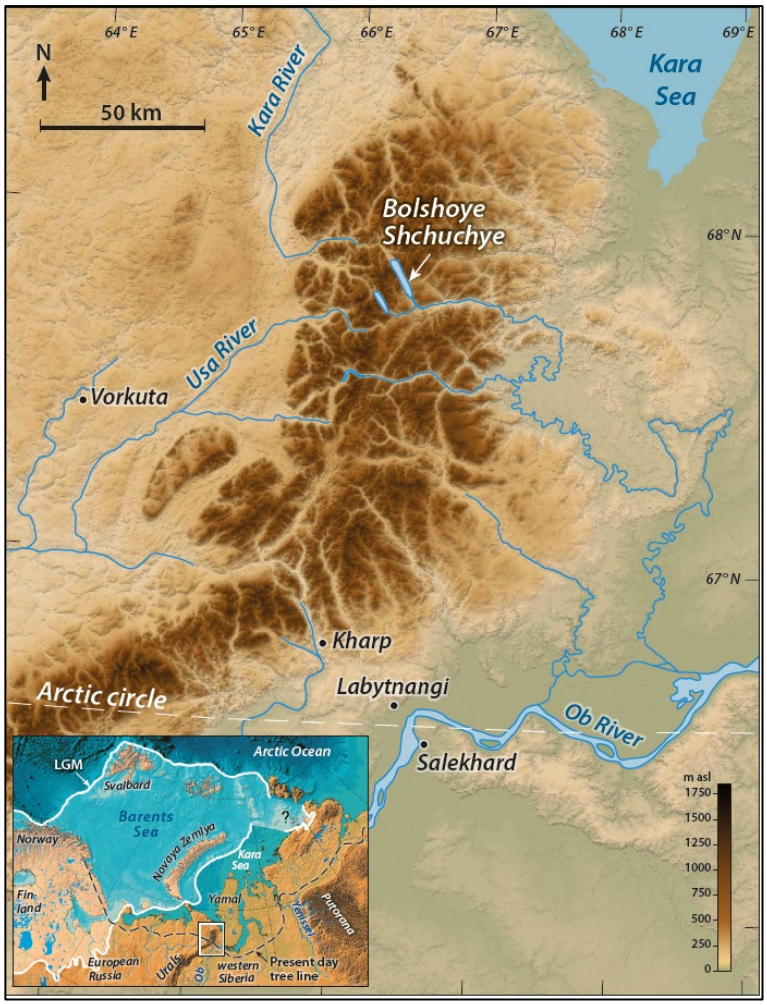

Figure 5.1 Location of Lake Bolshoye Shchuchye in the Polar Ural Mountains of Arctic Russia (from Clarke *et al*., 2019). Map inset shows the location (rectangle) of main map. The ice sheet limit during the Last Glacial Maximum (white line) from Svendsen *et al.* (2004) and the present-day boreal treeline (black dashed line) are also indicated.

Lake Bolshoye Shchuchye is considered to have been formed by glacial erosion during repeated past glaciations, following weaknesses along ancient NW-SE striking faults (Svendsen *et al.,* 2019). The lake and its catchment are located well outside the maximum extent of the Barents-Kara Ice Sheet during the LGM *ca.* 30,000 - 15,000 cal. years BP (Hughes *et al.,* 2016) and the region is thought to have remained ice-free for at least the last 50,000 cal. years BP (Svendsen *et al*., 2004). During time periods around 90,000 cal. years BP and 70,000 - 60,000 cal. years BP, large ice sheets formed over the Barents-Kara Sea region, creating major ice-dammed lakes that flooded the adjacent lowlands on both sides of the Ural Mountains chain (Mangerud *et al.,* 2004; Svendsen *et al.,* 2014). It remains controversial whether large ice caps formed over the Polar Urals, the northernmost part of the mountain chain, during this time (Astakhov 2013; 2014; 2018) but it is clear that smaller outlet glaciers reached the foothills of the Polar Urals *ca.* 60,000 cal. years BP (Svendsen *et al.,* 2014). In contrast to previous glaciations, the Barents-Kara Ice Sheet did not reach

the northern rim of the mainland during the LGM and almost the entire Arctic seaboard of Russia to the east of the Arkhangelsk region remained ice-free (Svendsen *et al.,* 2004). Cosmogenic (^{10}Be) exposure dating revealed that the small cirque glaciers that exist today within the Polar Urals were not much larger during the LGM than today (Mangerud *et al.,* 2008).

Present-day climate conditions at Lake Bolshoye Shchuchye are characterised as cold and continental, with a mean summer (June-July-August) temperature of 7 ^{o}C (Solomina *et al*., 2010). The catchment vegetation mosaic comprises dwarf shrub-tundra with patchy thickets of *Alnus viridis* growing on south-facing slopes up to an elevation of 300 m a.s.l. The lake is situated close to the northern distributional limit of *Larix sibirica*, with isolated trees observed a few kilometres to the southeast of the lake (Svendsen *et al*., 2019).

5.3 Material and Methods

5.3.1 Sediment Retrieval and Sampling

Six separate cores were retrieved from Bolshoye Shchuchye between 2007 and 2009, full details of which are provided by Svendsen *et al.* (2019). A 24-m core (number 506-48) retrieved in July 2009 from the southern end of the lake (67^{o}51'22.20"N, 66^{o}21'30.07"E) spans the last *ca.* 24,000 cal. years BP and is the focus of this study. Detailed sediment descriptions and the chronology of this core are presented by Regnéll *et al.* (2019) and Svendsen *et al.* (2019). In total, 26 samples of terrestrial macrofossils were radiocarbon (^{14}C) dated with Accelerator Mass Spectrometry (AMS) at the Poznań Radiocarbon Laboratory of the Adam Mickiewicz University, Poland, to create an age-depth model, which is supported by counting of a sequence of annual laminations (varves).

Subsampling of the core for *sed*aDNA was undertaken at *ca.* 15-cm resolution in a laminar flow cabinet in a clean laboratory at the Centre for Geobiology and Microbiology, Department of Earth Sciences, University of Bergen, Norway, using sterile tools, a full bodysuit, facemask, and gloves. Subsampling was undertaken in the presence of subsampling controls (open water samples) in order to detect potential laboratory contamination. Following the protocol described by Parducci *et al.* (2017), the outer 10 mm of sediment was avoided and an ~20 g subsample was retrieved from inside the freshly exposed core centre only.

5.3.2 Sedimentary Ancient DNA (*Sed*aDNA) Analysis

Full details of the extraction, PCR amplification, sequencing and taxonomic assignment of *sed*aDNA is presented in an earlier paper by Clarke *et al.* (2019). DNA was extracted from 153 sediment subsamples and 35 negative controls (17 extraction, nine subsampling and nine PCR negative

controls), which contained no sediment and were used to monitor for contamination at each step of the process. Each DNA extract and negative extraction control was independently amplified using uniquely-tagged generic primers that amplify the *trn*L P6 loop of the plant chloroplast genome (Taberlet *et al.,* 2007) in eight PCR replicates. In order to minimise any erroneous taxonomic assignments, only taxa with a 98 % match, or greater, to a reference sequence were retained. We further removed sequences which displayed higher average reads in the negative extraction or PCR controls than lake sediment samples.

5.3.3 Pollen Analysis

In total, 101 pollen samples were analysed from core 506-48. Subsamples of 1.0 cm^3 were prepared using standard methods (acid-base-acid-acetolysis; HF; Fægri and Iversen, 1989) and were mounted in glycerol. Four *Lycopodium* spore tablets (*n* = 18,584) were added to each sample to calibrate pollen concentration estimation. Where possible, at least 300 pollen grains of terrestrial taxa were identified per sample using taxonomic keys (Fægri and Iversen, 1989) and an extensive reference collection at the Department of Biological Sciences, University of Bergen. A few samples had low pollen concentrations and sums were therefore lower (< 100 grains). The pollen sum (∑P) includes all terrestrial pollen and spore taxa, thus excluding aquatic taxa.

5.3.4 Floristic Richness

A comparison of richness between samples with different count sizes can be biased, as the chance of detecting rare taxa increases with count size (Birks and Line, 1992). We therefore rarefied the *sed*aDNA and pollen data to estimate the number of terrestrial plant taxa that would have been detected if the count had been standardised among samples. Rarefaction analysis was performed using the minimum count size in the Vegan (Oksanen *et al*., 2017) package for R (R Core Team, 2017). The minimum count size for *sed*aDNA samples was 19,162 DNA reads and 206 grains for pollen samples. Due to very low counts (<100 grains), four pollen samples (860 cm, 1420 cm, 1630 cm and 1980 cm) were removed from the analysis.

5.4 Results

5.4.1 *Sed*aDNA Results

We obtained around 100 million raw reads for the 153 *sed*aDNA samples analysed from Lake Bolshoye Shchuchye. Following the post-identification filtering steps (removing sequencing artefacts, sequences with <98 % match to reference library and sequences which displayed higher

average frequency in negative controls than in lake sediment samples; described in Clarke *et al*. 2019), we retained around 19 million reads, representing 134 vascular plant and 28 bryophyte taxa. Of these, 40 % are identified to species level, 45 % to genus, 15 % to a higher taxonomic level (Appendix B Supplementary Figures B.3- B.5). Inferences to species level in the *sed*aDNA record were made for *Dryas octopetala* and *Empetrum nigrum* based on the present-day native distributions of individuals within these genera.

The full-glacial and early late-glacial period (24,000 - 17,000 cal. years BP) is characterized by herbaceous tundra taxa (Appendix C Supplementary Table C.1) There is a rich diversity (32 taxa) of arctic-alpine herbs such as *Draba pauciflora*, *Saxifraga cespitosa*, *Saxifraga oppositifolia*, *Papaver* and *Juncus biglumis,* more than half of which continue through the Holocene record. All arctic-alpine plant taxa are still present in the region today (Clarke *et al*., 2019). A distinct increase in *Carex* is apparent beginning around 17,000 cal. years BP and lasts until around 10,500 cal. years BP; this is broadly coincident with a rise in the diversity of bryophyte taxa (e.g. Polytrichaceae, Dicranaceae*, Andreaea*, *Aulacomnium turgidum*, *Bryum*) from around 16,000 cal. years BP. The mat-forming dwarf shrub *Dryas* becomes more common in the *sed*aDNA from 15,000 cal. years BP, and the *Dryas* increase is followed by an ordered arrival of additional dwarf shrubs and tall shrubs/deciduous trees (e.g. *Empetrum*, *Vaccinium* sp., *Betula*). Between *ca.* 9000 and 4000 cal. years BP, the coniferous trees *Picea* and *Larix* are common elements in the *sed*aDNA, alongside boreal herbs such as *Alchemilla*, *Anthriscus sylvestris*, *Filipendula ulmaria*, *Galium boreale* and ferns such as *Dryopteris fragrans* and *Gymnocarpium dryopteris*. Coniferous forest taxa (e.g. *Picea*, *Larix*, *Dryopteris fragrans*, *Gymnocarpium dryopteris*, *Filipendula ulmaria*, *Galium boreale*) disappear from the *sed*aDNA around 4000 cal. years BP, leaving dwarf-shrub tundra and herbaceous taxa dominant.

5.4.2 Pollen Results

Pollen analysis detected 114 vascular plant taxa, plus *Sphagnum*, across the 101 samples analysed from Bolshoye Shchuchye. Inferences to species level in the pollen record were made for several taxa, including *Picea abies*, *Dryas octopetala*, *Empetrum nigrum* and *Salix herbacea*-type based on current native distributions of species within these genera (Appendix C Supplementary Table C.2). Of the total 115 taxa, 29 % were identified and/or inferred to species level, 30 % to the genus, 10 % to the family and 31 % to a pollen type.

Similar to the *sed*aDNA record (see Clarke *et al*., 2019), the pollen stratigraphy from Lake Bolshoye Shchuchye (Appendix C Supplementary Figure C.1, Table C.1) suggests that a treeless landscape persisted in the full-glacial and early Lateglacial intervals between *ca.* 24,000 and 15,000 cal. years BP. High pollen percentages are observed for *Artemisia* (15 - 30 %), Poaceae (10 - 25 %)

and Chenopodiaceae (5 - 10 %) throughout this interval. The coniferous trees *Pinus sylvestris* and *Picea abies* are recorded but percentages do not exceed 20 % of total terrestrial pollen. By 16,000 cal. years BP, Cyperaceae and *Artemisia* pollen increases up to 30 %. This is later followed by a distinct rise in *Betula* pollen (up to 55 %) around 13,000 cal. years BP immediately before a short-lived interval of low values persisting until *ca.* 11,800 cal. years BP. By *ca.* 10,000 cal. years BP, rising percentages of tree and shrub taxa such as *Alnus*, *Betula*, *Corylus*, *Pinus sylvestris* and *Picea abies* with the fern taxa *Dryopteris*-type are observed, accompanied with a short-lived peak in *Juniperus communis* (up to 15 %) pollen and increasing values (5 - 10 %) of *Lycopodium annotinum* spores. *Betula and Picea abies* reach their highest percentages between *ca.* 10,000 and 4,000 cal. years BP, up to 58 % and 17 % of total terrestrial pollen respectively. After *ca.* 4000 cal. years BP, woody taxa such as *Alnus*, *Corylus* and *Pinus* maintain high percentages whilst *Picea* and *Betula* decline to low values.

5.4.3 Comparison Between the *Sed*aDNA and Pollen Records

A combined approach of *sed*aDNA and pollen analysis resulted in an additive total of 239 taxa of 89 families identified to varying taxonomic levels (Appendix C Supplementary Table C.2). In total, 31 families were shared between *sed*aDNA and pollen. Of the 119 vascular plant taxa, plus *Sphagnum*, detected by pollen analysis, 18 were also identified in the *sed*aDNA to the same taxonomic level.

In the *sed*aDNA record, a consistent, long-term increase in rarefied floristic richness is observed from *ca.* 24,000 cal. years BP until the early-Holocene (*ca.* 9,000 cal. years BP), where it peaks and then stabilises, with the exception of the four anomalously high peaks, over the remainder of the Holocene interval (Figure 5.2a). The same metric reconstructed from pollen shows no-long term change (Figure 5.2a). Mean floristic richness of LGM-aged samples (*ca.* 24,000- 17,000 cal. years BP; 2400 - 880 cm) derived from *sed*aDNA (19 taxa) were comparable to those based on pollen (17 taxa). After *ca.* 17,000 cal. years BP, a distinct and consistent rise in floristic richness is observed in the *sed*aDNA record, which is in contrast to pollen where values remain relatively constant (Stdev = 2.1) across the entire record (Figure 5.2). Mean floristic richness based on *sed*aDNA estimated for the three time-windows shows a 48 % increase in richness between LGM (*ca.* 24,000- 17,000 cal. years BP; 2400 - 880 cm) and Lateglacial (*ca.* 17,000 - 11,800 cal. years BP; 880 - 490 cm) aged samples. Floristic richness increases by a further 4 % between Lateglacial (*ca.* 17,000 - 11,800 cal. years BP; 880 - 490 cm) and Holocene (*ca.* 11,800 – 1300 cal. years BP; 490 – 0 cm) aged samples.

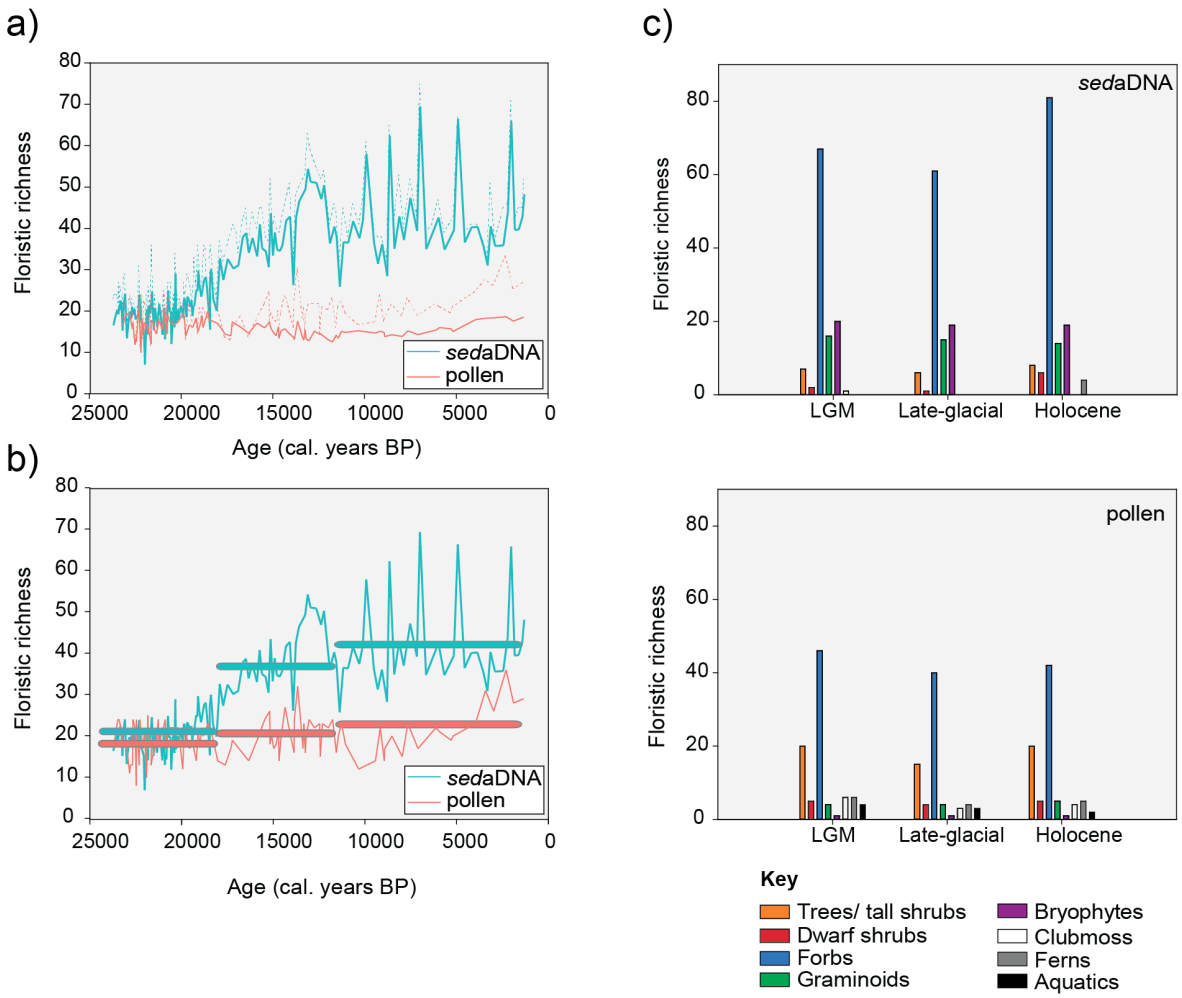

Figure 5.2 Measures of floristic richness based on *sed*aDNA and pollen from Lake Bolshoye Shchuchye. a) Total floristic richness pre-rarefaction (dashed line) and post-rarefaction (bold line) for all taxa detected by *sed*aDNA and pollen b) Floristic richness of angiosperms and gymnosperms pre-rarefaction (bold lines) with mean floristic for three time-windows; Last Glacial Maximum (LGM; 24,000 – 17,000 cal. years BP), Lateglacial (17,000 – 11,800 cal. years BP) and Holocene (11,800 – 1300 cal. years BP) based on *sed*aDNA and pollen and c) Total observed taxa within plant functional groups based on *sed*aDNA and pollen for three time-windows; LGM, Lateglacial and Holocene.

In each of the three time-windows investigated (Figure 5.2), *sed*aDNA detected a greater number of forbs (61 to 81 taxa) than pollen (40 to 46 taxa) and was also able to resolve more graminoid and bryophyte taxa. For woody taxa, *sed*aDNA identified more dwarf shrub taxa within the Holocene interval than were identified by pollen, yet pollen detected a number of trees and/or tall shrub taxa across all three time-windows (15 to 20 taxa) compared to those detected by *sed*aDNA (6 to 8 taxa).

Several shifts in the dominance of plant functional groups is observed in both the *sed*aDNA and pollen records from Lake Bolshoye Shchuchye over the past 24,000 years (Figure 5.3). Terrestrial forbs (e.g. *Papaver*, *Draba*, *Bistorta vivipara*) dominate the *sed*aDNA record, accounting for 60 % of total DNA reads on average, trees and/or tall shrubs (e.g. *Betula*, *Alnus* and Salicaceae) account for 32 % and graminoids (e.g. *Puccinellia*, Poaceae and Agrostidinae) account for 8 %. In contrast, trees and/or tall shrubs (e.g. *Betula*, *Alnus*, *Pinus, Artemisia*) are the most dominant functional group in the pollen record, accounting for 47 % of the total pollen sum, closely followed by forbs (e.g. *Artemisia*, Chenopodiaceae, Brassicaceae, *Rumex acetosa*, *Thalictrum*) at 23 % and graminoids (e.g. Poaceae, Cyperaceae) at 23 %.

Figure 5.3 Summary of the percentage abundance of plant functional groups in the *sed*aDNA and pollen records from Lake Bolshoye Schuchye. Where a) functional groups as a percentage of total *sed*aDNA reads per sample and b) functional groups as a percentage of the total sum of terrestrial pollen and spores (ΣP).

5.4.3.1 Woody Plant Taxa

High similarity is observed in the pattern of occurrence of woody taxa such as *Picea*, *Betula*, *Alnus* and *Dryas* (assumed here to be *D. octopetala* based on biogeographic inference) detected by *sed*aDNA in both pollen percentages versus proportion of *sed*aDNA PCR replicates (Figure 5.4) and pollen concentrations and total DNA reads (Appendix C Supplementary Figure C.2). The distinct, short-lived decline in *Betula* between *ca.* 12,600 - 11,500 cal. years BP revealed by *sed*aDNA is also observed in the pollen record. The scattered occurrences of *Picea* in the *sed*aDNA record between *ca.* 21,000 - 18,000 cal. years BP is mirrored in the pollen record at this time, in addition to the two finds of *Picea abies* stomata at *ca.* 20,400 cal. years BP and 18,800 cal. years BP, which overlap with this interval (Figure 5.4).

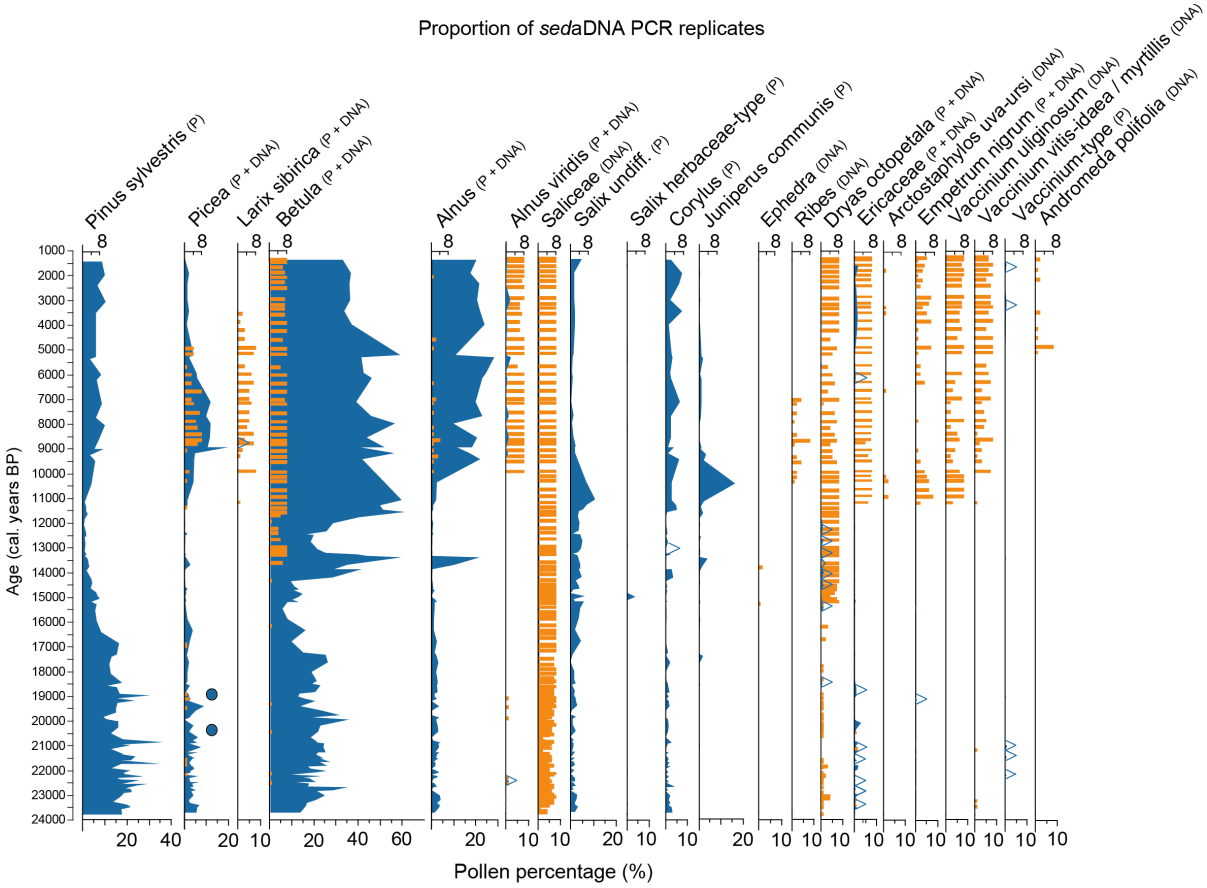

Figure 5.4 Selected woody plant taxa identified by pollen (P) and *sed*aDNA (DNA) at Lake Bolshoye Shchuchye. Taxa are presented as a proportion of *sed*aDNA PCR replicates (orange histogram bars; uppermost x-axis) and pollen percentage (filled blue silhouette; lowermost x-axis). Blue closed circle indicates presence of *Picea abies* stomata. A 10x exaggeration is given for low pollen occurrences (open blue silhouette).

*Sed*aDNA detected *Dryas* throughout the record, including prior to *ca.* 17,000 cal. years BP when this taxon first appears in the pollen record, but it displays its highest values in both the *sed*aDNA and pollen record between *ca.* 15,000 and 10,800 cal. years BP. The pollen record displays a similar pattern to that seen in the *sed*aDNA record for this taxon; very low values between 10,800 and 5000 cal. years BP and subsequent increase again at *ca.* 4,000 cal. years BP (Figure 5.4). Unlike the *sed*aDNA record which was able to resolve a range of individuals (e.g. *Arctostaphylos uva-ursi*, *Empetrum nigrum*, *Vaccinium uligonosum*, *V*. *vitis-idaea*/ *myrtillis*) within the Ericaceae family, only Ericaceae-type, *Empetrum nigrum* and *Vaccinium*-type were able to be resolved by pollen.

5.4.3.2 Herbaceous Plant Taxa

*Sed*aDNA detected a higher diversity of herbaceous plant taxa and, in general, identifications were at a higher taxonomic level than was achieved by pollen (Figure 5.5). Of the main herbaceous plant taxa identified by both *sed*aDNA and pollen (P+DNA on Figure 5.5), many show a similar timing of occurrence. For the grasses, only Poaceae was identified in the pollen record, whereas *sed*aDNA was better able to resolve taxa within this family and shows turnover in taxonomic dominance over time. Spores of the taxon *Dryopteris*-type were detected in high abundance across the record, whereas ferns (including *Dryopteris* sp.) were only detected by *sed*aDNA in the period between *ca.* 9,000 and 4,000 cal. years BP*,* when the coniferous trees *Picea* and *Larix* were also detected in the *sed*aDNA (Figure 5.4).

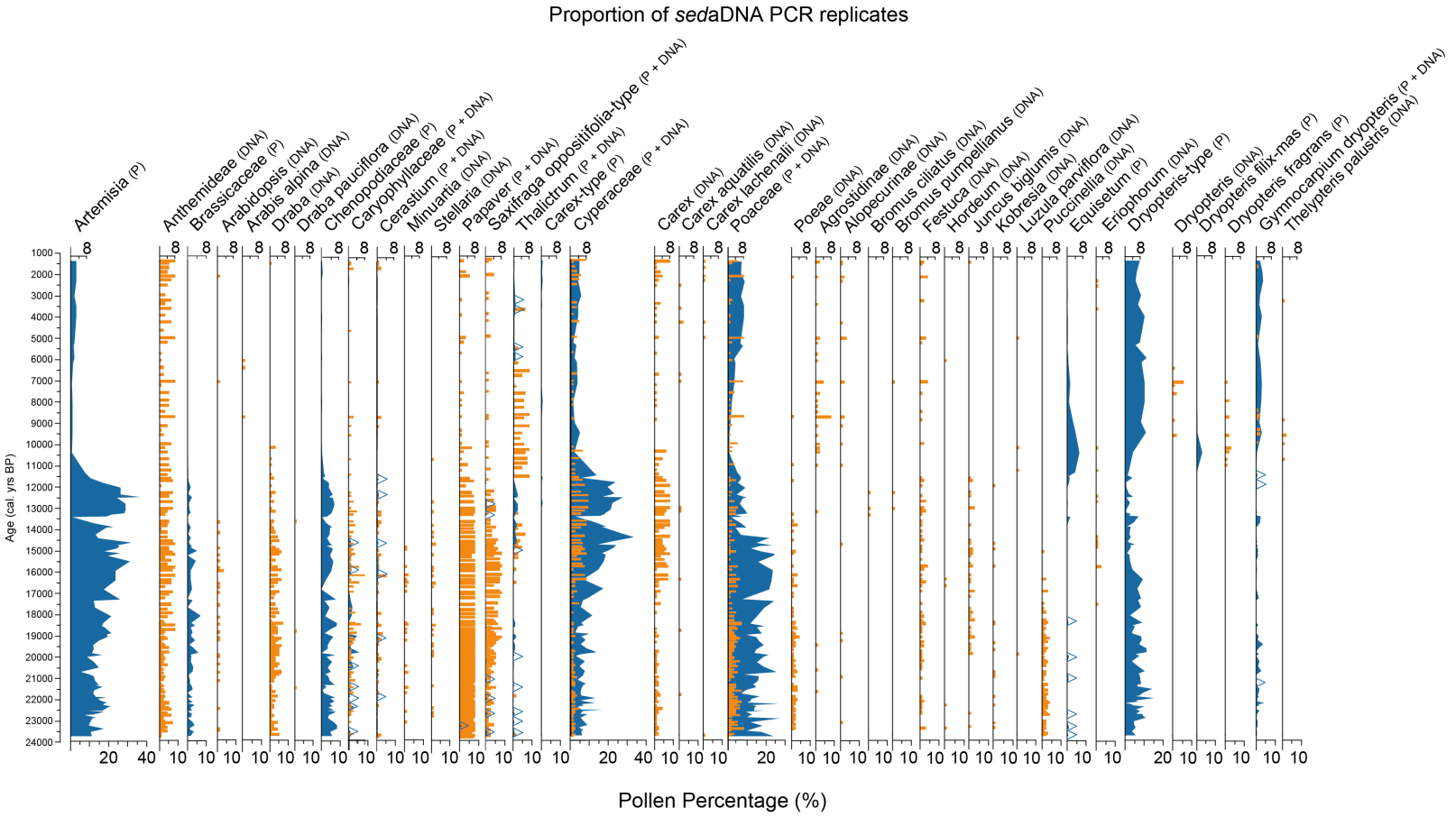

Figure 5.5 Selected forb, graminoid and fern taxa identified by pollen (P) and *sed*aDNA (DNA) at Lake Bolshoye Shchuchye. Taxa are presented as a proportion of *sed*aDNA PCR replicates (orange histogram bars; uppermost x-axis) and pollen percentage (filled blue silhouette; lowermost x-axis). A 10x exaggeration is given for low pollen occurrences (open blue silhouette).

5.4.3.3 Clubmoss and Bryophyte Taxa

In contrast to pollen, a diverse bryophyte flora, which varied over time, was detected by *sed*aDNA (Figure 5.6). A distinct increase in the proportion of PCR replicates for many bryophyte taxa (e.g. *Andreaea*, *Aulacomnium turgidum*, Dicranaceae, Polytrichaceae) is observed in the *sed*aDNA record between *ca.* 15,000 and 12,000 cal. years BP. For clubmosses, *sed*aDNA detected only Lycopodiaceae whereas pollen analysis detected five taxa (*Diphasiastrum*, *Selaginella selaginoides*, *Lycopodium annotinum*, *Lycopodium* undiff. and *Huperzia selago*), although many of them at low pollen abundances.

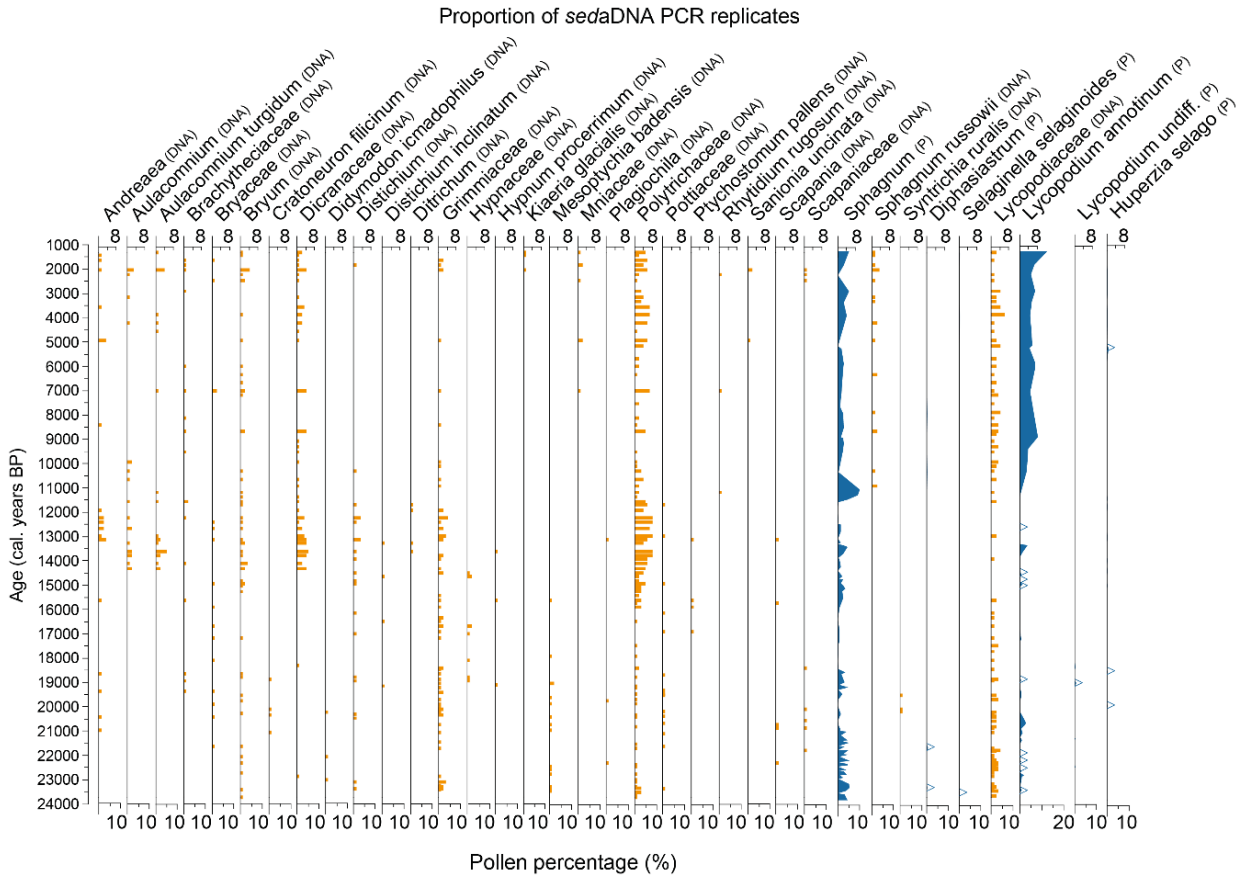

Figure 5.6 Selected clubmoss and bryophyte taxa identified by pollen (P) and *sed*aDNA (DNA) at Lake Bolshoye Shchuchye. Taxa are presented as a proportion of *sed*aDNA PCR replicates (orange histogram bars; uppermost x-axis) and pollen percentage (filled blue silhouette; lowermost x-axis). A 10x exaggeration is given for low pollen occurrences (open blue silhouette).

5.5 Discussion

5.5.1 Interpreting the *Sed*aDNA and Pollen Records

Lake Bolshoye Shchuchye has a large and topographically complex catchment with steep slopes, high rates of erosion and considerable riverine input (Svendsen *et al.,* 2019), all of which probably contribute to the floristically rich *sed*aDNA and pollen records obtained from this site. The DNA signal at Lake Bolshoye Shchuchye likely reflects sediment inputs from a much larger source area than lakes used previously in *sed*aDNA studies (e.g. Epp *et al*., 2015; Pansu *et al*., 2015; Alsos *et al*., 2016, 2018). Both the *sed*aDNA and pollen sources likely receive considerable inputs via the river and/or inwash events, representing catchment-scale rather than regional-scale inputs. In contrast to the *sed*aDNA, the pollen record from Lake Bolshoye Shchuchye also has inputs via long-distance transport of pollen from wind-pollinated, woody taxa which are not present in the lake catchment today but are in the regional flora (e.g. *Pinus*, *Picea*) and others that may be extra-regional (e.g. *Corylus*).

Previous comparisons between *sed*aDNA and pollen have shown limited overlap in taxonomic composition (e.g. Pedersen *et al.,* 2013; Parducci *et al.,* 2015). However, our data from Lake Bolshoye Shchuchye demonstrate considerable overlap in the dominance of plant functional groups over the past 24,000 years (Figure 5.3) and in temporal pattern of occurrence of key plant taxa such as *Picea, Betula, Alnus,* Cyperaceae, Poaceae, Caryllophylaceae, *Gymnocarpium dryopteris* and *Sphagnum* (Figures 5.4 to 5.6). Similar taxonomic resolution was achieved by *sed*aDNA and pollen at Lake Bolshoye Shchuchye, with a high diversity of taxa identified in both records. The two proxies differ, however, regarding the functional groups they are able to identify and/or resolve. *Sed*aDNA was found to be superior at detecting forb, graminoid, bryophyte and dwarf shrub taxa (Figure 5.2), functional groups which are typically poorly resolved and/or represented by their pollen. For example, *sed*aDNA was able to resolve taxa within the Ericaceae family (e.g. *Arctostaphylos uva-ursi*, *Vaccinium uligonosum*, *V. vitis-idaea*/ *myrtillis*) and these taxa were detected in the *sed*aDNA from *ca.* 11,000 cal. years BP which is considerably earlier than the main rise in Ericaceae pollen (*ca.* 5,000 cal. years BP). On the other hand, the pollen record detected a higher diversity of tall shrub and/or tree taxa than *sed*aDNA; this is, however, primarily the result of long-distance transport of wind-pollinated woody taxa. Thus, comparison of the patterns of occurrence of woody, anemophilous taxa in the pollen record with those in the *sed*aDNA record could help to differentiate between local/catchment presence and long-distance dispersal (i.e., probable local/catchment absence).

A number of taxa show limited overlap in their pattern of occurrence and/or representation in the *sed*aDNA and pollen records. Notably, two taxa (*Dryopteris*-type and *Larix*) that show this are known to have unusual representation in palynological records. For example, *Dryopteris*-type fern spores are detected, often in high abundance, throughout the record whereas fern *sed*aDNA was only detected between *ca.* 10,000 and 5,000 cal. years BP. Spores are often not representative of fern abundance as they are highly resistant to breakdown and often mark episodes of erosion (Wilmhurst and McGlone, 2005a,b). Limited overlap occurs between the *sed*aDNA and pollen records for *Larix sibirica* (Figure 5.4), with *sed*aDNA able to clearly resolve the establishment of this tree species in the vicinity of the lake between *ca.* 9,000 and 4,000 cal. years BP, whereas only one pollen grain of *Larix* was identified. In this case, *Larix* is notorious for its low pollen productivity and underrepresentation in pollen records.

Another difference between the *sed*aDNA and pollen records is their long-term trends in floristic richness (Figure 5.2); the pollen record demonstrates a relatively stable rarefied floristic richness with no long-term changes over the past 24,000 years whereas *sed*aDNA demonstrates a sustained, long-term increase in rarefied floristic richness until the early-Holocene (*ca.* 9,000 cal. years BP) where it peaks and then levels out into the later part of the Holocene (Figure 5.2). A dominance of taxa producing high amounts of pollen (e.g. *Pinus*, *Picea*, *Betula*) at the expense of insect-pollinated, entomophilous forbs is a common issue in pollen records and likely leads to an underestimation of floristic richness based on pollen (Lamb and Edwards, 1998; Gajewski, 2015).

Our results from Lake Bolshoye Shchuchye demonstrate that *sed*aDNA can significantly augment the information gained by pollen analysis and *vice versa*. In the following sections, we bring together the insights gained from the two records in terms of plant community composition during the LGM and how it changed over the late-Quaternary interval.

5.5.2 A Diverse Arctic-Alpine Herb Flora During the Last Glacial Maximum (LGM)

The high sedimentation rate (2.2 - 2.6 mm year^{-1}) observed between *ca.* 24,000 – 18,000 cal. years BP (Svendsen *et al*., 2019) in Lake Bolshoye Shchuchye allows an investigation of plant community composition at high temporal resolution during the LGM; something which earlier attempts of vegetation reconstruction in the Polar Urals have found challenging. A mean sampling interval of *ca.* 60 years and *ca.* 100 years was achieved with *sed*aDNA and pollen analysis, respectively, during this interval.

The *sed*aDNA and pollen records together reveal an open, treeless landscape persisted during the LGM and early late-glacial interval (*ca.* 24,000 – 15,000 cal. years BP) characterised by a dominance of arctic-alpine forbs plus grasses, with the sub-shrubs *Salix* and *Dryas* (assumed here

to be *D. octopetala*) also consistently present (Figures 5.3 to 5.6). The vegetation mosaic also included taxa (*Ephedra*, *Puccinellia* and *Artemisia*) that hint at a steppic element in the flora. In contrast, *Kobresia*, which occurs in this portion of the record, characterises a macrofossil fossil flora dated to *ca.* 20,000 cal. years BP from northwest Alaska described by Goetcheus and Birks (2001) as "dry tundra". Several *Kobresia* species are also dominant in high-elevation, herbaceous plant communities of the Tibetan Plateau: "artic-alpine pastures" and "alpine steppe" (Miehe *et al*., 2019), characterized by cool, dry growing seasons. At this far-northern site in the Polar Urals, the steppe-tundra ecosystem that likely covered much of the Russian Arctic throughout the last glacial period (Andreev *et al*., 2003; Kienast *et al*., 2005; Binney *et al*., 2017; Chytrý *et al*., 2019) is characterised more by tundra forbs than steppe forbs, although grass genera such as *Festuca* have large ecological ranges that encompass both biomes and are thus harder to characterise. The *sed*aDNA and pollen records show a similar taxonomic composition during this interval, with the exception that *sed*aDNA permitted the detection of several additional forb and graminoid taxa (Figure 5.2 and 5.6) that were sparse or even absent in the pollen record (e.g. *Papaver*, *Draba*, *Saxifraga oppositifolia*, *Bistorta vivipara*, *Puccinellia*, *Festuca* and *Juncus biglumis*).

In general, the pollen assemblages of this interval is comparable with those observed in the pollen record from Lake Gerdizty (pollen zones G-IIa and b), located in the eastern foothills of the Polar Urals to the southeast of Lake Bolshoye Shchuchye (Svendsen *et al*., 2014; see Appendix C Supplementary Figure C.3). This is the only record, to date, from this part of the northern Urals that extends back to the LGM, yet it has a low temporal resolution and the authors conclude that a hiatus during the LGM interval could not be ruled out (Svendsen *et al*., 2014). Thus, the combined *sed*aDNA and pollen records from Lake Bolshoye Shchuchye provide a unique insight into plant community composition within an area that most likely provided refuge for species that were displaced by ice during the peak of the last glacial period.

The scattered occurrence of *Picea* between 21,000 and 18,000 cal. years BP (~1700 - 1010 cm depth), which is replicated in the *sed*aDNA, pollen and the observation of two *Picea abies* stomata (Figures 5.4), raises questions. It is possible that the occurrence of *Picea* pollen during the LGM interval at Lake Bolshoye Shchuchye originates from long-distance dispersal. The present-day northern limit for *Picea* is approximately 40-50 km south of Lake Bolshoye Shchuchye, and isolated stands occur in the lowlands of the Pechora basin to the southwest of the lake (Pravdin, 1975; Kremenetski *et al*., 1998). Studies of *sed*aDNA, pollen and historical vegetation maps in Scotland suggest that *sed*aDNA of *Picea* does not derive from pollen (Sjögren *et al*., 2017), and finds of its stomata within sediments are considered to represent strong evidence for local growth (Ammann *et al*., 2014). Pollen grains of *Picea* do contain some cpDNA, however, which could be introduced into the sediment matrix. Alternatively, the *Picea sed*aDNA could represent contamination by DNA

reagents, laboratory materials or by pollen carried into the DNA laboratories. *Picea* DNA was found in the negative extraction and water controls (Appendix C Supplementary Table C.2). Unfortunately, the preservation of plant macrofossil remains is poor within the retrieved core from Lake Bolshoye Shchuchye, with examination yielding only fragmented, largely unidentifiable plant remains, so the *Picea* data remain equivocal.

A survey of plant macrofossils from across northern Eurasia demonstrate early finds of *Picea* from 25,000 cal. years BP in the southern part of the Ural Mountains, but sites in the Polar Urals do not show *Picea* presence until around 13,000 cal. years BP (Binney *et al*., 2009). Except for Saliceae, the *sed*aDNA detected no other tree and/or tall shrub taxa during this interval or other thermophilic taxa which could support the assertion of favourable growing conditions for *Picea* at this time. Re-sedimentation of older material might explain the scattered occurrences of its pollen, *sed*aDNA and stomata at roughly the same time (but not exactly the same sample depths), yet there is no evidence of re-sedimentation in the sedimentology of the retrieved core or even disturbance of the annual laminations (varves) during this interval (Regnéll *et al*., 2019).

5.5.3 Compositional Changes in Vegetation Through the Pleistocene-Holocene Transition

Large changes in the dominance of plant functional groups and numerous species additions accompanied the Pleistocene-Holocene transition at Lake Bolshoye Shchuchye. A distinct increase in Cyperaceae is observed in the *sed*aDNA and pollen record at *ca.* 17,000 cal. years BP (Figure 5.5), coincident with an increase in bryophyte taxa such as *Bryum*, *Aulacomnium,* Polytrichaceae and Dicranaceae in the *sed*aDNA record (Figure 5.6). This may reflect an early (by hemispheric standards) shift to moister conditions and the onset of a prolonged postglacial transition. At this time, recession of the Barents-Kara ice sheet was well underway. Ice had by then retreated from the mainland (Hughes *et al*., 2016), likely leading to an increase in moisture availability within the Polar Urals themselves.

By around *ca.* 15,000 cal. years BP, the number of taxa detected within the *sed*aDNA record greatly increases, reflecting the establishment of shrub-tundra communities of *Salix*, *Betula* and *Dryas octopetala* (Figure 5.4), with a diversity of forbs such as *Bistorta vivipara*, *Eritrichium, Pedicularis oederi* and *Oxytropis* (Figure 5.5). Dwarf shrubs reach their maximum proportional abundance in the *sed*aDNA between *ca.* 14,000 and 11,000 cal. years BP (Figure 5.3); however, this functional group is represented by only *Dryas* (*D. octopetala*), and thus the pattern is somewhat unrepresentative of the majority of dwarf-shrub taxa. *Ephedra*, *Puccinellia* and *Artemisia* decline in both the *sed*aDNA and pollen, perhaps reflecting an increase in moisture availability and/or competition from *Betula* and *Salix*.

A rapid yet short-lived decline in *Betula* between *ca.* 12,600 and 11,800 cal. years BP is evident in both the *sed*aDNA and pollen records from Lake Bolshoye Schuchye (Figure 5.4). At the same time, *Alnus* disappears from the pollen record and percentages of *Artemisia* increase. We also note a small increase in the *sed*aDNA of forbs such as *Geum*, *Cardamine*, *Valeriana*, *Hedysarum hedysaroides* and *Castilleja* during this interval (see Clarke *et al*., 2019). The timing of these changes coincides with the Younger Dryas (YD) chronozone (*ca.* 12,900 - 11,600 cal. years BP; Björck *et al.,* 1998), which, although a well-known climatic event in the Northern Hemisphere, differs in terms of its strength and extent across European Russia and Siberia (Andreev *et al*., 1997; 2003; Khotinsky and Klimanov, 1997; Paus *et al*., 2003; Henriksen *et al*., 2008; Svendsen *et al*., 2014; Väliranta *et al*., 2006).

The signals of the YD event are muted in the current biostratigraphic record from Lake Bolshoye Shchuchye, but the low temporal resolution of *sed*aDNA and pollen samples over this interval hinder the detection of clear vegetation changes in periods as short-lived as the YD chronozone. In the current analysis, only five *sed*aDNA samples and three pollen samples span the formally assigned ages of the YD given by Björck *et al*. (1998). The YD interval is represented by around 80 cm of sediment (Regnéll *et al.,* 2019; Svendsen *et al.,* 2019) and thus the sediments have the potential to provide a high-resolution record of vegetation change over this chronozone (but this was not the principal aim of the current study).

The recovery of *Betula* to high values in both the *sed*aDNA and pollen records around *ca.* 11,500 cal. years BP coincides with the first appearance of the dwarf shrubs *Arctostaphylos uva-ursi*, *Empetrum*, *Vaccinium uligonosum* and V. *vitis-idaea* / *myrtillis* in the *sed*aDNA (Figure 5.4). This probably reflects warmer climatic conditions and more stabilised soils near or at the onset of the Holocene. Detectability of dwarf shrubs in the pollen record is limited, although Ericaceae pollen is detected but shows its main increase much later than in the *sed*aDNA *ca.* 5,000 cal. years BP (Figure 5.4).

5.5.4 Expansion of Woody Plant Taxa in the Holocene

The early- to middle-Holocene interval (*ca.* 11,800 – 4,000 cal. years BP) saw an increasing dominance of woody taxa in both the *sed*aDNA and pollen records from Lake Bolshoye Shchuchye. It is during this interval where we can clearly see the valuable contribution *sed*aDNA can make for reconstructing past community composition: ability to identify a range of herbaceous taxa, little or no "swamping" by dominant woody taxa, estimates of floristic richness not limited by pollen-counting time, and the ability to resolve the establishment and duration of *Larix sibirica* populations.

The coniferous trees *Picea* and *Larix* established within the lake's catchment from *ca.* 9000 cal. years BP, along with several fern taxa (*Dryopteris fragrans*, *Gymnocarpium dryopteris* and *Thelypteris palustris*). At the same time, many boreal herbs such as *Aconitum lycoctonum*, *Alchemilla*, *Chamerion angustifolium*, *Filipendula ulmaria*, *Galium boreale* show first appearances in the *sed*aDNA, suggesting a change in the composition of the understory linked to conifer expansion. The timing of forest tree establishment (9000 cal. years BP and continuing until ~4000 cal. years BP) is concurrent with their known northward treeline expansion into the lowlands of the Polar Urals and surrounding areas, which many authors consider as representing warmer summer temperatures during the regional Holocene Thermal Maximum (Surova *et al*., 1975; Kremenetski *et al*., 1998; Kaakinen and Eronen, 2000; Salonen *et al*., 2011; Binney *et al*., 2017). Moreover, the timing of the increase in *Picea sed*aDNA and pollen is comparable with the main rise in *Picea* in nearby pollen records from the region (Appendix C Supplementary Figure C.3). During this period, when cold-adapted taxa would have been most disadvantaged, most arctic-alpine taxa were detected, at least in small quantities, in the *sed*aDNA, strongly suggesting their persistence in the lake catchment from full-glacial times (Clarke *et al*., 2019).

Coniferous forest (*Larix*, *Picea*) and certain elements of its understory vegetation (e.g. *Dryopteris fragrans*, *Gymnocarpium dryopteris*, *Filipendula ulmaria*, *Anthriscus sylvestris*, *Galium boreale*) withdrew from the catchment of Lake Bolshoye Shchuchye around 4000 cal. years BP and the vegetation subsequently reverted back to shrub tundra with *Betula*, *Alnus*, *Salix* and dwarf-shrubs such as *Arctostaphylos uva-ursi*, *Vaccinium uligonosum*, *Empetrum* and *Dryas*, similar to the present-day vegetation mosaic of the Polar Urals. A diverse herb flora persisted despite the withdrawal of forest, characterised by a dominance of forbs such as *Aconitum lycoctonum*, *Bistorta vivipara*, *Lagotis glauca*, *Oxyria digyna* and graminoids such as *Carex*, *Agrostidinae*, *Eriophorum* and *Festuca.* Palaeoenvironmental records from adjacent areas also indicate the loss of forest taxa, with the authors concluding that climatic deterioration occurred around this time (Surova *et al*., 1975; Andreev *et al*., 1998, 2001; Serebryanny *et al*., 1998; Koshkarova *et al*., 1999; Panova and Jankovska, 2000; Paus *et al*., 2003).

5.5.5 Dynamics of Plant Functional Groups and Compositional Turnover

Species and/or plant community composition is likely to play an important role in determining ecosystem function because species differ in terms of their functional traits. For example, the introduction of a specific species or functional group can alter plant community composition through varying competitive abilities and facilitative effects (Scherer-Lorenzen *et al*., 2003; Odling-Smee *et al.* 2013), which may in turn affect ecosystem functioning. The *sed*aDNA record from Lake Bolshoye Shchuchye brings new insights into the dynamics within and between plant functional

groups over the past 24,000 years, being able to resolve more forb, graminoid, dwarf shrub and bryophyte taxa in general than pollen (Figures 5.4 to 5.6).

In addition to shifts in the dominance of plant functional groups over time (Figure 5.3), the *sed*aDNA record reveals compositional turnover within plant functional groups themselves (Figures 5.4 to 5.6). For example, there is turnover between the different grass taxa detected; a dominance of *Puccinellia*, Poeae, *Kobresia* and *Festuca* in the full-glacial, *Juncus biglumis*, *Festuca* and *Bromus* spp. in the Lateglacial, and a dominance of Agrostidinae during the Holocene interval. This is rarely, if ever, detected in traditional palaeorecords as pollen of grasses are barely distinguishable below family level, as demonstrated in the pollen stratigraphy from Lake Bolshoye Shchuchye (Figure 5.4).

5.6 Conclusions

The 24-m long sediment sequence obtained from Lake Bolshoye Shchuchye enabled a detailed reconstruction of long-term plant community dynamics during and since the Last Glacial Maximum (LGM) without any apparent breaks in sedimentation over the 24,000-year period investigated. Contrary to earlier comparative *sed*aDNA studies, we show considerable overlap in the nature of compositional changes and the pattern of occurrence of key taxa identified by *sed*aDNA and pollen. We demonstrate that the information gained by *sed*aDNA and pollen augment each other, with each proxy differing in terms of their ability to detect and/or resolve individuals within different plant functional groups.

Herb-tundra vegetation with a rich diversity of forbs, such as *Papaver, Draba, Bistorta vivipara* and *Saxifraga oppositifolia,* and graminoids such as *Puccinellia, Festuca* and *Juncus biglumis* persisted throughout the Last Glacial Maximum (LGM; *ca.* 24,000 - 17,000 cal. years BP). By 17,000 cal. years BP, mesic sedge communities established, followed a little later by the establishment of shrub-tundra (*Salix*, *Betula*, *Dryas*) from 15,000 cal. years BP. This was interrupted by a short-lived response to Younger Dryas cooling around 13,000 cal. years BP, although the low temporal resolution of samples during this chronozone limits an interpretation of vegetation response. Forest trees *Larix* and *Picea* became dominant elements of the vegetation between 9000 and 4000 cal. years BP, alongside many boreal herbs. Coniferous forest withdrew around 4000 cal. years BP and the vegetation subsequently reverted to dwarf-shrub tundra with a diverse herb flora, comparable to the present-day vegetation mosaic of the Polar Urals.

The *sed*aDNA record revealed several features of late Quaternary vegetation dynamics which the pollen stratigraphy and earlier palaeorecords in the region typically failed to detect; a sustained, long-term increase in floristic richness since the LGM with the largest step change

occurring in the early Lateglacial interval, a turnover in grass and forb genera over the Pleistocene-Holocene transition, persistence of a diverse arctic-alpine flora, and a diverse and variable bryophyte flora through time.

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5.8 Author Contributions

C.L.C., A.B., I.G.A., M.E., J.I.S., H.H. and J.M. contributed to the concept and designed the study. J.I.S., H.H. and J.M. surveyed and cored Lake Bolshoye Shchuchye and constructed the age-depth model. Pollen analysis was conducted by A.B. with contributions from A.P. C.L.C. subsampled the sediment core and extracted the DNA and L.G. amplified the DNA and performed the initial sequence analysis and taxa assignment. C.L.C. further analysed the DNA data and performed the post-identification filtering with contributions from I.G.A and M.E. Ecological and methodological expertise was contributed by A.B., I.G.A., M.E., A.P. and P.D.M.H. J.I.S., H.H. and J.M. contributed their expertise on the geomorphological background and glacial history of the site. C.L.C. compared the *sed*aDNA and pollen data, created the visualizations and wrote the manuscript, which M.E., I.G.A., A.B. and P.D.M.H. commented on.

Chapter 6 Insights into Late-Pleistocene Vegetation of Interior Alaska from Sedimentary Ancient DNA (*Sed*aDNA) (Paper IV)

This paper is currently in the pre-submission stage of the publication process:

Clarke, C.L., Bigelow, N.H., Edwards, M.E., Alsos, I.G., Heintzman, P.D., Lammers, Y. and Reuther, J.D. 2019: Insights into late Pleistocene vegetation of interior Alaska from sedimentary ancient DNA (*sed*aDNA). *Manuscript in preparation*.

This work has been presented as:

Clarke, C.L., Bigelow, N.H., Edwards, M.E., Hildebrandt, K., Potter, B., Lopez, A., Olson, L., Lyon, B., Heintzman, P., Lammers, Y., Alsos, I.G and Reuther, J.D. 2019: Ancient DNA in unfrozen archaeological sediments from interior Alaska. In: *20th Congress of the International Union for Quaternary Research (INQUA)*. Dublin. 25th July. Poster presentation.

6.1 Preface

In this chapter, I present a manuscript which is currently in the pre-submission stage of the publication process. It provides an overview of a 4.5-m long *sed*aDNA record obtained from Chisholm Lake, interior Alaska, which spans the last *ca.* 15,000 cal. years BP. The manuscript has a particular focus on the composition of plant communities during the first 1000 cal. years BP of the record, prior to the establishment of *Betula* shrubs. I discuss the new insights gained by *sed*aDNA analysis for resolving plant community composition of the Lateglacial 'herb zone' in interior Alaska.

Due to various delays with obtaining funding for this work, additional radiocarbon ages for this record are pending. However, an initial age-depth model (based on five radiocarbon ages) is provided within this manuscript. The basic themes of the conclusions presented here will not change with additional radiocarbon ages, but the timings of key vegetation changes over the late-Quaternary interval will likely be better refined and thus able to be compared with other records. Chisholm Lake is located in close proximity to several key archaeological sites in the Tanana River Valley of interior Alaska; thus, further study of this site promises to improve our understanding of the natural resources available to early humans in interior Alaska.

6.2 Abstract

Knowledge of the composition of the late-Quaternary full-glacial flora within ice-free northern regions has often been constrained by issues of low pollen concentrations and the ability to taxonomically resolve herbaceous taxa below family level and/or genus level based on their pollen. Here we present a new record of plant community composition and floristic diversity spanning the past *ca.* 15,000 cal. years BP based on ancient DNA extracted from the sediments (*sed*aDNA) of Chisholm Lake, located within the Tanana Valley of interior Alaska. In total, we identified 167 vascular plant and 14 bryophyte taxa based on *sed*aDNA, of which 45 % were identified to the species level, 39 % to genus and 16 % to a higher taxonomic level. Prior to *ca.* 14,000 cal. years BP, herbaceous communities prevailed within the lake catchment which were characterised with a dominance of forbs (e.g. Anthemideae, *Hedysarum*, *Potentilla*, *Eritrichium*, *Plantago*, *Bupleurum*) and graminoids (e.g. Agrostidinae, *Bromus pumpellianus, Festuca, Calamagrostis* and *Equisetum*). The full-glacial plant community also contained two taxa (*Anemone patens* and *Puccinellia*) that can typify (though are not limited to) modern boreal steppe communities. After *ca.* 14,000 cal. years BP, shrub-tundra communities of *Betula, Arctostaphylos uva-ursi* and *Arctous* established and largely replaced the herb-tundra communities, likely in response to an increase in effective moisture. The *sed*aDNA record documents high turnover in plant community composition over the past *ca.* 15,000 cal. years BP with the lake catchment supporting a diverse range of plant communities over time.

6.3 Introduction

Interior Alaska, the central inland area between the Alaska Range to the south and the Brooks Range to the north, is an important region for understanding long-term vegetation dynamics due to its long and complex Quaternary history (Edwards and Brubaker, 1986; Anderson *et al*., 1988; Brubaker *et al*., 2005). Like much of Beringia, of which it forms a part, interior Alaska remained ice-free during the Pleistocene glaciations while many northern high latitude regions were covered by extensive ice sheets (Dyke *et al*., 2002; Clark *et al*., 2009; Hughes *et al*., 2016). The region thus provided refuge for a high latitude flora and fauna displaced by ice and may have even supported refugial populations of boreal trees during the last glacial interval (Hopkins *et al*., 1981; Brubaker *et al*., 2005; Anderson *et al*., 2010; Edwards *et al*., 2014).

Possibilities for a unique view into this full-glacial (*ca.* 25,000 - 15,000 cal. years BP) landscape and how it changed with climate warming in the Lateglacial (*ca.* 15,000 - 11,800 cal. years BP) have inspired palaeoecological studies in the region since the 1960's (e.g. Guthrie, 1968; Ager, 1975; Bigelow and Edwards, 2001; Carlson and Finney, 2004; Finkenbinder *et al*., 2014). In most

records, the floristic composition of the prevailing Pleistocene vegetation mosaic is poorly resolved, however, with reconstructions often being constrained by limits of pollen and plant macrofossil data (e.g. productivity, preservation, deposition and taxonomic resolution). Several detailed pollen records (i.e counted at a high pollen sum) have been developed from the region (Ager, 1975; Cwynar, 1982; Ritchie and Cwynar, 1982; Anderson *et al*., 1988) which have made important contributions to the ongoing debate concerning the composition of the Pleistocene flora. However, "swamping" of pollen records by dominant, anemophilous woody taxa at the expense of insect-pollinated herbs often limits interpretations of plant community composition derived from pollen records (Sugita, 1994; Gajewski, 1995). The objective of this study is to improve knowledge of the composition of the late-Pleistocene flora of interior Alaska with the use of sedimentary ancient DNA (*sed*aDNA) analysis from Chisholm (also called Lost) Lake, located in the Tanana River Valley (Figure 6.1).

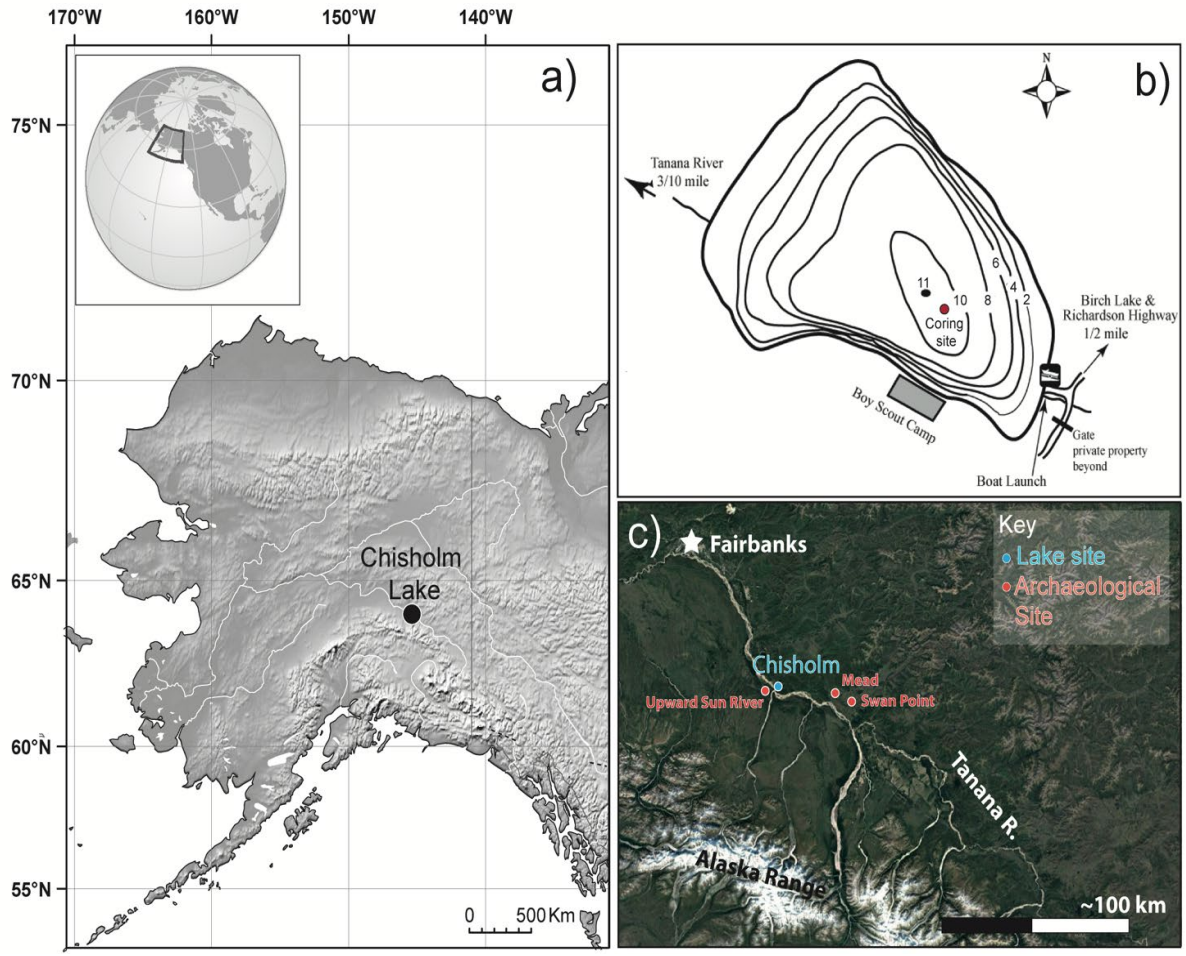

Figure 6.1 a) Location of Chisholm Lake within the Tanana River Valley of interior Alaska with b) a bathymetric map of the lake basin with lake depth (in metres) and location of the coring site for the Chis17A-B core indicated. Map sourced and adapted from the Alaska Department of Fish and Game (http://www.adfg.alaska.gov). c) Location of Chisholm

Lake in relation to key archaeological sites within the Tanana River Valley, including Upward Sun River (USR; Potter *et al*., 2014, 2017).

Between 18,000 and 15,000 cal. years BP, the climate of interior Alaska was cooler overall (Bartlein *et al*., 1991, 2014) and far drier than present, with precipitation possibly half that of modern values (Barber and Finney, 2000). Lake levels within the Tanana Valley were very low and some lake basins were entirely dry (Nakao and Ager, 1985; Abbott *et al*., 2000b; Edwards *et al*., 2000; Finkenbinder *et al*., 2014). Regionally, glaciers were restricted to the Alaska Range to the south of Chisholm Lake and a few, small glaciers within the Yukon-Tanana Uplands to the north (Coulter *et al*., 1965; Kaufman and Manley, 2004). The nature of the treeless vegetation which prevailed within unglaciated areas of Alaska during the late Pleistocene has been discussed widely in the literature (e.g. Colinvaux, 1964; Guthrie, 1982; Matthews, 1982; Ritchie and Cwynar, 1982; Anderson and Brubaker, 1994; Brubaker *et al*., 2005). Early studies suggested that vegetation across the Beringian landmass at this time resembled a productive grassland, which supported a diverse megafauna of grazers such as bison, horse and woolly mammoth, referred to as 'steppe-tundra' or 'mammoth steppe' (Guthrie, 1968, 1982, 1990; Matthews, 1982). The vegetation has no widespread modern analogue, but it might have contained elements of the modern topographic mosaic. This includes south-facing mountain slopes and river bluffs in interior Alaska and the Yukon that support boreal steppe (Murray *et al*., 1983; Lloyd *et al*., 1994; Walker *et al*., 2001; Zazula *et al*., 2006), or the steppe-tundra ecotone found (rarely) at higher elevation (Edwards and Armbruster, 1989).

Regarding, palaeo-productivity estimates, low pollen influx rates during the full-glacial interval at sites across interior Alaska and the Yukon Territory suggest a sparsely vegetated landscape which more likely resembles herb-tundra and fellfield habitats rather than temperate grassland communities (Cwynar and Ritchie, 1980; Cwynar, 1982). This led to the notion of a 'productivity paradox' (Hopkins *et al*., 1982; Yurtsev, 2001), namely, the discrepancy between a highly diverse herbivorous megafauna at this time and an unproductive ecosystem type which could not have supported it. To some extent, this debate still endures today, although there is now a general acceptance that the full-glacial flora in northern ice-free regions most likely comprised elements of both steppe and herb-tundra ecosystem types, and displayed local habitat heterogeneity, including an elevational mosaic (Goetcheus and Birks, 2001; Kienast *et al*., 2001, 2005; Zazula *et al*., 2006; Kurek *et al*., 2009).

Resolving the floristic composition and/or diversity of the Pleistocene palaeoenvironment is particularly challenging using traditional techniques of palynology. Pollen grains are variably

resolved taxonomically, and several diverse groups, including Poaceae and Cyperaceae, are often barely distinguishable below family level (Bigelow, 2013). Plant macrofossils, in contrast, are often identifiable to genus or species level, but preservation of identifiable remains is often highly variable among taxa and sites (Bigelow *et al*., 2013). Ancient DNA extracted from sediments (*sed*aDNA) has the potential to provide more detail on floristic composition and diversity of the full-glacial vegetation mosaic in interior Alaska, enhancing current knowledge derived by pollen and plant macrofossil records (e.g. Jørgensen *et al*., 2012; Willerslev *et al*., 2014; Alsos *et al*., 2016; Pedersen *et al*., 2016; Parducci *et al*., 2017; Zimmerman *et al*., 2017a; Clarke *et al*., 2018).

In a large-scale *sed*aDNA study of 242 samples from 21 sites across the circumpolar Arctic, Willerslev *et al*. (2014) concluded that the full-glacial Arctic vegetation consisted of dry steppe-tundra with a dominance of forbs. The samples analysed for *sed*aDNA derived from different sedimentary environments and temporal periods, with many representing only short snapshots in time, which were then combined to create a composite record of Pleistocene vegetation changes. Knowledge of whether similar trends of increasing floristic richness and patterns in plant community composition since the last glacial interval are seen within a single landscape represented by a single site is lacking.

More information on the full-glacial flora and the compositional changes which occurred during the transitional period between the Pleistocene and Holocene in interior Alaska is crucial for understanding the environmental context and nutrition of the Pleistocene megafauna (Guthrie, 1990; Mann *et al*., 2013) and the natural resource use of early human populations within interior Alaska (Dixon, 2001; Potter *et al*., 2017; Wygal *et al*., 2018). Chisholm Lake (Figure 6.1) is located in close proximity to several key archaeological sites, including Upward Sun River (USR) which, to date, contains the earliest human remains in North America, dated to 11,500 years ago (Potter *et al*., 2014, 2017). These early humans entered the region on the cusp of environmental changes over the Pleistocene-Holocene transition which affected the distribution of plants, animals and the location of water bodies (e.g. Barber and Finney, 2001; Edwards *et al*., 2000). The lake is therefore an ideal site for understanding landscape changes faced by early humans in interior Alaska and the availability of plant resources at this time.

This study represents the first *sed*aDNA-based investigation of floristic composition and diversity of the late-Pleistocene flora in interior Alaska, in this case from the sediments of Chisholm Lake within the Tanana River Valley. A pollen record from the same lake was published by Tinner *et al*. (2006) and documents low pollen concentrations between *ca.* 15,000 and 13,500 cal. years BP, prior to the establishment of *Salix* and *Betula* shrub-tundra. Around 15 terrestrial plant taxa, many at a low concentration, were detected within the lowermost pollen zones (LL-1 to 3) but of those

that were detected, graminoids such as Poaceae, Cyperaceae and *Artemisia* dominated, with some occurrences of forbs such as *Potentilla*, *Bupleurum*, *Plantago canescens* and Chenopodiaceae. This early period (zones LL-1 to 3 of Tinner *et al*., 2006) provides an interesting target to assess the value of the *sed*aDNA metabarcoding (Taberlet *et al*., 2007) approach. A new 4.5-m long sediment core retrieved in 2017 which spans the last *ca.* 15,000 cal. years BP is the focus of this study.

6.3.1 Study Site

Chisholm Lake (64°18'8.345" N, 146°41'17.705" W) is located in the Tanana River valley north of the Alaska Range at 240 m a.s.l. (Figure 6.1). The lake has a surface area of ~31 ha and a catchment area of ~780 ha. It is surrounded by bedrock ridges to the south, east and north, whereas the west end is formed by a dam of outwash sand and gravel deposited by the Tanana River during the late Pleistocene (Ager, 1975). Extensive Quaternary sand dune and loess deposits exist throughout the Tanana valley (Péwé, 1975) and the region is underlain by discontinuous permafrost (Jorgenson *et al*., 2008).

Present-day climate conditions are characterised as boreal and continental, with mean July and January temperatures of 15.6 °C and -20 °C, respectively, recorded at Big Delta (305 m a.s.l., ~5 km south of Chisholm Lake) for the period 1961-1990 (WorldClimate, 2012). The mean annual temperature is − 2.3 °C, and mean annual precipitation 304 mm. The catchment vegetation comprises boreal forest, dominated by *Picea glauca* (white spruce) with *Betula neoalaskana* (tree birch) *Populus tremuloides* (aspen) and a few isolated *Larix laricina* (tamarack) trees. In the flatlands, south of the Tanana River where permafrost is usually close to the soil surface, *Picea mariana* (black spruce) and *Larix laricina* dominate the forest composition.

6.4 Material and Methods

6.4.1 Sediment Retrieval and Sampling

A 4.5-m long sediment core (Chis17A-B) was retrieved in April 2017 from the winter ice surface using a combination of a Bolivia corer with 7.5-cm-diameter polycarbonate tubes for the uppermost sediments and 7.5-cm-diameter aluminium tubes for deeper sediments. For the basal sediments, a 5-cm-diameter modified Livingstone piston corer (Wright, 1967) was used and sediments were extruded, immediately wrapped and placed in acrylonitrile-butadiene-styrene (ABS) pipe halves. Lake depth at the coring site (WGS84, 64.30229 N, -146.68623 W) was 10.5 m, as measured by a single-beam echo-sounder (Echotest II Plastimo).

After retrieval of the sediments, all tubes and core sections were sealed immediately to minimise the risk of contamination by airborne or other modern environmental DNA. An exotic DNA tracer, comprising concentrated extracted DNA from a modern specimen of the cactus family (Cactaceae) mixed with DNA-free water, was sprayed on the surface of all coring equipment and core tubes. This was used as a means of detecting any issues of contamination by exogenous DNA present on coring equipment; we did not detect any Cactaceae DNA in any of the sampled analysed and conclude there was no contamination from any of the equipment or core tubes used.

The core sections were transported and stored at 4 °C in the cold storage facility at the Alaska Quaternary Center, University of Alaska Fairbanks (UAF). Core sections were opened by longitudinal splitting. One half was used for subsampling, and the other half kept for archival purposes. The 4.5-m core was sampled at around 4-cm resolution, with 2-cm resolution achieved within key periods of interest, within a dedicated ancient DNA clean-room facility at the University of Alaska Museum of the North (UAMN). Sterile tools, a full bodysuit, facemask and gloves were used. Following the protocol described by Parducci *et al*. (2017), the outer 10 mm of sediment was avoided or discarded and an ~20-g subsample was retrieved from inside the freshly exposed centre only.

6.4.2 Lithological Analyses

Upon longitudinal splitting of the core sections (see above), a basic lithostratigraphic core log was described based on visual interpretation. Subsamples of 2 cm^3 were taken for loss-on-ignition (LOI) analyses at contiguous 1-cm intervals throughout the core using a volumetric sampler. Samples were weighed in crucibles and dried overnight at 100 °C before dry weight was determined (Chambers *et al*., 2011). Samples were then ignited at 550 °C for 2 h, placed in a desiccator to cool to room temperature and reweighed. Total LOI was calculated as the percentage loss of dry weight after ignition (Heiri *et al*., 2001). Magnetic susceptibility was measured at 1-cm intervals on the archival core halves using a Bartington point sensor on the Geotek Ltd. Multi-Sensor Core Logger using a 10-s exposure time.

6.4.3 Radiocarbon Dating and Age-Depth Model Construction

Six samples of terrestrial plant macrofossils were picked and sent for accelerator mass spectrometry (AMS) radiocarbon (^{14}C) dating at the Keck-Carbon Cycle AMS Facility, University of California, Irvine. All radiocarbon ages were calibrated according to the terrestrial IntCal13 curve (Reimer *et al*., 2013), and an age-depth relationship was established using the Bayesian framework calibration software 'Bacon' (v. 2.2; Blaauw and Christen, 2011), which was implemented in R v. 3.2.4 (R Core Team, 2017).

6.4.4 Macroscopic Charcoal Analysis

Subsamples of 1 cm^3 were taken for macroscopic (>125 µm) charcoal analysis at 5-cm intervals using a volumetric sampler. Samples were prepared following standard protocols (Stevenson and Haberle, 2005), with the exception that they were left to soak in a 6 % hydrogen peroxide (H_2O_2) solution whilst being heated at 50 ^oC for 48 h. Following bleaching, samples were rinsed over a 125 µm sieve to isolate macroscopic fragments, transferred into a grooved perspex sorting tray (Bogorov tray with a groove 5 mm deep and 5 mm wide) and counted using a stereomicroscope, with samples initially scanned at 20 x magnification and identification of charcoal fragments confirmed at 40 x magnification. All macroscopic charcoal fragments present within the sample were counted. Concentrations (particles/ cm^3) were multiplied by sediment accumulation rate (cm/ yr^{-1}) to obtain charcoal accumulation rate (CHAR; particles cm^{-2} yr^{-1}).

6.4.5 DNA Extraction, Amplification, Library Preparation, and Sequencing

DNA extraction followed a modified version of the Qiagen DNeasy PowerSoil® DNA Isolation Kit which included the addition of Proteinase-K and Dithiothreitol (DTT) to the cell lysis mix with Solution C1 (Heintzman *et al*., in prep). DNA was extracted from 120 sediment subsamples and 12 negative extraction controls, which contained no sediment and were used to monitor for contamination, within a dedicated ancient DNA clean-room facility in the Palaeoecology Laboratory at the University of Southampton. Aliquots of DNA extract were then transferred to the ancient DNA facility at Tromsø University Museum (TMU), Norway, for metabarcoding. PCR amplification, PCR product pooling and purification, and sequencing follow the protocols of Alsos *et al*. (2016), unless otherwise stated. Each DNA extract and negative extraction control was independently amplified using uniquely tagged generic primers that amplify the *trn*L P6 loop of the plant chloroplast genome (Taberlet *et al*., 2007). Four negative PCR controls, which contained no DNA template, were run alongside the sediment samples and negative extraction controls. Every sample and all negative controls underwent eight individual PCR replicates.

Pooled and cleaned PCR products were then converted to three Illumina-compatible amplicon libraries using the single-indexed, PCR-free MetaFast method. These libraries were then sequenced on the Illumina HiSeq-2500 platform for 3 x 125 cycles at the NextGen Sequencing Facility (NGSF) at the Department of Medical Biology, UiT- The Arctic University of Norway.

6.4.6 Sequence Analysis and Taxonomic Assignments

Next-generation sequence data were filtered using the OBITools software (Boyer *et al*., 2016; http://metabarcoding.org/obitools/doc/index.html) implemented within R v. 3.2.4. (R Core Team,

2017). Taxonomic assignments were performed using the *ecotag* program (Boyer *et al*., 2016) by matching sequences against a local taxonomic reference library comprising 815 arctic and 835 boreal vascular plant taxa, and 455 bryophytes (Sønstebo *et al*., 2010; Willerslev *et al*., 2014; Soininen *et al*., 2015). Sequences were then matched to a second reference library generated after running *ecopcr* on the global EMBL database (release r117). In order to minimise any erroneous taxonomic assignments, only taxa with a match of 98 % or more to a sequence in the reference library were retained. We further considered a taxon to be undetected in a PCR replicate if it was represented by fewer than 10 DNA reads. Identified taxa were checked against the flora of Alaska (Hultén, 1969) and the Pan Arctic Flora (PAF) checklist (http://panarcticflora.org/). Sequences assigned to taxa which are not present within Alaska today were checked against the NCBI BLAST database for multiple or alternative taxonomic assignments (http:// www.ncbi.nlm.nih.gov/blast/).

6.5 Results

6.5.1 Chronology and Lithostratigraphy

In total, six out of the eight samples sent for AMS radiocarbon dating were successful; two of the samples failed to yield the minimum volume of carbon required for dating. Replacements for these failed samples and four new samples will be sent to the Keck-Carbon Cycle Radiocarbon AMS Facility, University of California, in Autumn 2019. For the six radiocarbon ages which proved successful, the ages span 12,740 ± 60 to 1,225 ± 25 ^{14}C years BP, which corresponds to a calibrated weighted-mean range of 15,248 – 1,144 cal. years BP (Table 6.1).

Table 6.1 Radiocarbon ages of plant macrofossil remains from Chisholm Lake with their 1σ error, calibrated weighted mean age, calibrated median age and calibrated 95 % confidence age ranges. All radiocarbon ages were calibrated against the IntCal13 curve (Reimer *et al*., 2013). One radiocarbon age, marked with an asterisk (*), is considered to be an outlier and omitted from the final age-depth model presented in Figure 6.2.

Core no.	**Lab ID**	**Depth below sediment surface (cm)**	**Age ± 1σ (^{14}C yr. BP)**	**Cal. weighted mean age (cal. yr BP)**	**Cal. median age (cal. yr BP)**	**Cal age, 2σ (cal. yr BP)**	**Sample size (mg of carbon)**	**Material**
Chis17 surface 37-38cm	UCIAMS-219312	38	1225±25	1144	1138	1022 - 1257	112	Wood fragments
Chis17A D2 44-46cm	UCIAMS-219309	187-188	6050±160	6356	6401	5770-6773	27	Wood and monocot stem
Chis17A D3 2-3cm*	UCIAMS-219310	237-238	6455±25	7376	7379	7223-7485	300	Charred wood fragments
Chis17A D4 27-28cm	UCIAMS-219313	303-304	10600±80	11831	11894	11011-12375	118	Monocot leaf and stem fragments
Chis17A D6 47-48cm	UCIAMS-219314	315-316	12740±60	15248	15241	14908-15657	241	Charred wood and charcoal fragments
Chis17B D4 16-17cm	UCIAMS-219311	394-395	12570±90	12424	12470		145	Monocot leaf and stem fragments

The age-depth relationship, lithostratigraphy, sediment properties and macroscopic charcoal results for core Chis17A-B are presented in Figure 6.2. One radiocarbon age (lab ID no. UCIAMS-219310; 237-238 cm depth) is considered to be an outlier based on the lithostratigraphy and comparison with the record obtained by Tinner *et al*. (2006) and adjacent lake sediment records from the Tanana Valley which document a sharp transition from minerogenic sediment to gytjja around 10,000 cal. years BP (Ager, 1975; Abbott et al., 2000b; Finkerbinder *et al*., 2014). Thus, we omitted this radiocarbon age from the final age-depth model (Figure 6.2).

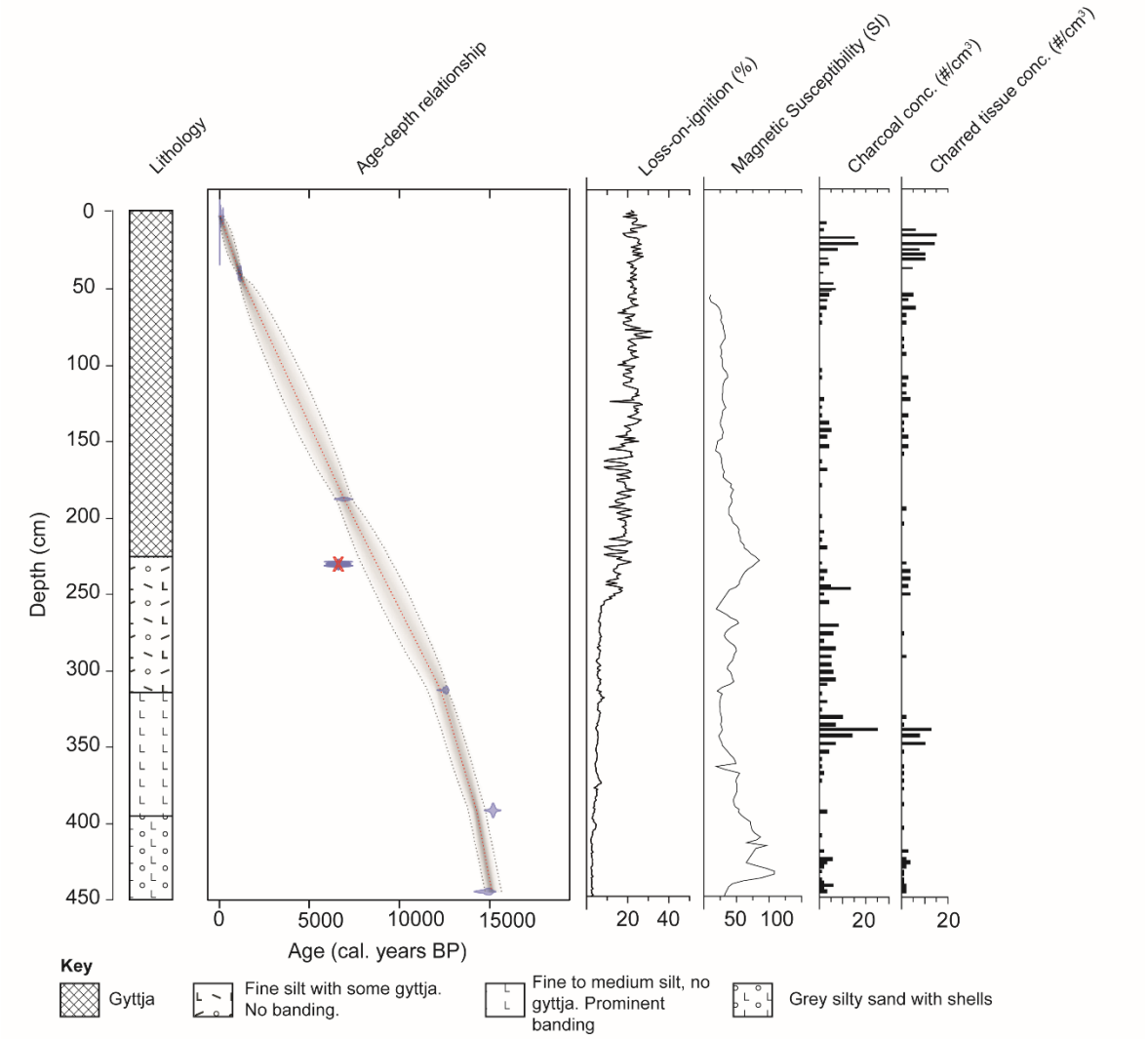

Figure 6.2 Sediment properties, age-depth relationship and macroscopic charcoal concentrations for Chis17A-B core from Chisholm Lake, interior Alaska. Loss-on-ignition (LOI) is given as a percentage of dry sediment weight. One radiocarbon age, marked with a red cross, is considered an outlier based on comparison with adjacent lake sediment records from the Tanana Valley (described in the main text).

The lithology of core Chis17A-B ranges from grey silty sand in the lowermost part to gyttja following a sharp transition around 225 cm depth, when LOI values increase from <5 % dry weight to up to 27 % (Figure 6.2). High magnetic susceptibility values (75 to 100 SI) are observed within the grey silt sand unit in the lowermost part of the core, with generally declining values observed through the gytjja units although several fluctuations are observed. Macroscopic charcoal fragments and charred plant tissue remains show similar trends throughout the core, with the highest concentrations observed between 350 and 275 cm depth and between 40 and 25 cm depth.

6.5.2 *Sed*aDNA Results

In total, we obtained around 100 million raw DNA reads for the 120 sediment samples analysed from Chisholm Lake. Following post-identification filtering, 15 million reads remained representing 167 vascular plant taxa and 14 bryophyte taxa (Appendix D Supplementary Table D.1). Of the total 181 taxa, 45 % were identified to the species level, 39 % to genus and 16 % to a higher taxonomic level (Appendix D Supplementary Figures D.1 – D.3). None of the exotic Cactaceae DNA tracer was detected within the sediment samples nor the negative extraction and PCR controls. Between 138 cm and 53 cm depth, 12 sediment samples failed (i.e. no plant taxa were detected by *sed*aDNA), although other samples taken from within these depths were successful. The failed samples were processed in two different DNA extraction batches that both contained successfully amplified samples.

6.5.2.1 Overall Patterns ~15,000 cal. years BP to Present

Aquatic plants (e.g. *Potamogeton praelongus*, *Myriophyllum sibiricum, Nuphar polysepala*, *Sparganium*) dominate the *sed*aDNA record, accounting for up to 93 % of total DNA reads, but here the focus is terrestrial plant community changes, so we excluded aquatic plants from the following analyses. Saliceae (willow tribe) is detected in every *sed*aDNA sample and nearly every PCR replicate across the record, and accounts for up to 95 % of total DNA reads between *ca.* 15,000 - 9,000 cal. years BP. Turnover in the dominance of terrestrial plant functional groups occurs over the past *ca.* 15,000 cal. years BP (Figure 6.3). Forb and graminoid taxa dominate the earliest period between 15,200 to 14,000 cal. years BP. From *ca.* 14,000 cal. years BP, *Betula* increases in the *sed*aDNA. Based on the chloroplast marker used, both Saliceae and *Betula* were identified to family and genus level only, respectively, and thus could represent sub-shrubs, shrubs and/or tree growth forms. Dwarf shrubs and/or sub-shrubs such as *Arctostaphylos uva-ursi*, *Arctous* and *Dryas* increase a little later *ca.* 13,000 cal. years BP, accounting for up to 37 % of total DNA reads, until a distinct decline in their proportional abundance from *ca.* 9,500 cal. years BP. At *ca.* 9,500 cal. years BP, there is a rapid and sustained increase in the proportional abundance of tall shrub and/or tree taxa (e.g. *Populus*, *Alnus alnobetula* (=*viridis*), *Shepherdia canadensis*, *Viburnum edule*), which is simultaneous with a decline in the proportional abundance of Saliceae (Figure 6.3).

Turnover also occurs among plant taxa within the same functional group over the past *ca.* 15,000 cal. years BP (Figure 6.4). The most notable change occurs at *ca.* 9,500 cal. years BP (~240 cm depth) when additional tree and shrub taxa (e.g. *Picea*, *Alnus alnobetula* (=*A.viridis*), *Populus*, *Rhododendron* and *Vaccinium* sp.), along with boreal forbs (e.g. *Pedicularis palustris*, *P. parviflora*, *Chamaenerion latifolium*, *Galium boreale*, *Cornus canadensis*, *Mertensia paniculata*, *Linnaea borealis*) first appear in the *sed*aDNA, the majority within the same *sed*aDNA sample (Figure 6.4).

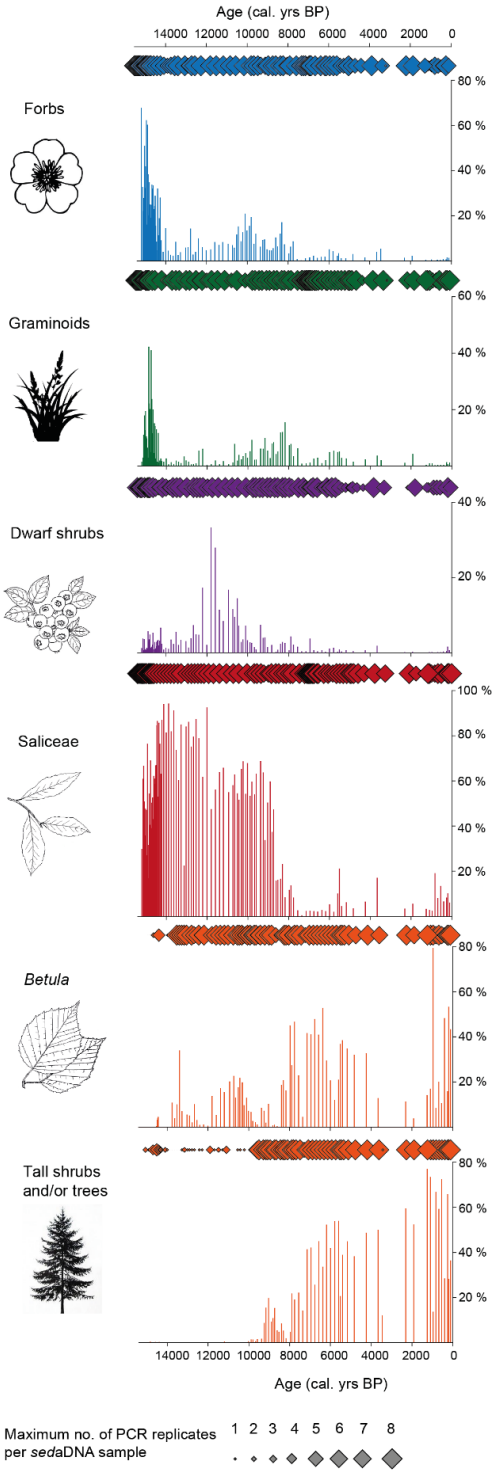

Figure 6.3 Selected terrestrial plant functional groups presented as a percentage of total DNA reads per sample (histogram) and maximum number of PCR replicates (out of eight) per sample (diamond symbols) for the Chisholm Lake *sed*aDNA record. Note that the height of the y-axis varies amongst panels.

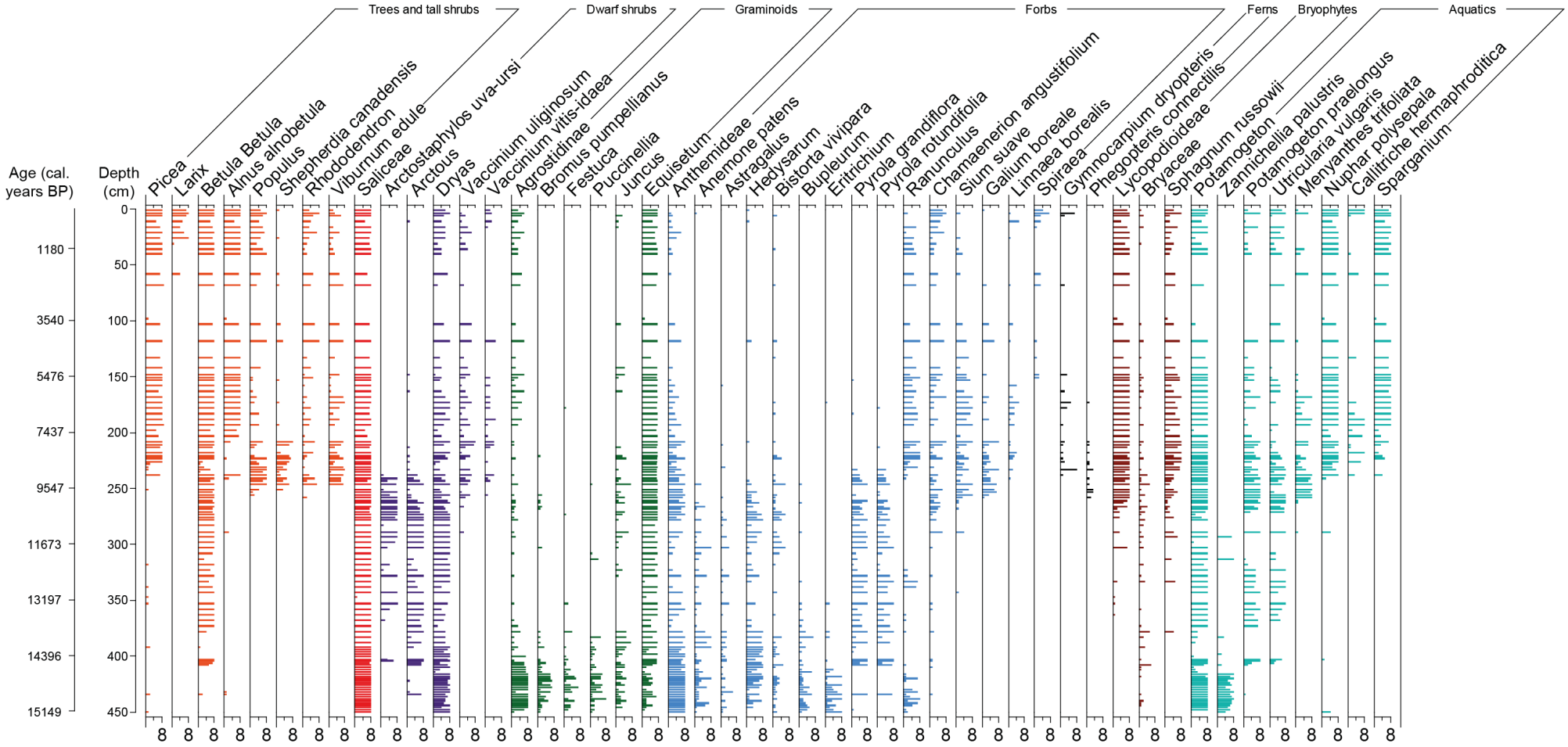

Figure 6.4 Summary figure of selected taxa detected by *sed*aDNA at Chisholm Lake, interior Alaska, sorted by functional group. The x-axis refers to the number of PCR replicates (out of eight) a taxon was detected within at 10 or more DNA reads. Only taxa with >98 % match to a reference sequence are included.

6.5.2.2 The Late-Pleistocene Record

In the earliest period between *ca.* 15,000 and 14,000 cal. years BP (450 - 408 cm depth), before *Betula* and *Arctous* establish, a diversity of forbs (e.g. Anthemideae, *Potentilla*, *Eritrichium*, *Bupleurum*) and graminoids e.g. (Agrostidinae, *Puccinellia*, *Bromus pumpellianus*, *Equisetum*) are detected within the *sed*aDNA at a high level of replication (Figure 6.5). Of the total identified forb and graminoid taxa identified by *sed*aDNA at Chisholm Lake, 54 % and 78 % are detected within the period between *ca.* 15,000 and 14,000 cal. years BP (~ 450 – 400 cm depth) respectively. Saliceae and the mat-forming *Dryas* are also detected within every *sed*aDNA sample within this interval. Tall shrub and/or tree taxa such as *Picea*, *Pinus* and *Alnus alnobetula* (=*viridis*) show sporadic occurrences in this interval.

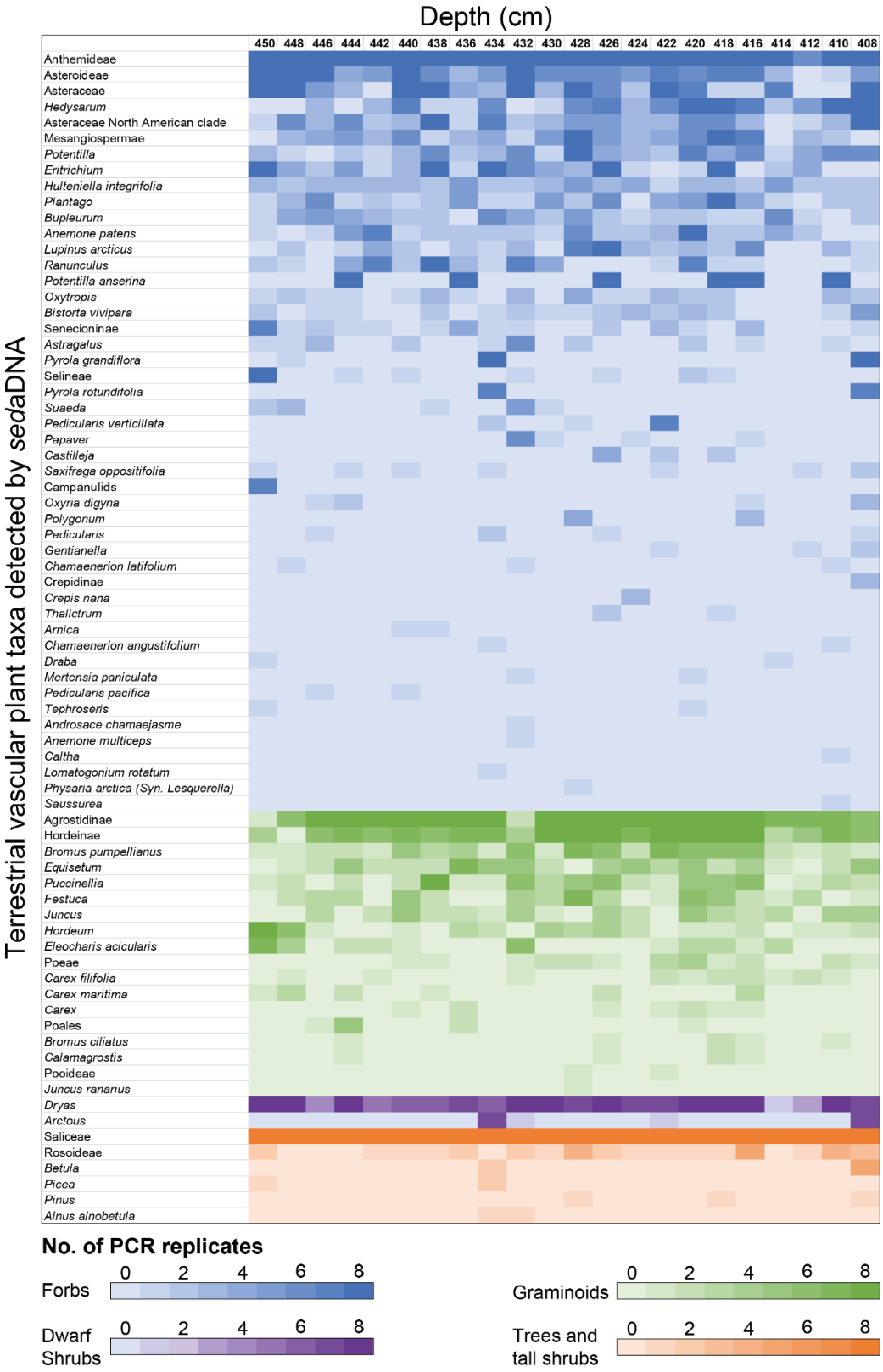

Figure 6.5 Incidence matrix of terrestrial vascular plant taxa detected by *sed*aDNA from Chisholm Lake during the Lateglacial herb zone (450 - 408 cm depth) between *ca.* 15,000 and 14,000 cal. years BP, prior to the main establishment of *Betula*. Plant taxa are presented as a proportion of number of PCR replicates (out of eight) per sample and are sorted according to their functional group.

6.6 Discussion

6.6.1 Lithostratigraphy and Chronology

The lithostratigraphical record from Chisholm Lake (Figure 6.2) indicates an increase in lake production *ca.* 10,000 cal. years BP, with an abrupt transition from minerogenic sediments, composed of grey silty sands with some laminae, low LOI and high magnetic susceptibility values, to gyttja with increasing LOI values. Following this transition, lithostratigraphical properties remain relatively stable with small fluctuations seen in LOI values. To date, the chronology for core Chis17A-B is based on five radiocarbon ages, following omission of the outlier (Table 6.1; Figure 6.2). Detailed inter-site comparisons and interpretations of the timing of key changes in plant community composition must wait until more radiocarbon ages are received, although we note that a shift from minerogenic sediments to a predominance of gyttja occurs at many sites within interior Alaska at *ca.* 10,000 cal. years BP and is linked to the establishment of the boreal forest (Ager, 1975).

6.6.2 Interpreting the *Sed*aDNA Record

A high number of vascular plant and bryophyte taxa were detected within the *sed*aDNA record from Chisholm Lake, with several shifts seen in the dominance of taxa between (Figure 6.3) and within (Figure 6.4) plant functional groups over time. In regards to the 12 *sed*aDNA samples which failed (138 – 53 cm depth), identifying the reasons behind their apparent failure requires further testing, but several hypotheses can be considered. Firstly, unusual sediment properties within these core depths (e.g. high calcium carbonate content, humic acids) could have increased PCR inhibitors and thus limited amplification of the starting DNA molecules. However, five samples spread intermittently between the failed samples worked well, which makes PCR inhibition related to sediment properties unlikely. There does not seem to be any bioinformatic issues; the individual tags were not detected in any of these samples, which suggests an issue with amplification of the starting DNA molecules (if any) from these samples. We further checked the results from other libraries on the sequencing run and the position of these failed samples on the PCR plate set-up, but neither seems to systematically explain the pattern seen in the failed samples. A second hypothesis is that there was an issue with DNA extraction (e.g. chemical inhibition due to incomplete removal of ethanol) and/or error with placing the DNA template on the PCR plates.

The sporadic occurrences of *Picea* and *Pinus sed*aDNA within the late full-glacial to early Lateglacial interval (~ 450 - 400 cm depth) at Chisholm Lake, prior to the main establishment of trees and shrubs (Figures 6.4 and 6.5), are not dissimilar to pollen records, and these taxa occur in other DNA records at times when it is unlikely the plants were present at the site. It is possible that

these occurrences originate from pollen; *Picea* and *Pinus* pollen is found consistently throughout the pollen stratigraphy by Tinner *et al*. (2006), and pollen from gymnosperms can contain some chloroplast DNA; this, theoretically, could be introduced into the lake sediment matrix (Suyama *et al*., 2006; Parducci *et al*., 2015). Current thinking from *sed*aDNA studies suggests that DNA extracted from sediments does not derive from pollen grains (Jørgensen *et al*., 2012; Pedersen *et al*., 2016; Sjögren *et al*., 2017; Wang *et al*., 2017). The extraction of cpDNA from fossil pollen grains has proved challenging, but not impossible (Bennett and Parducci, 2006), yet it requires an additional lysis step in the extraction of DNA (Kraaijeveld *et al*., 2015; Parducci *et al*., 2017). Another possibility is that these sporadic occurrences result from contamination, but these taxa show clear patterns of occurrence across the *sed*aDNA record and they are only present in low abundance within the negative controls.

It has long been suggested that ice-free northern regions likely supported refugial populations of boreal trees during the last glacial interval (e.g. Provan and Bennett, 2008; Parducci *et al*., 2012), and there is some genetic and palaeoecological evidence that suggests *Picea* may have persisted within lowland areas of interior Alaska and the Yukon Territory (Anderson *et al*., 2006, 2010; Zazula *et al*., 2006). However, fossil evidence for local presence of *Picea* within the region is relatively weak (Brubaker *et al*., 2005; Edwards *et al*., 2014) and the *sed*aDNA data from Chisholm Lake does not contribute much to the available supporting evidence, as these early sporadic occurrences warrant further testing.

The coniferous tree *Larix*, which is present within the lake catchment today, is only detected by *sed*aDNA from *ca.* 1,000 cal. years BP (~ 30 cm depth) and it is entirely absent from the lower levels (Figure 6.4). *Larix* has poor pollen productivity and transport characteristics compared with *Pinus* and *Picea* and consequently, very little is known about its late-Quaternary history within Alaska (Brubaker *et al*., 2005). Our results from Chisholm Lake show that *sed*aDNA could make an important contribution towards understanding the timing and nature of its establishment within interior Alaska.

6.6.3 Composition of the Full-Glacial to Early Lateglacial Flora

This new vascular plant *sed*aDNA record and the previous pollen stratigraphy of Tinner *et al*. (2006) from Chisholm Lake together provide an excellent opportunity to assess how *sed*aDNA augments and/or provides additional perspectives on the taxonomic composition of the flora which persisted within interior Alaska during the late full-glacial to Lateglacial interval. At the peak of the LGM (*ca.* 25,000 - 17,000 cal. years BP), many lakes in the Tanana Valley were either seasonally dry or desiccated for prolonged periods, indicating an arid climate (Abbott *et al*., 2000b; Edwards *et al*.,

2000; Bigelow and Edwards, 2001; Finkenbinder *et al*., 2014) and thus there is a scarcity of lake sediment records from the region covering this interval. Our record from Chisholm Lake starts at *ca.* 15,000 cal. years BP and provides insight into the composition of the flora prior to the establishment of *Betula* shrubs *ca.* 14,000 cal. years BP (~ 400 cm depth).

The *sed*aDNA record indicates that herb-dominated vegetation with a diverse range of forbs (e.g. Anthemideae, *Potentilla*, *Hedysarum*, *Eritrichium*, *Bistorta vivipara* and *Astragalus*) and graminoids (e.g. Agrostidinae, *Bromus pumpellianus*, *Puccinellia*, *Festuca* and *Equisetum*) prevailed in the vicinity of Chisholm Lake before *ca.* 14,000 cal. years BP (Figure 6.5). The *sed*aDNA record for this interval also includes a handful of taxa which likely indicate dry growing conditions: for example, salt-tolerant taxa such as *Puccinellia* and species that are common on dry, south-facing river bluffs dominated by boreal steppe or in dry tundra in interior Alaska today (*Anemone patens* and *Anticlea elegans*; Murray *et al*., 1983; Edwards and Armbruster, 1989; Lloyd *et al*., 1994). The grass *Bromus pumpellianus* is typical of dry habitats such as boreal steppe today but is not restricted to the steppe communities (Hultén, 1969). These taxa are in the minority, and most forbs are more typical of tundra communities today.

The *sed*aDNA record from Chisholm Lake is floristically rich compared to the earlier pollen stratigraphy by Tinner *et al*. (2006), particularly for the interval prior to the establishment of *Betula* shrubs at around 380 cm. *Sed*aDNA detected a high diversity (48 taxa) of forbs within this interval (Figure 6.5), the majority of which were not detected by pollen, likely due to their low pollen productivity and/or low overall pollen concentrations within this interval. *Sed*aDNA identified 60 additional terrestrial plant taxa that were not found as pollen, and the DNA was better able to resolve the identity of graminoids to species or genus level. The pollen stratigraphy suffers from very low pollen concentrations (pollen sum < 200 grains) within this early period (zones LL-1 to 3 of Tinner *et al*., 2006); 23 terrestrial plant taxa were detected in total, many at low percentages (<5 % of terrestrial pollen sum) and showing sporadic occurrences. The pollen stratigraphy shows a similar composition to the *sed*aDNA record for this interval (Figure 6.5), with the pollen largely comprising herbaceous taxa such as Poaceae, Cyperaceae, *Artemisia*, *Potentilla*, *Bupleurum*, *Plantago canescens*, Chenopodiaceae and *Equisetum*, with some *Dryas* and *Salix* and a minor signal from *Picea glauca* (Tinner *et al*., 2006).

Our results from Chisholm Lake align with detailed pollen records (e.g. Cwynar, 1982; Ritchie and Cwynar, 1982; Anderson *et al*., 1988; Ager, 1993) and macrofossil records (e.g. Zazula *et al*., 2006) that challenge the somewhat monolithic attribution of "steppe" to the vegetation that prevailed across ice-free northern regions during the last glacial interval (e.g. Guthrie, 1968; 1990; Matthewes, 1982; Yurtsev, 2001; Zimov *et al*., 2012). Importantly, the data are from an interior

lowland site, in a region known to have supported a mixed Pleistocene megafauna dominated by grazers (Guthrie, 1990). The grasses were taxonomically diverse, and they contain taxa that today are linked with boreal steppe as well as tundra, but the forb indicator taxa are predominantly found in tundra today, though some are typical of drier sites. Thus, as has been suggested by many authors, the flora was likely an admixture of tundra taxa, including those adapted to warm, dry conditions, plus very few taxa with a predominantly steppic distribution. Evidently, it formed vegetation that was capable of supporting the grazing megafauna.

Our results from Chisholm Lake are comparable to those of Willerslev *et al*. (2014), which are also derived from *sed*aDNA: they concluded that the full-glacial vegetation mosaic in ice-free northern regions was much more dominated by forbs than is suggested by many pollen records. However, we are cautious about making inferences on the percentage dominance of different functional groups as there is likely a bias in favour of forbs in the DNA signal (and biases associated with the polymerase used) at the expense of other growth forms (Yoccoz *et al*., 2018; Nichols *et al*., 2018)

Small amounts (<10 fragments/cm^3) of macroscopic charcoal and charred plant remains were found in this early period between 450 and 400 cm depth (Figure 6.2), suggesting herb-tundra fires occurred, but they were rare. This is in agreement with other records from unglaciated areas of Alaska (e.g. Eisner and Collinvaux, 1990; Earle *et al*., 1996; Chipman *et al*., 2015). Our record from Chisholm Lake demonstrates an increase in macroscopic charcoal concentrations and thus, local fire activity, following a shift from herb tundra to shrub-tundra communities of *Betula*, *Arctostaphylos uva-ursi*, *Arctous* and *Dryas* after *ca.* 14,000 cal. years BP. It is the general consensus that fires are one of the most important factors favouring the maintenance and regeneration of both grassland and tundra communities (Sauer, 1950; Guthrie, 1968; Higuera *et al*., 2008).

6.6.4 Late-Pleistocene Establishment of Shrubs

By *ca.* 14,000 cal. years BP (~400 cm depth), *Betula* shrubs established in the vicinity of Chisholm Lake, simultaneously with an increase in the dwarf shrubs *Arctostaphylos uva-ursi* and *Arctous* and a distinct decline in grasses such as Agrostidinae, *Bromus pumpellianus*, *Festuca*, Hordeinae and *Puccinellia* in the *sed*aDNA (Figures 6.3 and 6.4). At the same time, forbs such as *Pyrola* sp., *Pedicularis verticillata* and *Castilleja* first appear or increase in the *sed*aDNA, along with the first appearance of aquatic plants such as *Potamogeton praelongus*, *Utricularia vulgaris*, *Hippuris* and *Persicaria pectinata*. Timing of *Betula* establishment in the *sed*aDNA (*ca.* 14,000 cal. years BP) from Chisholm Lake is in accordance with the pollen (*ca.* 13,300 cal. years BP) stratigraphy from the same site (Tinner *et al*., 2006).

The replacement of herb-tundra by *Betula* shrub tundra by *ca.* 14,000 cal. years BP, probably reflects climatic amelioration at the beginning of the Lateglacial interval, which is coincident with reports of increases in northern hemisphere summer temperatures at this time (von Grafenstein *et al*., 1999; Lowe and Hoek, 2001; Heiri and Millet, 2005; Miller *et al*., 2010) and an increase in the rate of recession of the last glacial northern hemisphere ice sheets (Clark *et al*., 2009; Dyke *et al*., 2002; Hughes *et al*., 2016). Deglaciation led to a shift in atmospheric circulation patterns which introduced a moist, westerly air flow into Alaska, thus increasing precipitation levels (Bartlein *et al*., 1991). Several authors document increases in lake-levels within the interior during this time (Abbott *et al*., 2000b; Barber and Finney, 2000).

At *ca.* 10,000 cal. years BP (~250 cm depth), large changes are observed in the sediment properties of core Chis17A (Figure 6.2) and there is turnover in plant community composition (Figure 6.3). LOI values begin to rise, coincident with decreases in magnetic susceptibility values, reflecting increases in lake productivity. A suite of new woody plant taxa such as *Populus*, *Shepherdia canadensis*, *Comarum palustre* and *Vaccinium* sp. show first appearances in the *sed*aDNA at this time, followed a little later by the main rise in *Picea* and *Alnus alnobetula* (= *viridis*) *ca.* 9,200 cal. years BP (~240 cm depth) (Figure 6.4). Ferns (e.g. *Gymnocarpium dryopteris*, *Phegoteris connectilis* and *Polypodium vulgare*) also appear at the same time as the main establishment of *Picea*, along with a suite of new aquatic taxa (e.g. *Nuphar polysepala*, *Callitriche hermaphroditica* and *Sparganium*) and terrestrial shrub and forb taxa indicative of boreal forest (e.g. *Galium boreale*, *Linnaea borealis*, *Cornus*, *Rumex*; Figure 6.4; Appendix D Supplementary Figure D.1 – D.3). The timing and sequence of establishment of *Populus*, *Picea* and *Alnus alnobetula* (= *viridis*) in the *sed*aDNA is in accordance with the pollen stratigraphy (Tinner *et al*., 2006) and other pollen records from central Alaska (Ager, 1975; Anderson *et al*., 1990; Hu *et al*., 1993; Bigelow and Edwards, 2001).

6.7 Conclusions

The *sed*aDNA record from Chisholm Lake in interior Alaska was better able to resolve the composition of plant communities during the late full-glacial and Lateglacial interval than pollen. It provides new insights into a herb-tundra community which was more diverse than has been suggested by previous studies based on pollen, and was characterised by a dominance of forbs and graminoids with some dwarf shrubs. Herb-tundra prevailed within the lake's catchment prior to *ca.* 14,000 cal. years BP, after which it was largely replaced by shrub-tundra communities of *Betula*, *Arctostaphylos uva-ursi* and *Arctous*. By *ca.* 10,000 cal. years BP, a distinct and sustained change in plant community composition occurred, when *Picea* trees established along with a number of other woody taxa and boreal herbs that dominate the present-day vegetation mosaic of the region.

Our results show that the analysis of *sed*aDNA can make an important contribution towards resolving the composition of the last full-glacial to Lateglacial flora within ice-free northern regions and the rapidity of woody taxa establishment in the early- to middle-Holocene. Looking forward, a more detailed analysis of the *sed*aDNA record from Chisholm Lake could provide important information regarding the early human use of natural resources, the history of *Larix* within interior Alaska and, considering the detailed aquatic plant record obtained, changes in within-lake plant communities over time.

6.8 Acknowledgements

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6.9 Author Contributions

C.L.C., M.E., N.B., J.R. and P.D.M.H. contributed to the concept, designed the study and cored Chisholm Lake. C.L.C. subsampled the sediment core, extracted the DNA and performed the PCR amplifications. Initial sequence analysis and taxonomic assignment was performed by C.L.C., P.D.H. and Y.L. Post-identification filtering and analysis of the DNA data was performed by C.L.C. Ecological and botanical knowledge of the flora of interior Alaska was provided by M.E., along with suggestions on the interpretations. C.L.C. picked terrestrial macrofossils for ^{14}C dating and constructed the age-depth model. N.B. and J.R. contributed to the visual description of the lithostratigraphy. C.L.C. performed the LOI, magnetic susceptibility and macroscopic charcoal analyses and wrote the manuscript, on which M.E. and P.D.M.H. commented.

Chapter 7 Synthesis and Conclusions

7.1 Introduction

The primary aim of this thesis was to characterise long-term changes in plant community composition and floristic richness in northern regions since the last glacial interval using *sed*aDNA extracted from lake sediments. A further aim was to assess the power and limitation of *sed*aDNA analysis as a palaeoecological tool for reconstructing past floras. Towards this aim, five objectives were outlined in Section 1.5 and have been addressed throughout this thesis:

- Identify suitable sites and retrieve sediment cores from lakes both within previously glaciated northern regions and those which remained ice-free (i.e. unglaciated) during the last glacial interval;
- Extract, amplify and sequence vascular plant *sed*aDNA from those lake sediment cores;
- Reconstruct changes in plant community composition and floristic richness over time at each site;
- Compare the results with high-resolution and detailed pollen analysis;
- Assess the success and applicability of *sed*aDNA for reconstructing plant community composition and floristic diversity using qualitative and quantitative measures (described in Section 1.3).

The four works (Chapters 3 to 6) together form a contribution beyond their individual parts. Each chapter presents an investigation of the use of *sed*aDNA analysis from the sediments of three different lakes across the Arctic and Subarctic region. All four works taken together inform a new understanding of the composition of the last-glacial flora and how it responded to environmental changes over the Pleistocene-Holocene transition. In this synthesis section, I assess the contribution and limitation of *sed*aDNA analysis within this study and I bring together what we have learned in terms of late-Quaternary plant community dynamics and address the four research questions which were outlined in Section 1.4.

7.2 The Importance of Lake Selection

Despite early challenges, the field of *sed*aDNA has experienced several methodological and technological advances over recent years. Improvements in coring techniques, DNA extraction methods, bioinformatics pipelines for data filtering and development towards standardised protocols (Parducci *et al*., 2017; Shapiro *et al.,* 2019) have all taken place, even over the course of

this study. Moreover, modern calibration studies using surface (surficial) lake sediment samples have enhanced our understanding of the underlying taphonomic processes by which sedimentary DNA is transferred and incorporated into lake sediments (Giguet-Covex *et al*., 2019) and of how the signal of plant sedimentary DNA represents vegetation in terms of spatial scale (Alsos *et al*., 2018). However, from these earlier studies and the four works presented in this thesis (Chapters 3 to 6) it is clear that lake selection remains an important consideration for plant *sed*aDNA studies, as lake features act as a control on levels of DNA preservation, detection success and the representation and/or spatial scale of floristic composition.

In this study, I tested the use of *sed*aDNA analysis for reconstructing past floras at three different lakes across the northern high latitudes which differ greatly in terms of their lake and/or catchment area, hydrology, sediment properties, sediment age and glacial history. *Sed*aDNA was successfully extracted, amplified and sequenced from all three lakes. At each lake, a high diversity of plant taxa (>115 taxa) was identified at a similarly high taxonomic resolution (>40 % identified to species level, > 45 % to genus level) using the chloroplast *trn*L marker (Taberlet *et al*., 2007). Overall, *sed*aDNA analysis was found to be a success at each of the lake studies, in that identified plant taxa showed clear patterns of occurrence, high levels of replication across PCR replicates and high fidelity between identified taxa and their presence within the regional flora. At each lake, *sed*aDNA contributed new information on late-Quaternary plant dynamics, which are discussed later in this Synthesis in Sections 7.4 to 7.8.

The results presented in this thesis highlight the importance of lake selection on the success and representation of *sed*aDNA within lake sediments. At Uhca Rohči Lake (Chapter 3), the *sed*aDNA signal is considered to represent, for the most part, a highly local signal originating from within its small (<2 km^2) hydrologic catchment. The lake may have received inflow from the Komagelva River during the early- and middle-Holocene which, if it occurred, would have greatly changed the source of *sed*aDNA to the lake at that time. The majority of identified plant taxa show low levels of replication per sample (i.e. present in 1-3 replicates across the eight PCR replicates). Sporadic occurrences could result from a low abundance of *sed*aDNA within the sediment and/or sub-optimal sediment properties for DNA preservation and amplification, including a relatively high organic content (20 to 47 % LOI), and possible humic acids and/or other inhibitors within the sediment.

In contrast to Uhca Rohči, Lake Bolshoye Shchuchye (Chapter 4 and 5) has a large (>200 km^2) hydrologic catchment, and the lake receives considerable slope-wash and riverine input. Thus, its *sed*aDNA signal is thought to derive from a large source area within its catchment and potentially more distant sources upstream, capturing plant communities over a large spatial area and

elevational range. Most of the plant taxa identified by *sed*aDNA at Lake Bolshoye Shchuchye are well-represented across the eight PCR replicates per sample, and the low organic content (<3 % LOI) and fine-grained nature of the sediment likely contributed to the rich floristic record retrieved from this site.

Chisholm Lake (Chapter 6) sits in the middle of the gradient in lake size and catchment area formed by the three lakes analysed as part of this study. The *sed*aDNA signal is thought to derive from within the lake and from its intermediate-sized catchment (<8 km^2), with a high proportion of all DNA reads being from aquatic plants. As at Lake Bolshoye Shchuchye, identified plant taxa at Chisholm Lake are well represented in the PCR replicates, showing clear patterns of arrival and/or occurrence throughout the record. However, 12 *sed*aDNA samples from Chisholm Lake failed completely (i.e. no plant taxa detected) and the cause of which is yet to be determined. Inhibition due to carbonates or humic acids, inefficient extraction or issues with the PCR plate set-up were suggested as potential causes for these failed samples but further testing is required.

The ideal lake and/or catchment size for *sed*aDNA research is dependent upon the ecological research questions being asked. For studies focusing on local, small-scale compositional changes, a small lake with a highly local *sed*aDNA signal is recommended. For studies within an interest instead on the dynamics between different plant communities on a landscape scale, a large lake with a large source area for sediment inputs is more suitable. Similarly, if the interest is instead on changes in within-lake aquatic plants rather than terrestrial communities, lakes with a reasonable depth where sunlight can penetrate and sustain aquatic plant growth is more of a priority.

7.3 Power and Limitation of *Sed*aDNA for Reconstructing Past Floristic Composition

All four works (Chapters 3 to 6) presented in this thesis demonstrate that *sed*aDNA from lake sediments can add new perspectives on late-Quaternary plant community dynamics, a summary of which is presented later in this Synthesis in Sections 7.4 to 7.8. In each chapter, plant identification based on *sed*aDNA was achieved at a higher taxonomic resolution in general than was possible with pollen. When pollen analysis was performed at a high temporal resolution using a high pollen count at Lake Bolshoye Shchuchye (Chapter 5), *sed*aDNA and pollen showed a similar taxonomic resolution, but *sed*aDNA detected more plant taxa per sample (>20 taxa) on average than pollen, particularly within the Lateglacial and Holocene intervals. The findings presented in this thesis show that *sed*aDNA from lake sediments can make an important contribution towards reconstructions of floristic richness based on pollen, particularly in the Holocene, when woody plant taxa established,

as it is less sensitive than pollen to "swamping" by anemophilous taxa at the expense of entomophilous and low pollen-producing herbs.

The value of *sed*aDNA analysis for identifying 'fossil-silent' plant taxa is particularly evident for functional types such as graminoids, bryophytes, and dwarf shrubs, which are underrepresented and/or poorly resolved by pollen and plant macrofossils. Moreover, *sed*aDNA permitted the documentation of *Larix* sp. establishment at Lake Bolshoye Shchuchye and Chisholm Lake and the long-term persistence of arctic-alpine plants at Lake Bolshoye Shchuchye; these taxa are known for their low pollen productivity and are commonly under-represented in traditional palaeorecords. Furthermore, the works presented in this thesis demonstrate that *sed*aDNA can be successfully extracted, amplified and sequenced to generate a floristically rich species and/or taxon list even from fine-grained, minerogenic sediments with low organic matter content. This sediment type is often particularly challenging for obtaining sufficient and well-preserved pollen grains or plant macrofossil remains for meaningful interpretations.

The findings presented in this thesis also help to elucidate several limitations or constraints for the use of *sed*aDNA as a tool for reconstructing past changes in plant community composition. Firstly, all four studies highlight the challenge of distinguishing individual species amongst the Saliceae (willow) family based on the chloroplast *trn*L marker (Taberlet *et al*., 2007). In this instance, pollen was superior at resolving taxa within this taxonomic group as it differentiates between two important genera (*Salix* and *Populus*) with different growth forms and ecology. In other cases, neither pollen nor *sed*aDNA was able to differentiate among important taxa (e.g. *Vaccinium vitis idaea* and V. *myrtillis*).

Earlier *sed*aDNA studies (e.g. Jørgensen *et al*., 2012; Parducci *et al*., 2015; Pedersen *et al*., 2015; Sjögren *et al*., 2017) concluded that *sed*aDNA analysis augments information gained by pollen and/or plant macrofossil analysis. In a comparative study, Jørgensen *et al*. (2012) concluded that *sed*aDNA was a complementary technique to pollen and plant macrofossils, enabling the identification of plant taxa which were underrepresented or poorly preserved as fossils. All four studies (Chapters 3 to 6) demonstrate that *sed*aDNA can help to identify 'fossil-silent' or low pollen-producing plant taxa, such as *Larix* sp., entomophilous arctic-alpine herbs (e.g. *Draba pauciflora*, *Papaver*, *Saxifraga oppositifolia*, *S*. *cespitosa*, *Arabis alpina*), and taxa which cannot be distinguished below family level based on their pollen (e.g. Poaceae, Cyperaceae and some taxa within Ericaceae). The detailed record of bryophytes and aquatic plants obtained from Lake Bolshoye Shchuchye and Chisholm Lake, respectively, further demonstrates the value of *sed*aDNA for resolving 'fossil-silent diversity' (Parducci *et al*., 2017).

In contrast to results from earlier studies that compared *sed*aDNA and pollen, the findings from Lake Bolshoye Shchuchye (Chapter 5) show that when a high pollen sum is used, as advocated by Birks and Birks (2016), *sed*aDNA and pollen can show considerable overlap. This is in both overall taxonomic composition and the pattern of occurrence of higher plants, pteridophytes and bryophytes, such as *Picea*, *Betula*, *Alnus* and Cyperaceae, Caryophyllaceae, *Gymnocarpium dryopteris* and *Sphagnum*. The *sed*aDNA and pollen records from this site show a similar level of taxonomic resolution and diversity, but they differ in the functional groups and taxa within those groups that they can identify. *Sed*aDNA was superior at resolving a diversity of taxa within the forbs, graminoids, dwarf shrubs and bryophyte groups, whereas pollen analysis detected a higher number of tall shrub and/or tree taxa across the record, many of which are likely long-distance dispersed. Thus, the addition of *sed*aDNA analysis to pollen records can help to confirm which plant taxa were actually growing in the vicinity of the lake at a given time versus those which are exhibiting long-distance dispersal of their pollen.

Biases resulting from the poorly understood relationship between plant biomass on the landscape and DNA abundance within lake sediments limit reconstructions of plant biomass changes over time based on *sed*aDNA data (Alsos *et al*., 2018). To date, the method accurately depicts general vegetation types and moderately detailed community composition, but it is not comprehensive enough to be used as a biomonitoring tool due to issues with detectability of certain plant taxa and/or biases related to PCR-based methods (Alsos *et al*., 2018; Parducci *et al*., 2017). Due to numerous studies on pollen representation and pollen productivity estimates (PPE's), pollen analysis currently remains superior at providing a more precise estimation of plant abundances (Webb *et al.*, 1981; Sugita, 1994; Hellman *et al.*, 2008; Birks and Birks, 2016; Parducci *et al.*, 2017).

An ongoing challenge with the interpretation of *sed*aDNA data is distinguishing between true presences, false presences and/or intermittent absences. Key questions remain concerning the representation of *Picea* sp. within full-glacial aged samples at Lake Bolshoye Shchuchye and Chisholm Lake and *Pinus* sp. within early to middle Holocene aged sediments at Uhca Rohči Lake. Whether these *sed*aDNA occurrences represent local presence or instead originate from long-distance dispersal of their pollen, reworking of older material and/or contamination cannot be answered based on the current datasets. An important conclusion from this thesis is the demonstration of the potential pitfall of relying on *sed*aDNA metabarcoding techniques for documenting past treeline dynamics and/or for identifying ephemeral, isolated populations of boreal trees in northern localities during the last glacial interval.

7.4 Postglacial Colonisation and Development of Plant Communities Within Previously Glaciated Regions

The investigation at Uhca Rohči Lake (Chapter 3) has one obvious limitation; that the retrieved sediment sequence fails to capture the interval when plants initially colonised this previously glaciated region within northeast Norway. The *sed*aDNA record from Uhca Rohči Lake begins at *ca.* 10,700 cal. years BP, yet the region was probably deglaciated around 17,000 cal. years BP and was certainly ice-free by 15,000 cal. years BP (Tolgensbakk and Sollid, 1981; Olsen *et al*., 1996; Stokes *et al*., 2014; Stroeven *et al*., 2016). The record nevertheless provides an important insight into the early-Holocene development of plant communities within this region. Postglacial colonisation must have taken place during the Pleistocene-Holocene transition, Younger Dryas stadial or earlier, as erect shrub-tundra and riparian grassland communities had already established in the vicinity of Uhca Rohči Lake by the beginning of the *sed*aDNA record at *ca.* 10,700 cal. years BP. The *sed*aDNA record shows no long-term change in floristic richness over the period investigated. There is no evidence of sequential addition of plant taxa that might be explained by differential migration rates.

7.5 Community Composition of the Last-Glacial Flora Within Ice-Free Regions

The sediment records retrieved from Lake Bolshoye Shchuchye in the Polar Urals (Chapters 4 and 5) and Chisholm Lake in interior Alaska (Chapter 6) offer a unique view into the already well-established (i.e. not successional) flora of these regions during the last glacial interval. Despite numerous earlier attempts, the composition and attributes of plant communities of ice-free regions during the last glacial interval remain poorly resolved due to constraints (e.g. preservation, productivity, taxonomic resolution) on the palaeoecological proxy records (Lamb and Edwards, 1988; Gajewski, 2015). Here, the *sed*aDNA records from Lake Bolshoye Shchuchye and Chisholm Lake have provided important contributions concerning which plant taxa were present and what plant communities were dominant in these regions during the last glacial interval.

Both records show that the full-glacial (*ca.* 24,000 – 15,000 cal. years BP) flora in these regions comprised what can best be called a herb-tundra vegetation dominated by forbs (e.g. *Papaver*, *Draba*, *Saxifraga* sp., *Astragalus*, *Bistorta vivipara*, *Lupinus arcticus*) with some graminoids (e.g. *Puccinellia*, *Festuca*, *Juncus biglumis, Bromus pumpellianus, Calamagrostis*). Some shrub or sub-shrubs of *Salix* and the mat-forming *Dryas* were also present, although these were the only shrub (sub-shrub) taxa detected in the full-glacial interval. This is consistent with cold and dry climate conditions (Kaplan *et al*., 2003) and a limited extent of shrub-tundra suggested by earlier

palaeoecological records from these regions (e.g. Bigelow *et al*., 2003; Binney *et al*., 2009, 2017). In general, the floristic composition suggested by *sed*aDNA was similar to that suggested by pollen at the same sites, but *sed*aDNA permitted the detection of >47 additional forb and >15 additional graminoid taxa than pollen at these sites.

The *sed*aDNA records from these sites help shed new light on the long-standing debate as to the nature of the full-glacial steppe-tundra or mammoth steppe ecosystem (Cwynar and Ritchie, 1980; Cwynar, 1982; Matthewes, 1982; Guthrie, 1968; 1990; Zimov *et al*., 2012; Hopkins *et al*., 2013; Willerslev *et al*., 2014). The *sed*aDNA records from Lake Bolshoye Shchuchye and Chisholm Lake contain a handful of plant taxa which may typify the steppe or temperate grassland communities suggested by Guthrie (1968, 1990) and others, namely *Ephedra*, *Puccinellia* and *Anemone patens*. The majority of plant taxa detected within this interval are typical of Arctic herb communities, more like dry tundra or possibly fellfield habitats than temperate grassland, thus supporting the more nuanced conclusions of most plant palaeoecologists, who envision a mosaic of herbaceous plant communities ranging from fellfield to cold steppe (Cwynar and Ritchie, 1982; Anderson *et al*., 2004; Kaplan *et al*., 2003).

The *sed*aDNA records from Lake Bolshoye Shchuchye and Chisholm Lake suggest a predominance of forbs (>60 % of DNA reads) with some graminoids (>30 % of DNA reads) and sub-shrubs (>10 % of DNA reads) in the full-glacial interval. This finding supports the conclusions of Willerslev *et al*. (2014) who questioned the predominance of graminoids within the full-glacial vegetation mosaic and argued instead that it consisted of dry steppe-tundra dominated by forbs. However, current thinking from DNA calibration studies suggests that forbs are overrepresented in the DNA signal within sediments and a bias with the polymerase used during PCR which occurs in favour of forbs (Yoccoz *et al*., 2012; Nichols *et al*., 2018). Moreover, many graminoid taxa that typify steppe environments are currently lacking in the reference libraries, or they are included but cannot be distinguished below family level based on the chloroplast *trn*L marker (Willerslev *et al*., 2014; pers. comm. M. Edwards 2019, 23 July). Interpretations concerning the dominance of certain functional groups within the full-glacial flora are therefore constrained by biases related to representation within DNA signal and/or reference libraries.

Many of the plant taxa detected within the *sed*aDNA at Lake Bolshoye Shchuchye and Chisholm Lake within the full-glacial interval are still present within the local vegetation mosaic of these regions today, suggesting long-term persistence. In particular, the *sed*aDNA record from Lake Bolshoye Shchuchye documents the long-term persistence of arctic-alpine plants in this region over the past 24,000 cal. years BP. These findings support the conclusions from local-scale modelling which suggests that spatially heterogeneous landscapes can facilitate long-term persistence via

their provision of a range of habitats for different plant communities (e.g. Patsiou *et al*., 2014; Niskansen *et al*., 2017, 2019).

7.6 Compositional Changes in Plant Communities Over the Pleistocene-Holocene Transition

Knowledge of how the composition of plant communities responded to climate and environmental changes over the Pleistocene-Holocene transition (*ca.* 15,000 to 11,000 cal. years BP) can inform understanding of biotic response (including biotic turnover) to large-magnitude climate changes. At Lake Bolshoye Shchuchye (Chapters 4 and 5) and Chisholm Lake (Chapter 6), this transition is characterised with the sequential addition of woody plant taxa over time and thus, turnover in the dominance of plant functional groups.

The dwarf-shrub *Dryas (*assumed here to be *D. ocopetala*) increases in the *sed*aDNA at Lake Bolshoye Shchuchye from *ca.* 15,000 cal. years BP, followed a little later by an increase in *Betula*. The establishment of *Betula* shrubs and/or sub-shrubs from *ca.* 13,500 cal. years BP reflects favourable growing conditions for shrubs in the Lateglacial interval which is probably related to a shift to warmer and moister climatic conditions. At Chisholm Lake (Chapter 6), *Dryas* sp*.* was present during the full-glacial interval from the beginning of the record and remained dominant throughout the Lateglacial interval; shrubs and/or sub-shrubs of *Betula* and *Arctous* established from *ca.* 14,000 cal. years BP. At both sites, the Lateglacial is characterised by increasing floristic richness and the sequential addition of new plant taxa and thus new plant communities. The *sed*aDNA records from both regions document a shift from a predominantly treeless, open ecosystem dominated by forbs and graminoids in the full-glacial interval to one that supported some elements of woody vegetation in the Lateglacial.

In addition to shrub establishment, there is turnover and the arrival of new taxa amongst the graminoids and forbs within the Lateglacial interval at both sites. At Lake Bolshoye Shchuchye (Chapters 4 and 5), sedges such as *Carex* sp. and *Eriophorum* increase in dominance and new forb taxa (e.g. *Caltha*, *Eritrichium*, *Lagotis glauca*, *Oxygraphis glacialis*, *Oxytropis*, *Rhodiola rosea*, *Thalictrum* and *Valeriana*) appear from *ca.* 17,000 cal. years BP. At Chisholm Lake (Chapter 6), grasses such as Agrostidinae, *Bromus pumpellianus*, *Festuca* and *Puccinellia* decline when *Betula* and *Arctous* shrubs establish around 14,000 cal. years BP. This is coincident with an increasing dominance of *Equisetum* and the appearance of several new forb taxa (e.g. *Pyrola grandiflora*, *P. rotundifolia*, *Pedicularis verticillata*, *Castilleja* and *Tephroseris*).

Overall, the *sed*aDNA records from these sites suggest distinct changes in plant community composition over the Pleistocene-Holocene transition with both lake catchments supporting a growing set of different plant communities over time.

7.7 Timing and Nature of the Holocene Thermal Maximum (HTM)

The early-Holocene interval is characterised by the establishment of additional tall shrub and dwarf-shrub taxa (e.g. *Betula*, *Alnus*, *Dryas*, *Arctostaphylos uva-ursi*, *Empetrum nigrum*, *Vaccinium* sp.) within the catchments of Lake Bolshoye Shchuchye (Chapters 4 and 5) and Chisholm Lake (Chapter 6). At Uhca Rohči Lake (Chapter 3), shrub-tundra communities of *Betula*, *Empetrum nigrum*, *Vaccinium* sp. were already in place by the start of the *sed*aDNA record at *ca.* 10,700 cal. years BP.

At all three lakes, the *sed*aDNA record suggests growing-season temperatures that were warmer than present during all or part of the early- to middle-Holocene between *ca.* 11,000 to 4,000 cal. years BP. At Uhca Rohči Lake, presence of the extra-limital aquatic plant *Callitriche hermaphroditica* in the *sed*aDNA between *ca.* 10,200 and 9,600 cal. years BP is coincident with abundant *Pinus* sp. *sed*aDNA and pollen, although we cannot say with certainty whether the latter taxon represents local presence at this time. At Lake Bolshoye Shchuchye the coniferous trees *Picea* sp. and *Larix* sp. were present in the vicinity of the lake between 9,000 and 4,000 cal. years BP. The data that relate to western Eurasia support the conclusions of Väliranta *et al*. (2015), which are derived from plant macrofossil evidence, that summer temperatures were warmer than present in the early- to middle-Holocene between *ca.* 11,700 and 7,500 cal. years BP. At Chisholm Lake, the pattern is more complicated, as the evergreen forest established about 10,000 years ago and persisted to the present. Warmer than present growing-season temperatures during the early-Holocene interval in interior Alaska is, however, shown by other proxy indicators (Kaufmann *et al*., 2004).

7.8 Late-Quaternary Changes in Floristic Richness

Taken together, the *sed*aDNA records from Uhca Rohči Lake (Chapter 3), Lake Bolshoye Shchuchye (Chapters 4 and 5) and Chisholm Lake (Chapter 6) provide important insights into changes in floristic richness within these northern regions over the past 24,000 years (Figure 7.1). The *sed*aDNA record from Uhca Rohči Lake shows no long-term trends in floristic richness over the Holocene time period investigated. We infer that postglacial colonisation occurred prior to the full-Holocene, likely during the Pleistocene-Holocene transition or earlier, at Uhca Rohči Lake (see Chapter 3). Thus, the *sed*aDNA record from this site gives insight into an already established vegetation, with no evidence

for increased floristic richness over time that might be due to successional arrival and/or delayed immigration of species.

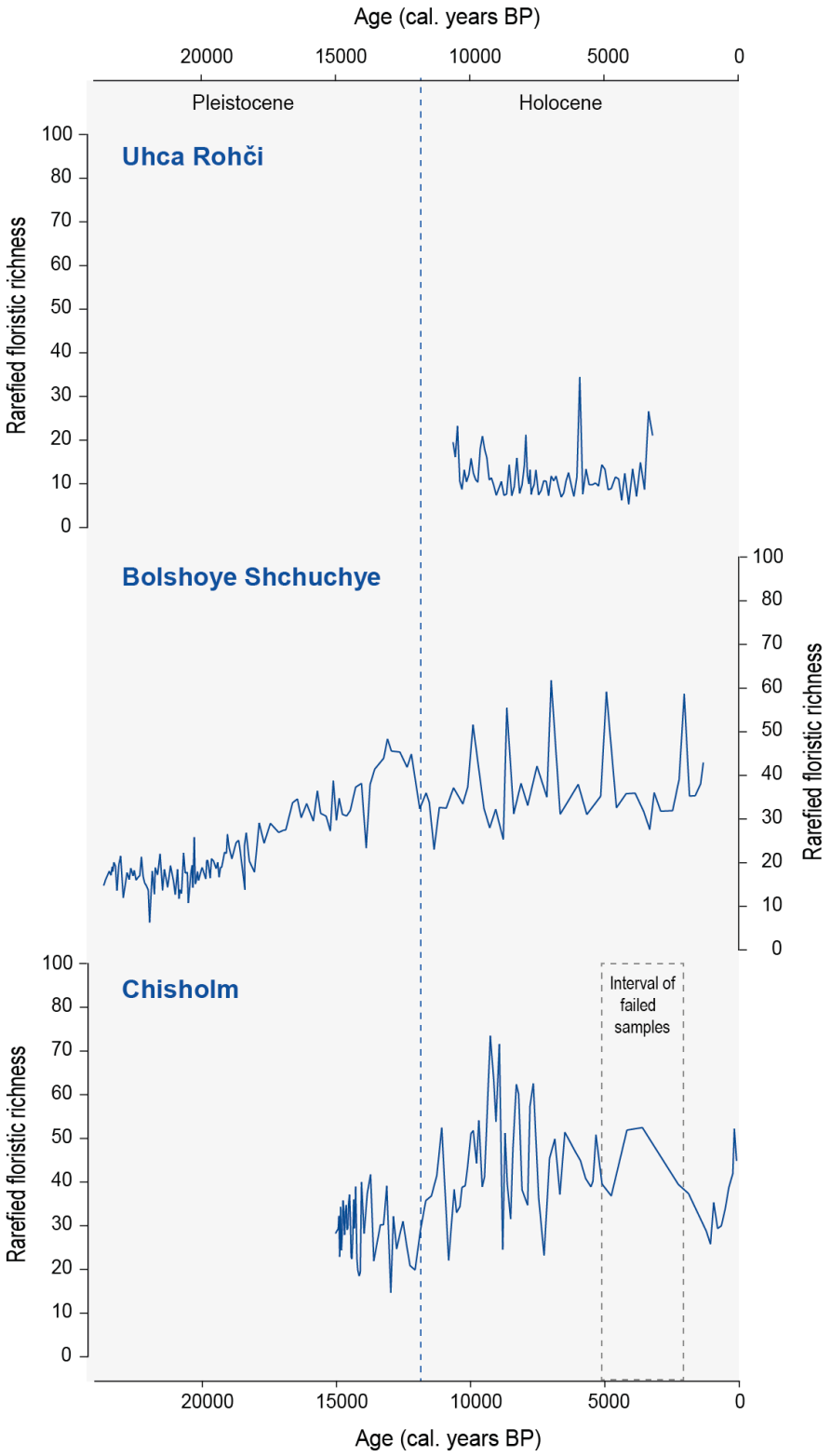

Figure 7.1 Rarefied floristic richness based on *sed*aDNA at the three lakes studied in this thesis. Dashed blue line indicates the Pleistocene-Holocene boundary at 11,800 cal. years BP. Grey dashed box indicates the interval where 12 *sed*aDNA samples failed (i.e. no plant taxa detected) in the Chisholm Lake record (between 138 and 53 cm depth).

The *sed*aDNA record from Uhca Rohči Lake confirms the conclusions of Normand *et al*. (2011) and Giesecke *et al*. (2012), based on pollen, that there is little change in floristic richness in previously glaciated regions over the Holocene. In this thesis, I expand these conclusions to include regions that were ice-free (i.e. unglaciated) during the last glacial interval (Polar Urals and interior Alaska). In contrast, floristic richness in these regions has undergone a long-term, sustained increase since the last glacial until the middle-Holocene, when richness appears to stabilise (Figure 7.1)

7.9 Future Research Directions

Chapters 3 to 6 have highlighted the importance of lake selection, including the size and physiographic characteristics of the lake and/or catchment and sediment type, in plant *sed*aDNA studies using lake sediments. Future research could involve testing a range of different lake and/or lake sediment types to improve knowledge of the mechanisms controlling the success and representation of *sed*aDNA from lakes. Moreover, new studies focusing to improve knowledge of DNA taphonomy from lake sediments is a crucial next step in understanding factors affecting *sed*aDNA preservation and thus detection success, such as temperature, pH, adsorption on mineral components within the sediments and/or oxygen availability.

Like many palaeoecological records, the *sed*aDNA records presented in this thesis are based on a single sediment sequence retrieved from each of the lakes studied. Therefore, there remains a question as to whether there is spatial variation in the *sed*aDNA signal obtained across a single lake basin and whether there are issues with focusing of *sed*aDNA. Is the *sed*aDNA signal retrieved from the deepest part of a lake the same as the *sed*aDNA signal from a shallower part of the lake? And is there any difference in the occurrence of dominant and/or rare plant taxa in the *sed*aDNA across a lake basin? I am currently involved in a project testing these questions within two lakes in western Russia. We have collected a series of surface samples along several transects across the lakes and aim to test the degree of spatial variation in the taxonomic composition and diversity of *sed*aDNA samples within a single lake basin and will compare the results to detailed vegetation surveys.

Whilst this study has provided the first plant *sed*aDNA records from lakes in the Polar Urals, northeast Norway and interior Alaska with insights into late-Quaternary plant community dynamics, there is a wealth of additional analyses that could be undertaken on these records. Whether insights into past climate can be gained from plant *sed*aDNA records requires further investigation. For the Polar Urals in particular, a high-resolution, proxy-based reconstruction of palaeoclimate is currently lacking. However, there are currently no calibration datasets available

and to date, quantitative estimates of plant abundances based on *sed*aDNA are cautioned against (Parducci *et al*., 2017). Insights into past climate could potentially be gained with the use of indicator species if they have small climate and environmental tolerances.

In Chapter 4, the plant taxa identified by *sed*aDNA at Lake Bolshoye Shchuchye were classified as "arctic-alpine", "boreal" or "arctic-boreal" based on their present-day biogeographic distribution. Similarly, identified plant taxa could be classified based on certain ecological traits to assess changes in surface and/or below ground properties such as mycorrhizae (e.g. Zobel *et al*., 2018), palatability or growth forms over time for example. As part of the work involved for Chapters 4 and 5 in this thesis, I compiled a range of ecological indicator values defined by Landolt (1977) and minimum July temperature requirements, where possible, for plant taxa identified by *sed*aDNA at Lake Bolshoye Shchuchye. For the purpose of this thesis, the distributional classification of taxa, which was used to investigate the persistence of arctic-alpine plants over time (Chapter 4; Clarke *et al*., 2019) proved a timelier and thus more interesting analysis to focus upon. However, an assessment of their ecological indicator values over time for parameters such as light, moisture and temperature and moisture generated some interesting patterns (Appendix E). Further analyses of relevant indicator values and/or plant traits would allow a more comprehensive study of long-term changes in plant communities and their underlying environmental variables.

The work presented in this thesis has provided new insights into the composition of flora within ice-free northern regions during the last glacial interval and how it subsequently changed over the Pleistocene-Holocene transition. The ability to detect plant taxa using *sed*aDNA is ultimately dependent on what sequences are available in the reference library. Further custom catalogues, like the arctic and boreal vascular plant reference libraries (Sønstebø *et al*., 2010; Willerslev *et al*., 2014), are needed to expand the possibilities for new applications and/or research questions. This study has highlighted the value of *sed*aDNA from lake sediments in resolving bryophyte and graminoid communities, in particular, compared to traditional palaeoecological techniques. Reference libraries, which include a range of different metabarcodes (e.g. *trn*L P6-loop, ITS; see Taberlet *et al*., 2018), for these functional groups are far from complete and thus the potential is high for *sed*aDNA to provide more detailed floristic data. To further improve *sed*aDNA detection success, future work should focus on increasing the extensiveness of reference libraries. This is currently a major goal for *sed*aDNA studies from regions outside the Arctic, particularly temperate and tropical regions where reference libraries are sparse or lacking.

Lastly, the principal aim of this thesis was to test the use of plant *sed*aDNA to understand and resolve changes in floristic composition over time. However, there is also a great potential to retrieve information on the composition of faunal communities over time using these sediment

cores. This was the aim of an associated study, which I was involved in, which tested the success of a universal mammal 16S ribosomal DNA marker (Giguet-Covex *et al*., 2014) on the same samples analysed in this study from Lake Bolshoye Shchuchye and Uhca Rohči Lake, full details of which are given in Appendix F. While some mammals were recorded using the marker, overall the recovery and detection of mammal *sed*aDNA using this marker was rather poor and instead, worm extracellular DNA 'bycatch' was rather high (Lammers *et al*., 2019). We concluded that the poor detection of mammals was likely due to a lack of template material in the sediments, potentially caused by the low amounts of mammalian DNA deposited in the lakes, the age of the sediments or the size of the target amplicon.

Future research could focus on the exploration of alternative primers and/or methods for the detection of mammals in ancient sediments. I am currently involved in a project to test species-specific primers for key mammal and fish taxa for detecting their *sed*aDNA within ancient archaeological sediments. Other alternatives are to bypass the usage of primers altogether by either shotgun sequencing sediment extracts (Pedersen *et al.*, 2016; Seersholm *et al.*, 2016) or by using DNA target capture methods (Slon *et al.*, 2017).

7.10 Conclusions

This thesis has provided the first *sed*aDNA-based record of plant community composition and floristic richness for northern Finnmark, the Polar Ural Mountains of northern Russia and for interior Alaska. It provides a characterisation of late-Quaternary dynamics of northern plant communities in three specific regions, each of which is represented by a single lake site. It was shown that the last full-glacial flora, which is represented by two of the lake sites, comprised herb-tundra vegetation, dominated by forbs with some graminoids. A sustained increase in floristic richness, associated with the sequential addition of new plant taxa over time, characterises the Lateglacial interval and Pleistocene-Holocene transition. The *sed*aDNA record from the site in the Polar Urals provides the first robust *sed*aDNA-based evidence of long-term persistence of arctic-alpine taxa through several large-magnitude climate changes within a spatially heterogeneous landscape. This *sed*aDNA record was also compared with a high-resolution and detailed pollen record (i.e. counted with a high pollen sum) from the same site and provides the first comprehensive comparison of the taxonomic overlap and detection success of the two proxies. Contrary to the conclusions of earlier *sed*aDNA studies, the findings presented in this thesis demonstrate considerable overlap in the pattern of occurrence of key plant taxa between the *sed*aDNA and pollen records. *Sed*aDNA was also able to document fossil-silent plant taxa which are poorly represented and/or taxonomically resolved within pollen records, including the coniferous tree *Larix*, a diversity of arctic-alpine herbs and turnover amongst bryophyte and grass genera over time.

The findings presented in this thesis contribute towards an understanding of how the composition of northern plant communities has changed in response to climate and environmental changes over long timescales, including a period during the early- to middle-Holocene when mean summer temperatures were likely warmer than present. In general, the *sed*aDNA records from all three sites demonstrate a degree of stability of the flora to environmental changes over the past *ca*. 25,000 years. Yet, it is important to remember that the nature and magnitude of future climate changes is likely to be different to those experienced in the past. The persistence of arctic-alpine plant taxa in the *sed*aDNA record from the Polar Urals demonstrates the buffering capacity of spatially heterogeneous mountain regions via their provision of a range of plant habitats that confer resilience to regional climate changes. This provides a new perspective for conservation strategies, suggesting that current conservation priorities should go beyond protecting individual species or regions which are deemed important today and instead move towards protecting areas that have shown to be resilient to past climate changes. A distinct decline in the proportional abundance and diversity of arctic-alpine plants began as soon as woody taxa started to expand, however, suggesting that in a future warming scenario, local species loss may occur long before tree establishment occurs. This supports the need for conservation awareness of potential biodiversity loss in northern environments, particularly those with less topographic buffering capacity.

At each northern lake site studied as part of this thesis, *sed*aDNA was found to contribute new information on late-Quaternary plant community dynamics. The results presented in this thesis confirm that lake selection remains an important consideration in plant *sed*aDNA studies. Techniques of *sed*aDNA analysis are rapidly developing and promise all kinds of new and exciting future applications and/or research questions to be addressed.

Appendix A Supporting Information: Paper I

Here we include three supplemental figures which present the full taxon list identified by *sed*aDNA analyses at Uhca Rohči Lake to augment those in the main text. We also include four supplemental tables, three containing summary data and one containing published *Pinus* pollen influx rates from northern Norway and Finland.

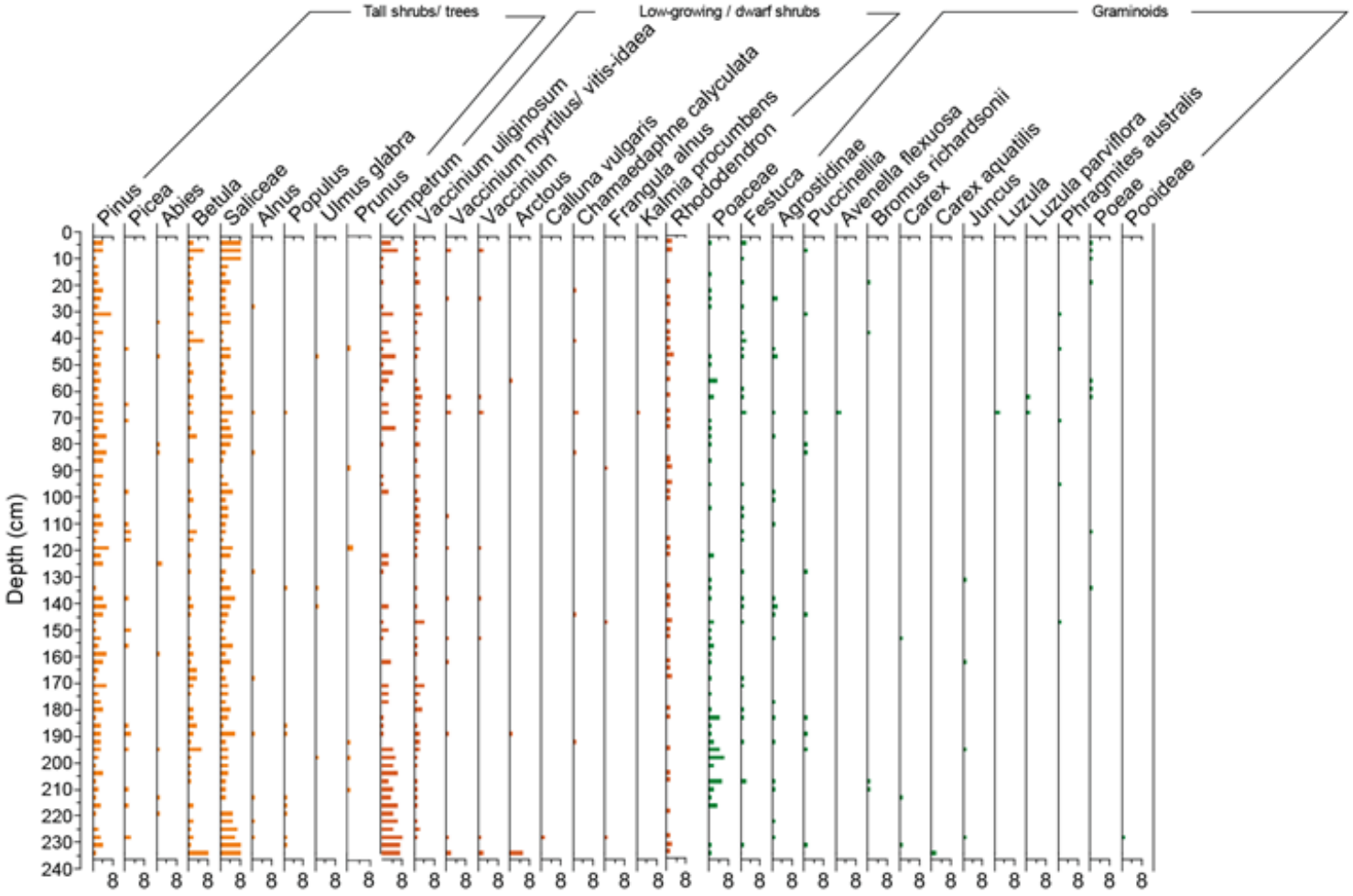

Figure A.1 Woody and graminoid taxa detected by *sed*aDNA in Uhca Rohči Lake. The x-axis refers to number of PCR repeats with 10 or more reads. Only taxa which survived post-identification filtering are included.

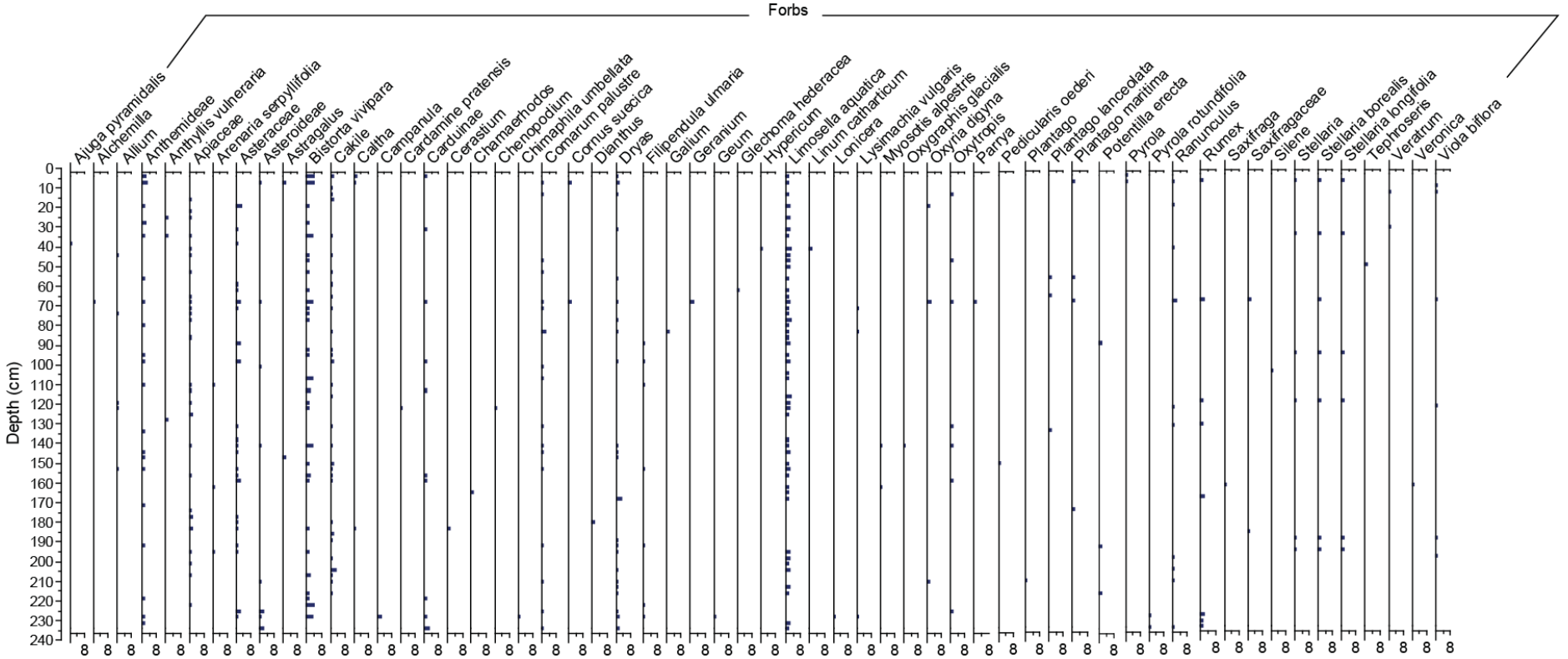

Figure A.2 Forb taxa detected by *sed*aDNA in Uhca Rohči Lake. The x-axis refers to number of PCR repeats with 10 or more reads. Only taxa which survived post-identification filtering are included.

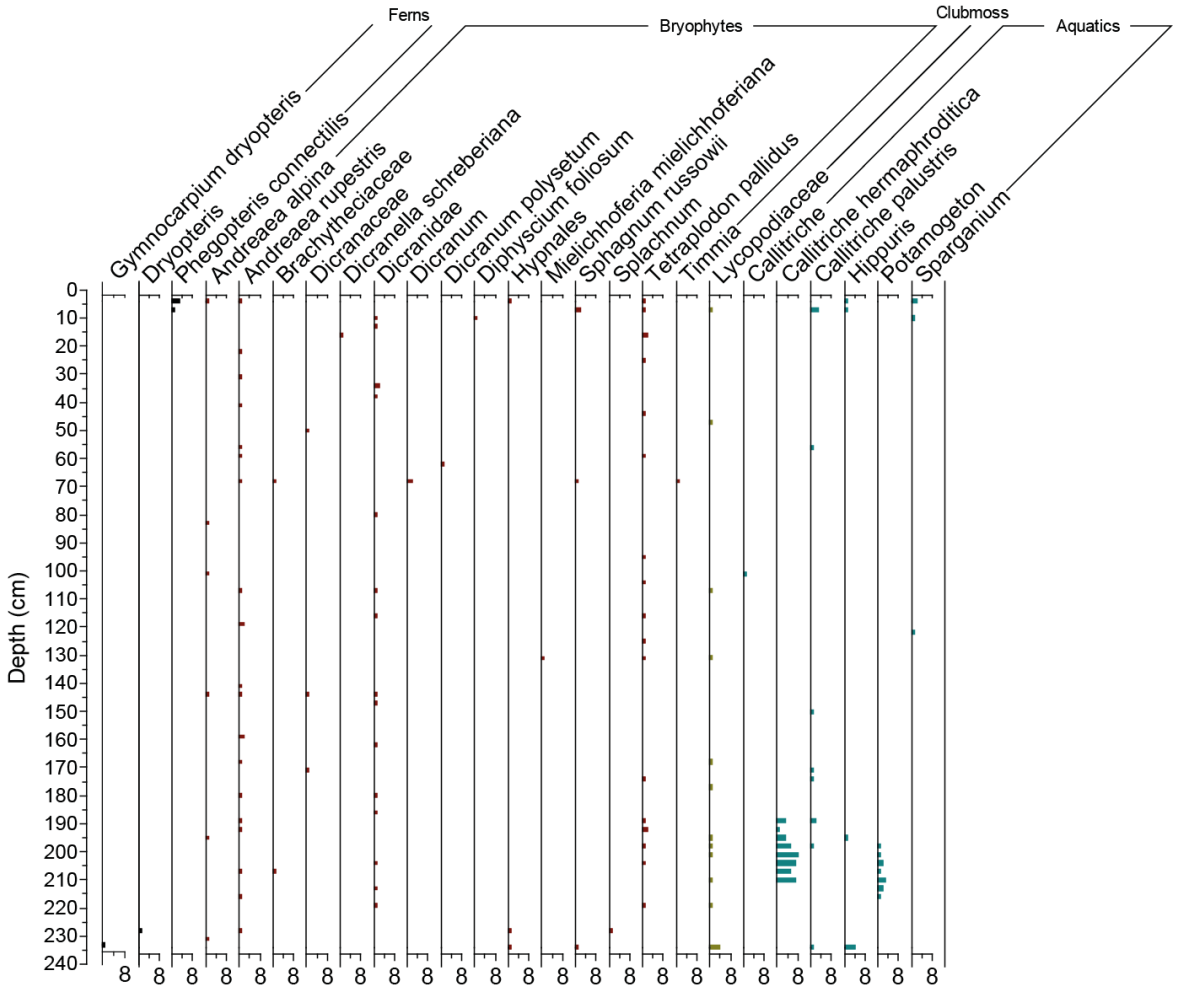

Figure A.3 Fern, bryophyte, clubmoss and aquatic plant taxa detected by *sed*aDNA in Uhca Rohči Lake. The x-axis refers to number of PCR repeats with 10 or more reads. Only taxa which survived post-identification filtering are included.

Table A.1 Total DNA read numbers remaining after each filtering step described in the material and methods section.

Filtering step	Total DNA reads
Pre-identification (raw)	72 355 976
Removing sequences not assigned to samples	5 597 221
Remove sequences < 100 % match to reference sequence in arcborbryo data	4 187 031
Remove sequences with fewer average reads in samples vs. negative contr	1 926 928
Minimum 10 reads in PCR replicate criterion	1 925 757
Sequences belonging to domestic food plants and/or assumed false positives (The identity of which could not be distinguished from NCBI BLAST search)	1 727 409

Table A.2 All taxa detected from *sed*aDNA (orange) and pollen (blue) analyses, sorted according to family within groups: vascular plants, bryophytes and clubmosses, aquatics and algae. All *sed*aDNA data is based on final dataset after post-identification filtering steps. Local is recorded within the Komagdalen valley, regional is recorded within Finnmark and/or Kola Peninsula which spans a bioclimatic gradient from boreal pine forest to low arctic shrub tundra.

Family	Taxa *sed*aDNA	Taxa pollen	Max. repeats per sample (out of 8)	Sum repeats across samples (out of 640)	Sum reads across samples	Max pollen %	Sum pollen grains across samples	Distrib. E=Exotic, F=assumed false positive, L=Local, R=regional	Sum reads across negative controls	Sum repeats across negative controls (out of 176)	Ratio reads across negative controls/ reads across samples
Amaranthaceae	Chenopodium		1	1	516			L	47	1	0.09
Amaryllidaceae	Allium		1	5	1009			R	0	0	0.00
Apiaceae	Apiaceae	Apiaceae	2	29	22287	0.6	2	R	2972	5	0.13
Asteraceae	Anthemideae		3	24	8761			E	405	2	0.05
		Artemisia				3.2	68	R			
	Asteraceae	Asteraceae	3	30	13248	1.9	31	L	1776	5	0.13
	Asteroideae		2	10	2573			L	9	0	0.00
	Carduinae		3	12	7344			L	1269	2	0.17
	Tephroseris		1	1	165			R	0	0	0.00
Betulaceae	Alnus	Alnus	1	9	4168	1.7	31	R	12	1	0.00
	Betula	Betula	8	107	42867	82.8	3832	L	6895	17	0.16
		Corylus				3.1	53	E			
Boraginaceae	Myosotis apestris		1	2	1143			R	0	0	0.00
Brassicaceae	Cakile		3	37	44432			R	9852	7	0.22
	Cardamine pratensis		1	1	1987			L	5	1	0.00
	Parrya		1	1	450			E	0	0	0.00

Table A.2 cont.

Family	Taxa *sed*aDNA	Taxa pollen	Max. repeats per sample (out of 8)	Sum repeats across samples (out of 640)	Sum reads across samples	Max pollen %	Sum pollen grains across samples	Distrib. E=Exotic, F=assumed false positive, L=Local, R=regional	Sum reads across negative controls	Sum repeats across negative controls (out of 176)	Ratio reads across negative controls/ reads across samples
Campanulaceae	Campanula		2	2	1354			L	0	0	0,00
Caprifoliaceae	Lonicera		1	1	44			R	0	0	0,00
Caryophyllaceae	Arenaria serpyllifolia		1	3	804			R	0	0	0,00
	Cerastium		1	1	119			L	0	0	0,00
		Caryophyllaceae				1,3	10	L			
	Dianthus		1	1	63			R	0	0	0,00
	Silene		1	1	472			R	0	0	0,00
	Stellaria		1	6	2914			L	0	0	0,00
	Stellaria borealis		1	7	492			L	0	0	0,00
	Stellaria longifolia		1	6	179			R	0	0	0,00
Chenopodiaceae		Chenopodiaceae				3,9	72	R			
Cornaceae	Cornus suecica		1	2	27			R	0	0	0,00
Cupressaceae		Juniperus				0,9	12	L			
Cyperaceae	Carex		1	3	57			L	0	0	0,00
	Carex aquatilis		2	2	54			L	0	0	0,00
		Cyperaceae				1,9	21	L			
Dryopteridaceae	Dryopteris	Filicales/ Dryopteris	1	1	332	8,2	219	L	0	0	0,00

Table A.2. cont.

Family	Taxa *sed*aDNA	Taxa pollen	Max. repeats per sample (out of 8)	Sum repeats across samples (out of 640)	Sum reads across samples	Max pollen %	Sum pollen grains across samples	Distrib. E=Exotic, F=assumed false positive, L=Local, R=regional	Sum reads across negative controls	Sum repeats across negative controls (out of 176)	Ratio reads across negative controls/ reads across samples
Ericaceae	Arctous		5	7	403			R	0	0	0.00
	Calluna vulgaris	Calluna	1	1	19	0.9	19	R	0	0	0.00
	Chamaedaphne calyculata		2	7	1158			R	268	4	0.23
	Chimaphila umbellata		1	1	22			E	0	0	0.00
	Empetrum	Empetrum	8	143	96227	18.9	370	L	15029	18	0.16
		Ericales				2	53	E			
	Kalmia procumbens		1	1	14			R	0	0	0.00
	Pyrola		1	2	149			L	0	0	0.00
	Pyrola rotundifolia		1	2	106			R	0	0	0.00
	Rhododendron tomentosum complex		3	51	22846			R	4191	9	0.18
	Vaccinium		2	12	1012			L	79	1	0.08
	Vaccinium vitis-idaea / myrtilis		2	16	2109			L	83	1	0.04
	Vaccinium uliginosum		4	85	61712			L	12200	20	0.20
Equisetaceae		Equisetum				3.3	50	L			
Fabaceae	Anthyllis vulneraria		1	3	3861			R	0	0	0.00
	Astragalus		1	2	471			R	90	0	0.19
	Oxytropis		1	7	2201			R	48	1	0.02
Geraniaceae	Geranium	Geranium	2	2	2026	0.6	5	L	79	2	0.04
Hypericaceae	Hypericum		1	1	350			R	1	1	0.00
Juncaceae	Juncus		1	4	643			L	0	0	0.00
	Luzula		2	2	470			L	0	0	0.00
	Luzula parviflora		1	2	92			R	0	0	0.00

Table A.2 cont.

Family	Taxa *sed*aDNA	Taxa pollen	Max. repeats per sample (out of 8)	Sum repeats across samples (out of 640)	Sum reads across samples	Max pollen %	Sum pollen grains across samples	Distrib. E=Exotic, F=assumed false positive, L=Local, R=regional	Sum reads across negative controls	Sum repeats across negative controls (out of 176)	Ratio reads across negative controls/ reads across samples
Lactuceae		Lactuceae				0,5	8	R			
Lamiaceae	Ajuga pyramidalis		1	1	172			R	0	0	0,00
	Glechoma hederacea		1	1	768			E	0	0	0,00
	Thymus		2	25	17817			R	2532	5	0,14
Linaceae	Linum catharticum		1	1	433			E	0	0	0,00
Melanthiaceae	Veratrum		1	2	172			R	0	0	0,00
Myricaceae		Myrica gale				1,7	27	E			
Onagraceae		Epilobium				2	47	L			
	Pedicularis oederi		1	1	174			E	0	0	0,00
Pinaceae	Abies		2	10	53856			E	2178	2	0,04
	Picea		2	21	14132	0,6	5	E	1301	4	0,09
	Pinus	Pinus	7	203	349969	15,2	508	E	97869	61	0,28
Plantaginaceae	Plantago		1	1	1034			R	0	0	0,00
	Plantago lanceolata		1	3	1547			R	0	0	0,00
	Plantago maritima		1	4	2663			R	33	1	0,01
	Veronica		1	1	217			L	0	0	0,00
Poaceae	Agrostidinae		2	22	19380			L	5089	5	0,26
	Avenella flexuosa		2	2	177			L	0	0	0,00
	Bromus richardsonii (NCBI BLAST suggests sequence similarity to Bromus inermis)		1	4	1626			F	256	1	0,16
	Festuca		2	31	25154			L	6776	8	0,27
	Phragmites australis		1	5	6316			R	514	1	0,08
	Poaceae	Poaceae	6	68	72794	11,5	344	L	6688	12	0,09

Table A.2 cont.

Family	Taxa *sed*aDNA	Taxa pollen	Max. repeats per sample (out of 8)	Sum repeats across samples (out of 640)	Sum reads across samples	Max pollen %	Sum pollen grains across samples	Distrib. E=Exotic, F=assumed false positive, L=Local, R=regional	Sum reads across negative controls	Sum repeats across negative controls (out of 176)	Ratio reads across negative controls/ reads across samples
Poaceae	Poeae		1	9	3895			L	1104	3	0.28
	Pooideae		1	1	135			L	0	0	0.00
	Puccinellia		1	11	18317			R	3071	4	0.17
Polygonaceae	Bistorta vivipara		4	52	30753			L	8555	18	0.28
	Oxyria digyna	Oxyria	2	4	527	1.5	41	L	0	0	0.00
	Rumex	Rumex- type	2	11	5415	3	75	L	373	1	0.07
Primulaceae	Lysimachia vulgaris		1	3	618			E	61	1	0.10
Ranunculaceae	Caltha		2	4	2122			L	0	0	0.00
	Oxygraphis glacialis		1	1	57			E	0	0	0.00
	Ranunculus		2	11	7035			L	421	2	0.06
		Thalictrum				0.6	3	R			
Rhamnaceae	Frangula alnus		1	3	1317			E	136	1	0.10
Rosaceae	Alchemilla		1	1	506			L	0	0	0.00
	Chamaerhodos (NCBI BLAST suggests sequence similarity to Dasiphore fruticosa)		1	1	161			F	0	0	0.00
	Comarum palustre		2	18	6176			L	985	9	0.16
	Dryas		3	28	8687			R	460	2	0.05
	Filipendula ulmaria	Filipendula	1	7	2392	3.2	58	L	0	0	0.00
	Geum		1	1	34			L	0	0	0.00
		Rosaceae-type				1	9				
Rosaceae	Potentilla erecta		1	3	1007			R	0	0	0.00
	Prunus		2	7	3916			R	0	0	0.00
Rubiaceae	Galium		1	1	545			R	0	0	0.00

Table A.2. cont.

Family	Taxa *sed*aDNA	Taxa pollen	Max. repeats per sample (out of 8)	Sum repeats across samples (out of 640)	Sum reads across samples	Max pollen %	Sum pollen grains across samples	Distrib. E=Exotic, F=assumed false positive, L=Local, R=regional	Sum reads across negative controls	Sum repeats across negative controls (out of 176)	Ratio reads across negative controls/ reads across samples
Salicaceae	Populus		1	9	2781			L	0	0	0.00
	Salicaceae	Salix	8	244	322954	10.2	240	L	40633	45	0.13
Saxifragaceae	Saxifraga	Saxifraga	1	1	59	1.3	7	L	0	0	0.00
	Saxifragaceae		2	3	265			L	0	0	0.00
Selaginellaceae		Selaginella selaginoides				0.3	4	L			
Thelypteridaceae	Phegopteris connectilis		3	4	1648			R	0	0	0.00
Ulmaceae	Ulmus glabra	Ulmus	1	4	952	0.9	6	E	253	1	0.27
Violaceae	Viola biflora		1	6	1087			L	0	0	0.00
Woodsiaceae	Gymnocarpium dryopteris		1	1	286			R	0	0	0.00
Aquatics											
Haloragaceae		Myriophyllum spicatum				1.7	30	E			
Menyanthaceae		Menyanthes				0.6	2	R			
Plantaginaceae	Callitriche	Callitriche	1	1	639	0.6	3	L	0	0	0.00
	Callitriche hermaphroditica		8	39	26542			R	0	0	0.00
	Callitriche palustris		3	11	3571			R	0	0	0.00
	Hippuris		4	7	4116			R	0	0	0.00
Potamogetonaceae	Potamogeton		3	11	1478			L	0	0	0.00
Scrophulariaceae	Limosella aquatica		3	71	56340			E	1478	15	0.03
Typhaceae	Sparganium	Sparganium	2	4	1198	4.2	98	R	0	0	0.00

Table A.2 cont.

Family	Taxa *sed*aDNA	Taxa pollen	Max. repeats per sample (out of 8)	Sum repeats across samples (out of 640)	Sum reads across samples	Max pollen %	Sum pollen grains across samples	Distrib. E=Exotic, F=assumed false positive, L=Local, R=regional	Sum reads across negative controls	Sum repeats across negative controls (out of 176)	Ratio reads across negative controls/ reads across samples
Bryophytes and Clubmosses											
Andreaeaceae	Andreaea alpina		1	6	2790			L	127	1	0.05
	Andreaea rupestris		2	21	15733			R	3078	9	0.20
Brachytheciaceae	Brachytheciaceae		1	2	1014			R	231	1	0.23
Dicranaceae	Dicranaceae		1	3	789			R	1	1	0.00
Dicranidae	Dicranidae		2	16	1739			R	127	2	0.07
	Dicranella schreberiana		1	1	118			R	0	0	0.00
	Dicranum		2	2	70			R	0	0	0.00
	Dicranum polysetum		1	1	276			R	0	0	0.00
Diphysciaceae	Diphyscium foliosum		1	1	53			R	0	0	0.00
Isoetaceae		Isoetes				10.2	95	R			
Lycopodiaceae	Lycopodiaceae		4	15	6474			R	0	0	0.00
Mniaceae	Mielichhoferia mielichhoferiana		1	1	51			R	0	0	0.00
Hypnaceae	Hypnales		1	3	542			R	1	1	0.00
Pelliaceae	Pellia		1	1	40			R	0	0	0.00
Polypodiaceae		Polypodium				1.6	30	R			
Sphagnaceae	Sphagnum russowii	Sphagnum	2	4	1281	0.8	14	L	303	1	0.24
Splachnaceae	Splachnum		1	1	53			L	0	0	0.00
	Tetraplodon pallidus		2	19	3135			R	854	5	0.27
Timmiaceae	Timmia		1	1	279			L	0	0	0.00
Algae											
Hydrodictyaceae		Pediastrum					27238	R			

Table A.2 cont.

Family	Taxa *sed*aDNA	Taxa pollen	Comment
Amaranthaceae	Chenopodium		
Amaryllidaceae	Allium		Allium schoenoprasum common on Varanger Peninsula
Apiaceae	Apiaceae	Apiaceae	Angelica archangelica, Anthriscus sylvestris, common on Varanger Peninsula, Ligusticum, Carum, Conioselinum in coastal locations
Asteraceae	Anthemideae		
		Artemisia	A. vulgaris most likely species
	Asteraceae	Asteraceae	
	Asteroideae		
	Carduinae		
	Tephroseris		
Betulaceae	Alnus	Alnus	
	Betula	Betula	
		Corylus	
Boraginaceae	Myosotis apestris		
Brassicaceae	Cakile		
	Cardamine pratensis		
	Parrya		Eastern, occurs on Kanin Peninsula, Novaya Zemlya
Campanulaceae	Campanula		

Table A.2 cont.

Family	Taxa *sed*aDNA	Taxa pollen	Comment
Caprifoliaceae	Lonicera		Eastern, Lonicera coerulea, rare on Kola Peninsula
Caryophyllaceae	Arenaria serpyllifolia		
	Cerastium		
		Caryophyllaceae	
	Dianthus		
	Silene		
	Stellaria		
	Stellaria borealis		
	Stellaria longifolia		
Chenopodiaceae		Chenopodiaceae	
Cornaceae	Cornus suecica		Chenopodium suecicum, C. album, Atriplex spp.
Cupressaceae		Juniperus	
Cyperaceae	Carex		
	Carex aquatilis		
		Cyperaceae	
Dryopteridaceae	Dryopteris	Filicales/ Dryopteris	

Table A.2 cont.

Family	Taxa *sed*aDNA	Taxa pollen	Comment
Ericaceae	Arctous		
	Calluna vulgaris	Calluna	
	Chamaedaphne calyculata		
	Chimaphila umbellata		Southern
	Empetrum	Empetrum	
		Ericales	
	Kalmia procumbens		
	Pyrola		
	Pyrola rotundifolia		
	Rhododendron tomentosum complex		Southeast
	Vaccinium		
	Vaccinium vitis-idaea/ myrtilis		
	Vaccinium uliginosum		
Equisetaceae		Equisetum	
Fabaceae	Anthyllis vulneraria		
	Astragalus		
	Oxytropis		
Geraniaceae	Geranium	Geranium	
Hypericaceae	Hypericum		Southern, rare on Kola Peninsula

Table A.2 cont.

Family	Taxa *sed*aDNA	Taxa pollen	Comment
Juncaceae	Juncus		
	Luzula		
	Luzula parviflora		
Lactuceae		Lactuceae	Lactuca alpina
Lamiaceae	Ajuga pyramidalis		Southern, only two records in Finnmark
	Glechoma hederacea		Southern, introduced in north
	Thymus		Eastern, Thymus serpyllum
Linaceae	Linum catharticum		Southern
Melanthiaceae	Veratrum		V. album and V. lobelianum found in region mainly NW of site (Ifjorden)
Myricaceae		Myrica gale	Southern
Onagraceae		Epilobium	
Orobanchaceae	Pedicularis oederi		Southern and eastern with distribution gaps
Pinaceae	Abies		Southeastern
	Picea		Southeastern, planted in18 km from lake today (Komagvær)
	Pinus	Pinus	Southeastern, planted in 30 km from lake today (Vadsø)
Plantaginaceae	Plantago		
	Plantago lanceolata		Southern, rare on Kola Pen.
	Plantago maritima		
	Veronica		

Table A.2 cont.

Family	Taxa *sed*aDNA	Taxa pollen	Comment
Poaceae	Agrostidinae		
	Avenella flexuosa		
	Bromus richardsonii (NCBI BLAST suggests sequence similarity to Bromus inermis)		
	Festuca		
	Phragmites australis		Southern
	Poaceae	Poaceae	
	Poeae		
	Pooideae		
	Puccinellia		Coastal
Polygonaceae	Bistorta vivipara		
	Oxyria digyna	Oxyria	
	Rumex	Rumex- type	
Primulaceae	Lysimachia vulgaris		Southern, bordering Kola Peninsula
Ranunculaceae	Caltha		
	Oxygraphis glacialis		Eastern, scattered from Polar Ural eastwards, Syn. Ficaria glacialis
	Ranunculus		
		Thalictrum	
Rhamnaceae	Frangula alnus		Southern, bordering Kola Peninsula

Table A.2 cont.

Family	Taxa *sed*aDNA	Taxa pollen	Comment
Rosaceae	Alchemilla		
	Chamaerhodos (NCBI BLAST suggests sequence similarity to Dasiphore fruticosa)		
	Comarum palustre		
	Dryas		
	Filipendula ulmaria	Filipendula	
	Geum		
		Rosaceae-type	
	Potentilla erecta		Southern, only one record from Varanger
	Prunus		Southern, only two record from Varanger
Rubiaceae	Galium		
Salicaceae	Populus		
	Salicaceae	Salix	
Saxifragaceae	Saxifraga	Saxifraga	
	Saxifragaceae		
Selaginellaceae		Selaginella selaginoides	
Thelypteridaceae	Phegopteris connectilis		
Ulmaceae	Ulmus glabra	Ulmus	Southern
Violaceae	Viola biflora		
Woodsiaceae	Gymnocarpium dryopteris		

Table A.2 cont.

Family	Taxa *sed*aDNA	Taxa pollen	Comment
Aquatics			
Haloragaceae		Myriophyllum spicatum	
Menyanthaceae		Menyanthes	
Plantaginaceae	Callitriche	Callitriche	
	Callitriche hermaphroditica		
	Callitriche palustris		
	Hippuris		
Potamogetonaceae	Potamogeton		
Scrophulariaceae	Limosella aquatica		Southern, nearest location Neiden 70 km south
Typhaceae	Sparganium	Sparganium	
Bryophytes and Clubmosses			
Andreaeaceae	Andreaea alpina		
	Andreaea rupestris		Not on Varanger Peninsula but otherwise common in Finnmark
Brachytheciaceae	Brachytheciaceae		
Dicranaceae	Dicranaceae		
Dicranidae	Dicranidae		
	Dicranella schreberiana		Southern. Only two records in Finnmark (Pasvik and Kautokeino)
	Dicranum		
	Dicranum polysetum		Mainly continental part of Finnmark

Table A.2 cont.

Family	Taxa *sed*aDNA	Taxa pollen	Comment
Diphysciaceae	Diphyscium foliosum		
Isoetaceae		Isoetes	
Lycopodiaceae	Lycopodiaceae		
Mniaceae	Mielichhoferia mielichhoferiana		
Hypnaceae	Hypnales		
Pelliaceae	Pellia		
Polypodiaceae		Polypodium	
Sphagnaceae	Sphagnum russowii	Sphagnum	
Splachnaceae	Splachnum		
	Tetraplodon pallidus		
Timmiaceae	Timmia		
Algae			
Hydrodictyaceae		Pediastrum	

Table A.3 Published *Pinus* pollen influx values from Finnmark, northern Norway (administrative region in brackets) and adjacent sites in Finland.

Site	% TLP	Influx gr cm^{-2} a^{-1}	Date (BP or calendrical)	Interpretation or known flora	Reference
Uhca Rohči (Vardø)	6-8	200-800	8.0-7.3	?	This paper
liten Cap'pesjav'ri (Nordkapp)	20-50	200-2700	8.5-4.3		Huntley *et al.* (2013)
over Kobbkrokvatnet (Berlevåg)	20-30	400-1300	8.5-4.3		Huntley *et al.* (2013)
over Gunnarsfjorden (Mehamn)	30-50	200-1000	8.5-4.3		Allen *et al.* (2007); Huntley *et al.* (2013)
Melkøya (Hammerfest)	20-30	200	8.5-8.3, 6.3 Ka	local presence	Jensen (2004)
Mortensnes (Nesseby)	0-75	2000	10-0 Ka	Local presence	Høeg (2000)
Trollvatnet (Alta)		500-2000	8.5 Ka	local presence	Hyvärinen (1985)
Lampemyr (Alta)	55	200	5.5-0.6 Ka	local presence	Høeg (2000)
Østervatnet (Vadsø)	35	400-650	8-8.5 Ka	local presence	Prentice (1982)
Orijarvi (Kymenlakso)		3000	9-7 Ka	pine forest 20-24 t ha	Seppa *et al*. (2009)
Orijavri (Kymenlakso)		2000	2.5-1.5 Ka	pine forest 12-13 t ha	Seppa *et al*. (2009)
Nautajärvi (Pirkanmaa)	25-65	3000	9-7 Ka	pine forest 20-24 t ha	Ojala *et al*. (2008), Seppa *et al*. (2009)
Gauptjern, lake (Målselv)	7	Av. 400 (max 900)	1945-1996	scattered pines around site 6 pine forest at 200m	Jensen *et al*. (2002)
Gauptjern, mire (Målselv)	14	Av. 260 (max 800)			
N-C Sweden, lakes	-	1000-7000	9-1 Ka	pine abundant in forest surrounding lakes	Giesecke and Fontana (2008)
Modern pollen studies in N Fennoscandia and Greenland Ice Sheet					
N Finland Ke8	-	960	1982-98	birch with scattered pine	Hicks and Hyvärinen (1999)
N Finland P9	-	1300	1982-98	birch & pine	
N Finland	-	2160	1982-98	pine forest	
N Finland (threshold value)	-	500	-	pine presence	Hyvärinen (1985); Hicks (1994)
N-C Sweden traps	-	200-19,500	2001-2004	pine present locally	Giesecke and Fontana (2008)
South Greenland Ice Sheet	23	0.08-0.299	1990	long distance transport	Bourgeois (1990)

Table A.4 *Pinus* and *Betula* pollen influx values for Uhca Rohči Lake for cores UR-1 and UR-2.

Core no.	Sample depth (cm)	Age (cal. years BP)	*Betula* pollen concentration (grains g^{-1})	*Pinus* pollen concentration (grains g^{-1})	*Betula* pollen influx (grains cm^{-2} $year^{-1}$)	*Pinus* pollen influx (grains cm^{-2} $year^{-1}$)
UR-2	4	85	30441	7637	848	125
UR-2	41	1363	33211	8592	977	153
UR-1	5	3500	30220	7407	602	148
UR-1	13	3756	58837	13168	1172	262
UR-1	30	4534	28429	9287	740	242
UR-1	34	4693	34024	8592	859	217
UR-1	50	5323	31993	5610	782	137
UR-1	71	6135	46751	7328	1328	208
UR-1	89	6719	54932	3380	1766	109
UR-1	109	7342	102725	8339	3368	273
UR-1	129	7855	82986	5972	5684	409
UR-1	138	7988	129440	13448	8297	862
UR-1	150	8275	52525	4134	1673	132
UR-1	162	8664	44769	1809	1399	57
UR-1	183	9327	44556	1473	1531	51
UR-1	198	9748	29584	1611	1020	56
UR-1	216	10258	19671	1017	693	36
UR-1	234	10710	79407	2989	5294	100

Appendix B Supporting Information: Paper II

Here we include five supplemental figures, two images for providing context to the study site, and three figures presenting the full taxon list identified by *sed*aDNA analyses at Lake Bolshoye Shchuchye to augment those given in the main text. We also include two supplemental tables containing summary *sed*aDNA data and details of the distributional classifications.

Figure B.1 Overview photograph of Lake Bolshoye Shchuchye taken on 14 July 2009 from the northern end of the lake. Image courtesy of John-Inge Svendsen at the University of Bergen. The dark green bushes growing on the alluvial fan and on the slope are green alder (*Alnus viridis*). Note the alluvial fan in the foreground. Arctic-alpine forbs such as *Myosotis* and *Chamerion* occupy fellfield habitats at the highest elevations.

Figure B.2 Overview photograph detailing the topographic characteristics of Lake Bolshoye Shchuchye's catchment taken on 14 July 2009 from the highest elevations on the eastern side of the lake looking towards the deltaic inlet on the northern shore. Image courtesy of John-Inge Svendsen at the University of Bergen. Mountain peaks in the catchment range between 50 and 1,100 m a.s.l.

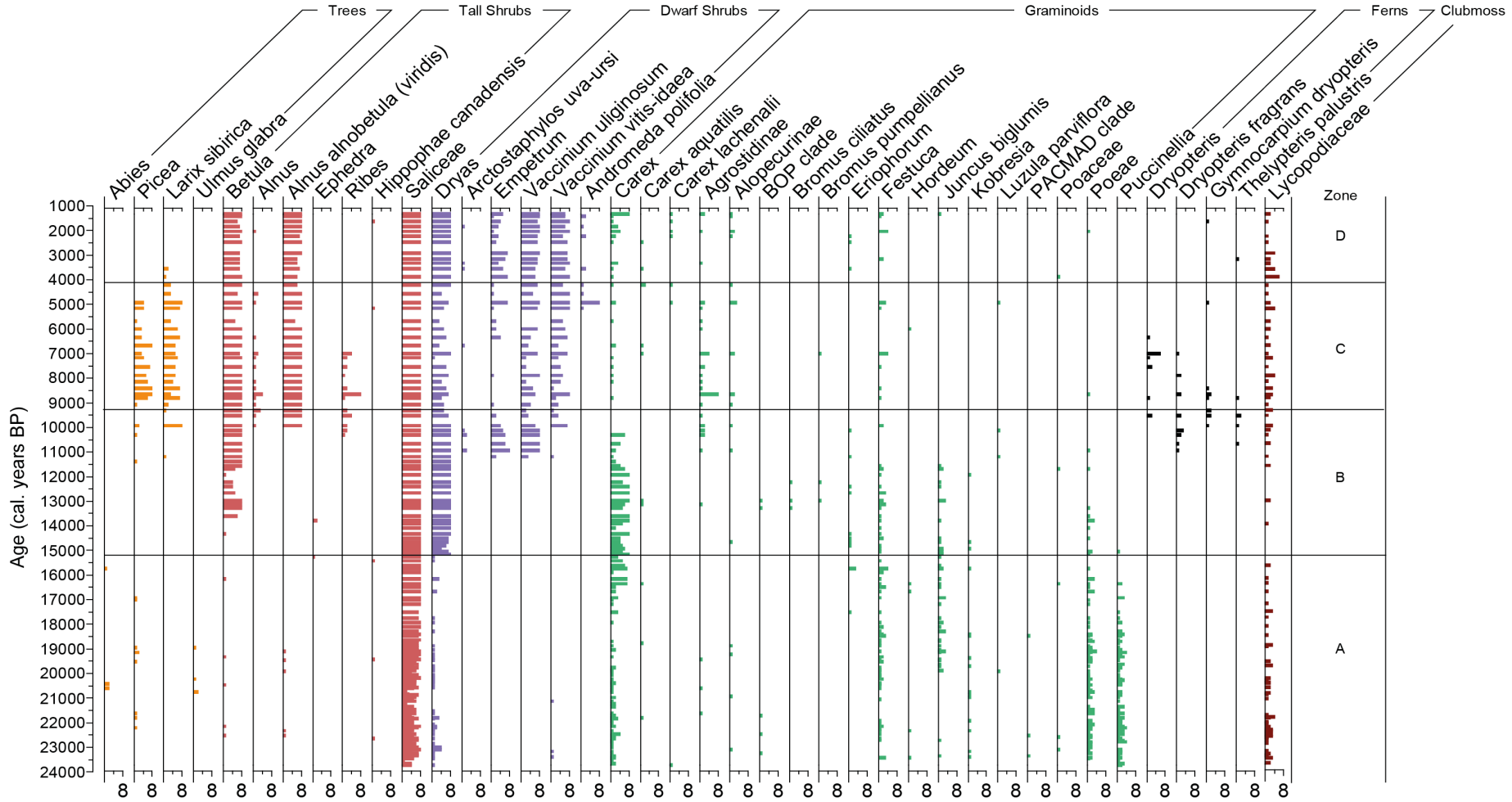

Figure B.3 Woody, graminoid, fern and clubmoss taxa detected by *sed*aDNA at Lake Bolshoye Shchuchye. The x-axis refers to number of PCR replicates (out of eight) a taxon was detected within per sample.

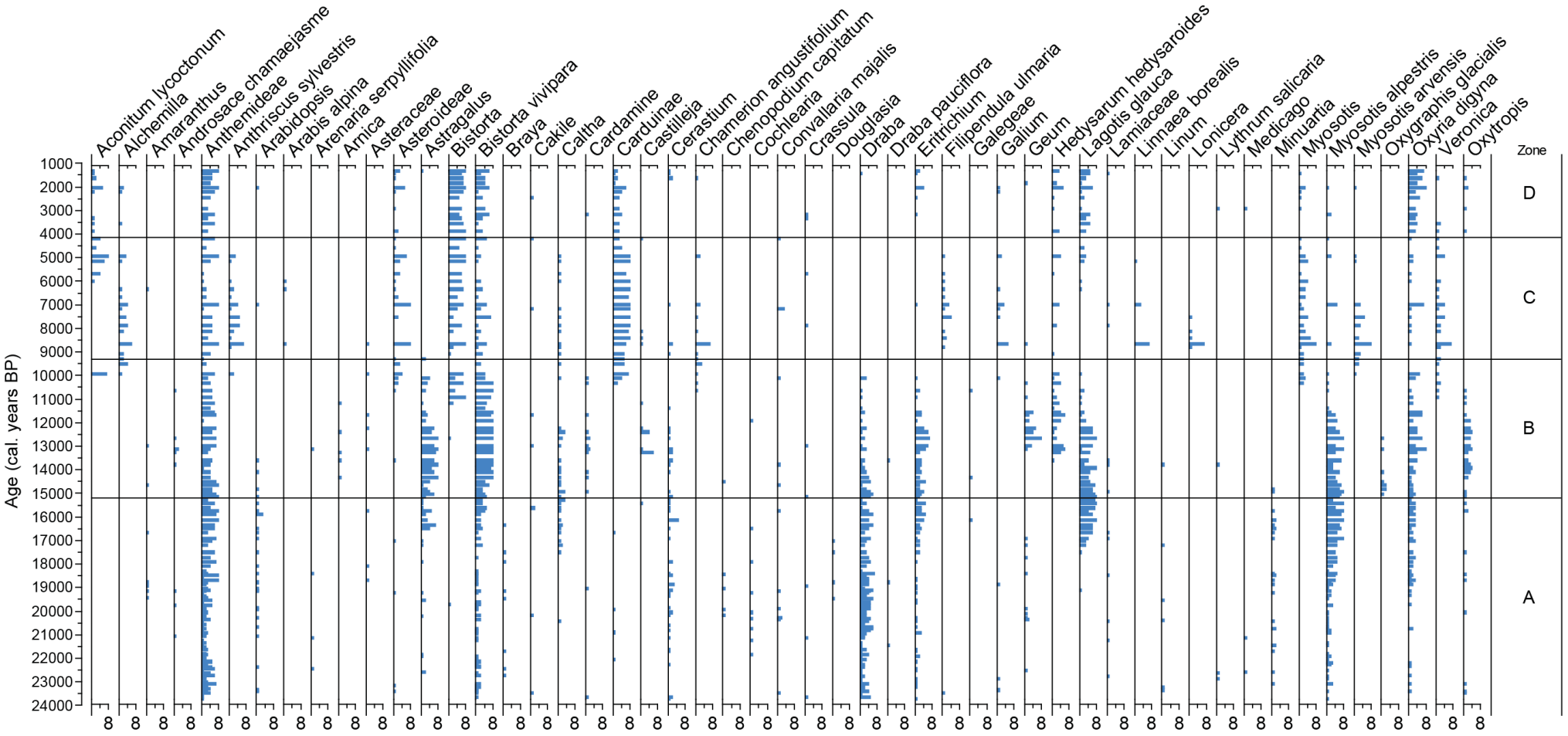

Figure B.4 Forb taxa detected by *sed*aDNA at Lake Bolshoye Shchuchye. The x-axis refers to number of PCR replicates (out of eight) a taxon was detected within per sample.

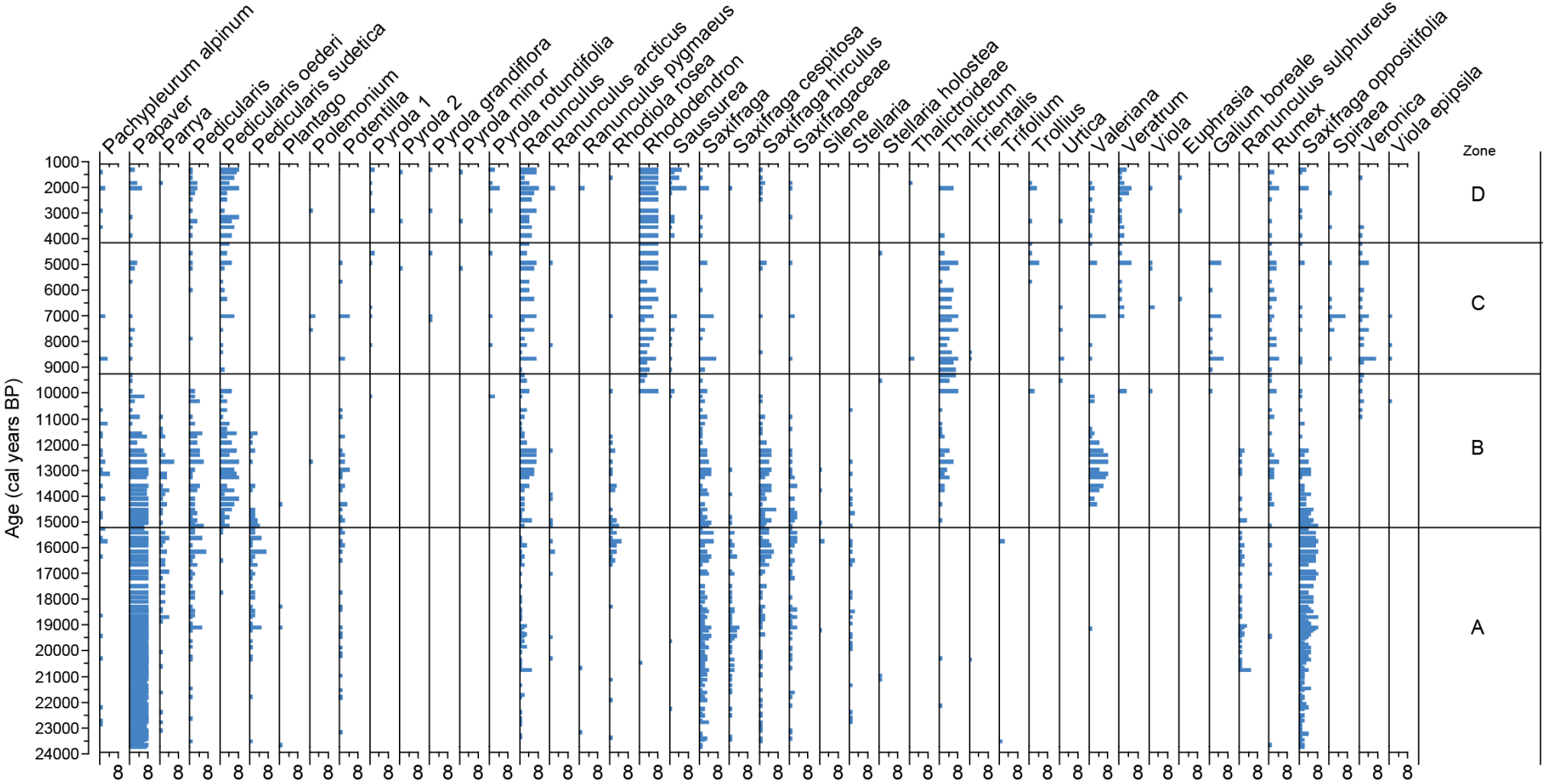

Figure B.4 cont.

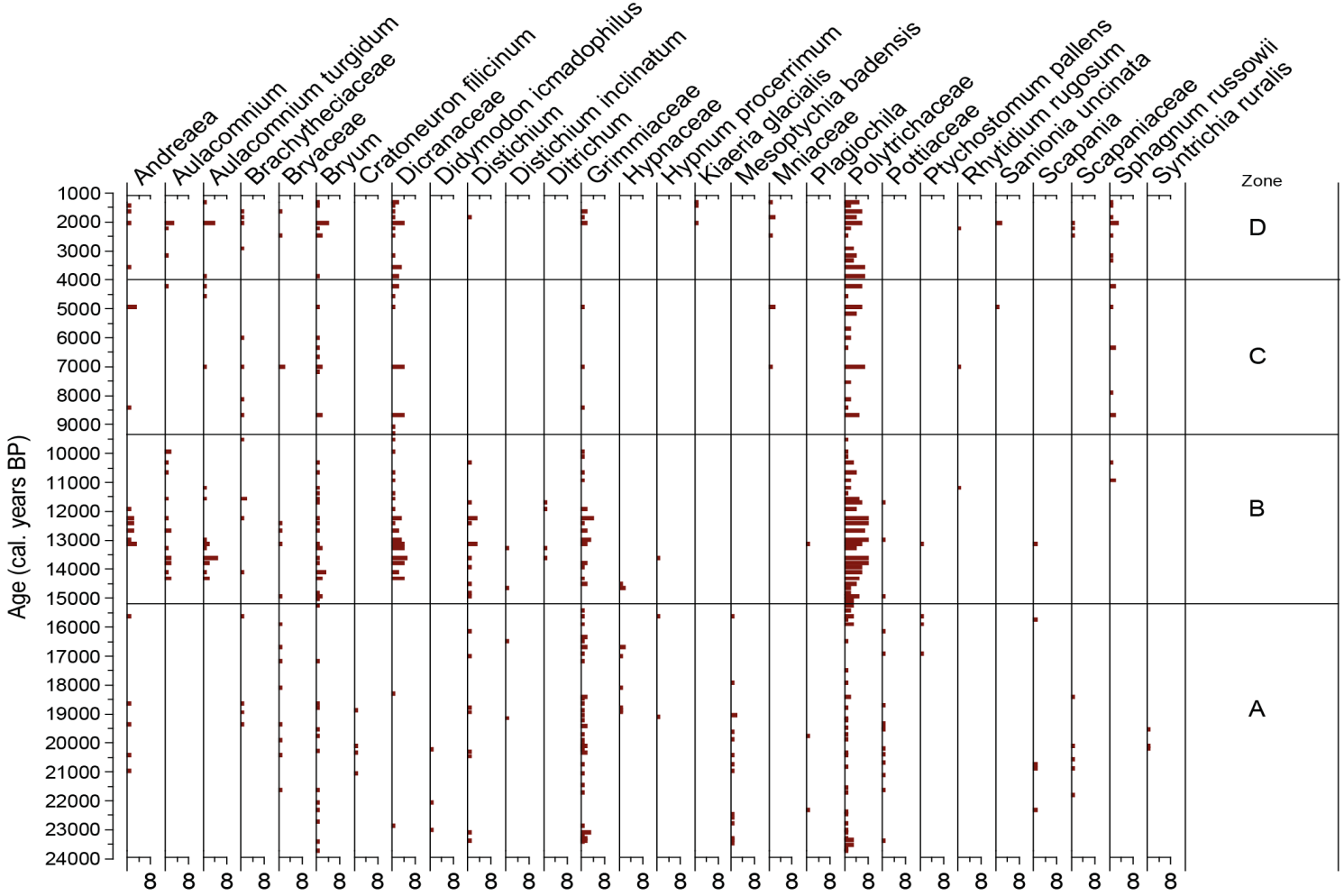

Figure B.5 Bryophyte and clubmoss taxa detected by *sed*aDNA at Lake Bolshoye Shchuchye. The x-axis refers to number of PCR replicates (out of eight) a taxon was detected within per sample.

Table B.1 Total *sed*aDNA read numbers remaining after each filtering step as described in the Material and Methods

Filtering step	Total DNA reads
Pre-identification (raw)	103,047,941
Remove sequences not assigned to samples and with < 98 % match to reference sequence in arcborbryo database	39,248,878
Remove sequences with fewer average reads in samples vs. negative controls	22,242,042
Minimum 10 reads in PCR replicate criterion	22,240,148
Remove sequences belonging to domestic food plants and/or assumed false positives (the identity of which could not be distinguished from NCBI BLAST search)	18,836,201

Table B.2 All taxa detected by *sed*aDNA at Lake Bolshoye Shchuchye with summary statistics of *sed*aDNA data and details of the assigned distribution classification. Taxa marked with an asterisk (*) are inferred to species level based on the biogeographic distribition and comments from botanical surveys in the region. Note that *Arabis alpina* is the only taxa classified as arctic-alpine which does not show occurrences in the full-glacial or LateGlacial interval. This species has a peculiar post-glacial colonization pattern in NW Europe, as the entire region seems to be recently colonized by one small population (Erhich *et al*., 2007), a genetic/dispersal pattern contrasting with most other species studied (Alsos *et al*., 2015).

Taxon name	Family	Functional group	Similarity to reference sequence (%)	Occurrence in present-day vegetation	Occurrence in present day vegetation of Polar Urals according to Pan-Arctic Flora (PAF)	Distribution classification	Max. repeats in sediment samples	Sum repeats in sediment samples	Sum reads in sediment samples	Sum reads in negative controls	Max. repeats in negative controls	Sum repeats in negative controls	Ratio reads negative/ sample
				Occurrence (0 = absent, 1= rare, 2= scattered, 3= common)									
Amaranthus	Amaranthaceae	Forb	98.33	1	Y	NA	1	8	1301	0	0	0	0
Chenopodium capitatum	Amaranthaceae	Forb	100.00	0	N	Boreal	1	5	3039	404	1	1	0.52153
Cratoneuron filicinum	Amblystegiaceae	Bryophyte	100.00	NA	NA	NA	1	4	659	0	0	0	0
Sanionia uncinata	Amblystegiaceae	Bryophyte	100.00	NA	NA	NA	2	3	72	0	0	0	0
Andreaea	Andreaeaceae	Bryophyte	100.00	NA	NA	NA	3	24	8504	0	0	0	0
Anthriscus sylvestris	Apiaceae	Forb	100.00	2	Y	Boreal	7	36	10492	0	0	0	0
Pachypleurum alpinum	Apiaceae	Forb	100.00	3	Y	Arctic-alpine	4	43	23326	0	0	0	0
Convallaria majalis	Asparagaceae	Forb	100.00	0	N	Boreal	3	14	11063	0	0	0	0
Anthemideae	Asteraceae	Forb	100.00	3	Y	Intermediate	8	538	397045	2536	1	4	0.02506
Arnica	Asteraceae	Forb	100.00	1	Y	Intermediate	1	5	124	0	0	0	0

Table B.2 cont.

Taxon name	Family	Functional group	Similarity to reference sequence (%)	Occurrence in present-day vegetation	Occurrence in present day vegetation of Polar Urals according to Pan-Arctic Flora (PAF)	Distribution classification	Max. repeats in sediment samples	Sum repeats in sediment samples	Sum reads in sediment samples	Sum reads in negative controls	Max. repeats in negative controls	Sum repeats in negative controls	Ratio reads negative/ sample
Asteraceae	Asteraceae	Forb	98.00		Y	NA	1	8	194	0	0	0	0
Asteroideae	Asteraceae	Forb	100.00	2	Y	NA	8	68	32208	624	1	2	0.07601
Carduinae	Asteraceae	Forb	100.00	3	Y	Boreal	8	170	207382	6819	1	2	0.129
Saussurea	Asteraceae	Forb	100.00	2	Y	Intermediate	7	41	24396	0	0	0	0
Aulacomnium	Aulacomniaceae	Bryophyte	100.00	NA	NA	NA	3	22	4347	36	1	1	0.03249
Aulacomnium turgidum	Aulacomniaceae	Bryophyte	100.00	NA	NA	NA	5	25	11640	11	1	1	0.00371
Alnus	Betulaceae	TreesShrubs	98.36	3	Y	Boreal	4	22	3805	0	0	0	0
Alnus alnobetula (viridis)	Betulaceae	TreesShrubs	100.00	0	Y	Boreal	8	248	508143	42	1	1	0.00032
Betula	Betulaceae	TreesShrubs	100.00	3	Y	Boreal	8	353	388895	3883	1	2	0.03917
Eritrichium	Boraginaceae	Forb	100.00	2	Y	Arctic-alpine	7	145	125906	0	0	0	0
Myosotis	Boraginaceae	Forb	100.00	2	Y	NA	8	66	18821	0	0	0	0
Myosotis alpestris	Boraginaceae	Forb	100.00	0	Y	Arctic-alpine	8	278	345234	0	0	0	0
Myosotis arvensis	Boraginaceae	Forb	100.00	0	N	Boreal	8	32	7203	0	0	0	0
Brachytheciaceae	Brachytheciaceae	Bryophyte	100.00	NA	NA	NA	2	17	5223	38	1	2	0.02854

Table B.2 cont.

Taxon name	Family	Functional group	Similarity to reference sequence (%)	Occurrence in present-day vegetation	Occurrence in present day vegetation of Polar Urals according to Pan-Arctic Flora (PAF)	Distribution classification	Max. repeats in sediment samples	Sum repeats in sediment samples	Sum reads in sediment samples	Sum reads in negative controls	Max. repeats in negative controls	Sum repeats in negative controls	Ratio reads negative/ sample
Arabidopsis	Brassicaceae	Forb	100.00	1	Y	Arctic-alpine	3	29	19546	0	0	0	0
Arabis alpina	Brassicaceae	Forb	100.00	1	Y	Arctic-alpine	1	3	433	0	0	0	0
Braya	Brassicaceae	Forb	100.00	1	Y	Arctic-alpine	1	8	13264	0	0	0	0
Cakile (Brassicaceae in NC	Brassicaceae	Forb	100.00	0	Y	NA	2	9	15881	34	1	1	0.0084
Cardamine	Brassicaceae	Forb	100.00	2	Y	Arctic-alpine	2	16	7126	0	0	0	0
Cochlearia	Brassicaceae	Forb	100.00	1	Y	Arctic-alpine	1	9	8127	0	0	0	0
Draba	Brassicaceae	Forb	100.00	2	Y	Arctic-alpine	7	302	659078	522	1	1	0.00311
Draba pauciflora	Brassicaceae	Forb	100.00		Y	Arctic-alpine	1	3	803	0	0	0	0
Parrya	Brassicaceae	Forb	100.00	1	Y	Arctic-alpine	6	75	75227	0	0	0	0
Bryaceae	Bryaceae	Bryophyte	100.00	NA	NA	NA	2	16	1956	0	0	0	0
Bryum	Bryaceae	Bryophyte	100.00	NA	NA	NA	4	55	15647	0	0	0	0
Ptychostomum pallens	Bryaceae	Bryophyte	100.00	NA	NA	NA	1	4	96	0	0	0	0
Linnaea borealis	Caprifoliaceae	Forb	100.00	2	Y	Boreal	7	11	1684	0	0	0	0
Lonicera	Caprifoliaceae	Forb	100.00		Y	Boreal	7	11	1009	0	0	0	0

Table B.2 cont.

Taxon name	Family	Functional group	Similarity to reference sequence (%)	Occurrence in present-day vegetation	Occurrence in present day vegetation of Polar Urals according to Pan-Arctic Flora (PAF)	Distribution classification	Max. repeats in sediment samples	Sum repeats in sediment samples	Sum reads in sediment samples	Sum reads in negative controls	Max. repeats in negative controls	Sum repeats in negative controls	Ratio reads negative/ sample
Valeriana	Caprifoliaceae	Forb	100.00	3	Y	Boreal	8	91	41059	0	0	0	0
Arenaria serpyllifolia	Caryophyllaceae	Forb	100.00	0	N	Intermediate	1	4	19398	973	1	1	0.19678
Cerastium	Caryophyllaceae	Forb	100.00	2	Y	NA	5	59	45832	0	0	0	0
Minuartia	Caryophyllaceae	Forb	98.36	2	Y	Arctic-alpine	2	24	18193	0	0	0	0
Silene	Caryophyllaceae	Forb	100.00	2	Y	NA	2	6	2125	0	0	0	0
Stellaria	Caryophyllaceae	Forb	100.00	1	Y	NA	2	33	26204	558	1	1	0.08354
Stellaria holostea	Caryophyllaceae	Forb	100.00	0	N	Boreal	1	4	2655	0	0	0	0
Crassula	Crassulaceae	Forb	100.00	0	N	Boreal	1	9	5232	0	0	0	0
Rhodiola rosea	Crassulaceae	Forb	100.00	3	Y	Intermediate	5	49	52316	0	0	0	0
Carex	Cyperaceae	Graminoid	100.00		Y	NA	8	280	23960	0	0	0	0
Carex aquatilis	Cyperaceae	Graminoid	100.00	1	Y	Intermediate	2	11	272	0	0	0	0
Carex lachenalii	Cyperaceae	Graminoid	100.00	2	Y	Arctic-alpine	1	7	1735	0	0	0	0
Eriophorum	Cyperaceae	Graminoid	100.00	3	Y	Intermediate	3	15	466	0	0	0	0
Kobresia	Cyperaceae	Graminoid	100.00	2	Y	Intermediate	1	15	848	0	0	0	0

Table B.2 cont.

Taxon name	Family	Functional group	Similarity to reference sequence (%)	Occurrence in present-day vegetation	Occurrence in present day vegetation of Polar Urals according to Pan-Arctic Flora (PAF)	Distribution classification	Max. repeats in sediment samples	Sum repeats in sediment samples	Sum reads in sediment samples	Sum reads in negative controls	Max. repeats in negative controls	Sum repeats in negative controls	Ratio reads negative/ sample
Dicranaceae	Dicranaceae	Bryophyte	100.00	NA	NA	NA	5	72	30353	238	1	2	0.03076
Distichium	Ditrichaceae	Bryophyte	100.00	NA	NA	NA	3	23	3547	0	0	0	0
Distichium inclinatum	Ditrichaceae	Bryophyte	100.00	NA	NA	NA	1	4	91	0	0	0	0
Ditrichum	Ditrichaceae	Bryophyte	100.00	NA	NA	NA	1	4	180	0	0	0	0
Dryopteris	Dryopteridaceae	Fern	100.00	2	Y	Boreal	6	13	12281	0	0	0	0
Dryopteris fragrans	Dryopteridaceae	Fern	100.00		Y	Boreal	3	14	2256	0	0	0	0
Shepherdia canadensis /sy	Elaeagnaceae	TreesShrubs	98.08	0	N	Boreal	1	5	8249	1100	1	1	0.52314
Ephedra	Ephedraceae	TreesShrubs	100.00	0	N	Intermediate	2	3	7898	0	0	0	0
Andromeda polifolia	Ericaceae	TreesShrubs	100.00	3	Y	Boreal	8	18	4676	0	0	0	0
Arctostaphylos uva-ursi	Ericaceae	TreesShrubs	98.00	1	Y	Boreal	2	9	1587	0	0	0	0
Empetrum nigrum*	Ericaceae	TreesShrubs	100.00	3	Y	Intermediate	8	100	36851	0	0	0	0
Pyrola 1	Ericaceae	Forb	100.00	2	Y	NA	2	13	935	0	0	0	0
Pyrola 2 alpina (lump into P	Ericaceae	Forb	100.00	0	Y	NA	1	3	173	0	0	0	0
Pyrola grandiflora	Ericaceae	Forb	100.00	2	Y	Arctic-alpine	1	5	427	0	0	0	0

Table B.2 cont.

Taxon name	Family	Functional group	Similarity to reference sequence (%)	Occurrence in present-day vegetation	Occurrence in present day vegetation of Polar Urals according to Pan-Arctic Flora (PAF)	Distribution classification	Max. repeats in sediment samples	Sum repeats in sediment samples	Sum reads in sediment samples	Sum reads in negative controls	Max. repeats in negative controls	Sum repeats in negative controls	Ratio reads negative/ sample
Pyrola minor	Ericaceae	Forb	100.00	2	Y	Boreal	1	3	177	0	0	0	0
Pyrola rotundifolia	Ericaceae	Forb	100.00	0	N	Boreal	4	13	1077	0	0	0	0
Rhododendron (Ledum palu	Ericaceae	Forb	100.00	0	Y	Intermediate	8	206	269315	0	0	0	0
Vaccinium uliginosum	Ericaceae	TreesShrubs	100.00	3	Y	Intermediate	8	210	233939	172	1	1	0.00288
Vaccinium vitis-idaea	Ericaceae	TreesShrubs	100.00	3	Y	Boreal	8	184	36321	0	0	0	0
Astragalus	Fabaceae	Forb	100.00	3	Y	Arctic-alpine	8	148	76564	0	0	0	0
Galegeae (Astragalus umbe	Fabaceae	Forb	98.33		Y	Intermediate	1	3	1602	0	0	0	0
Hedysarum hedysaroides	Fabaceae	Forb	100.00	2	Y	Intermediate	6	76	11369	0	0	0	0
Medicago	Fabaceae	Forb	100.00	0	N	Boreal	1	3	1778	0	0	0	0
Oxytropis	Fabaceae	Forb	100.00	3	Y	Arctic-alpine	4	58	31442	0	0	0	0
Trifolium	Fabaceae	Forb	100.00	0	Y	Boreal	2	3	7264	0	0	0	0
Grimmiaceae	Grimmiaceae	Bryophyte	100.00	NA	NA	NA	4	74	31752	0	0	0	0
Ribes	Grossulariaceae	TreesShrubs	100.00		Y	Boreal	8	31	5385	0	0	0	0
Hypnaceae	Hypnaceae	Bryophyte	100.00	NA	NA	NA	2	10	1067	0	0	0	0

Table B.2 cont.

Taxon name	Family	Functional group	Similarity to reference sequence (%)	Occurrence in present-day vegetation	Occurrence in present day vegetation of Polar Urals according to Pan-Arctic Flora (PAF)	Distribution classification	Max. repeats in sediment samples	Sum repeats in sediment samples	Sum reads in sediment samples	Sum reads in negative controls	Max. repeats in negative controls	Sum repeats in negative controls	Ratio reads negative/ sample
Hypnum procerrimum	Hypnaceae	Bryophyte	100.00	NA	NA	NA	1	3	69	0	0	0	0
Juncus biglumis	Juncaceae	Graminoid	100.00	2	Y	Arctic-alpine	3	52	26684	0	0	0	0
Luzula parviflora	Juncaceae	Graminoid	100.00	3	Y	Intermediate	1	4	1574	0	0	0	0
Mesoptychia badensis	Jungermanniaceae	Bryophyte	100.00	NA	NA	NA	2	15	554	0	0	0	0
Lamiaceae	Lamiaceae	Forb	100.00	2	Y	Boreal	1	12	1937	0	0	0	0
Linum	Linaceae	Forb	100.00	1	Y	Boreal	1	6	515	0	0	0	0
Lycopodiaceae	Lycopodiaceae	Clubmoss	100.00		Y	NA	6	147	137144	55021	2	20	1.5739
Lythrum salicaria	Lythraceae	Forb	100.00	0	N	Boreal	1	4	540	0	0	0	0
Veratrum	Melanthiaceae	Forb	100.00	2	Y	Boreal	5	41	7367	0	0	0	0
Mniaceae	Mniaceae	Bryophyte	100.00	NA	NA	NA	2	7	199	0	0	0	0
Chamerion angustifolium	Onagraceae	Forb	100.00	1	Y	Boreal	7	22	4794	0	0	0	0
Castilleja	Orobanchaceae	Forb	100.00	2	Y	Intermediate	6	18	3597	0	0	0	0
Euphrasia	Orobanchaceae	Forb	100.00	2	Y	Intermediate	1	3	368	0	0	0	0
Pedicularis	Orobanchaceae	Forb	100.00	1 OR 2	Y	NA	7	146	42743	0	0	0	0

Table B.2 cont.

Taxon name	Family	Functional group	Similarity to reference sequence (%)	Occurrence in present-day vegetation	Occurrence in present day vegetation of Polar Urals according to Pan-Arctic Flora (PAF)	Distribution classification	Max. repeats in sediment samples	Sum repeats in sediment samples	Sum reads in sediment samples	Sum reads in negative controls	Max. repeats in negative controls	Sum repeats in negative controls	Ratio reads negative/ sample
Pedicularis oederi	Orobanchaceae	Forb	100.00	2	Y	Arctic-alpine	8	219	79457	0	0	0	0
Pedicularis pacifica (syn: P	Orobanchaceae	Forb	100.00	2	Y	Arctic-alpine	7	82	30116	50	1	1	0.00651
Papaver	Papaveraceae	Forb	100.00	1	Y	Arctic-alpine	8	919	4017331	0	0	0	0
Abies	Pinaceae	TreesShrubs	100.00	0	N	Boreal	2	5	83458	0	0	0	0
Larix sibirica	Pinaceae	TreesShrubs	100.00	2	Y	Boreal	8	99	110463	0	0	0	0
Picea	Pinaceae	TreesShrubs	100.00		Y	Boreal	8	84	83039	34525	2	9	1.63109
Plagiochila	Plagiochilaceae	Bryophyte	100.00	NA	NA	NA	1	3	75	0	0	0	0
Lagotis glauca	Plantaginaceae	Forb	100.00	0	Y	Arctic-alpine	8	224	268394	0	0	0	0
Plantago	Plantaginaceae	Forb	100.00	0	Y	Boreal	1	4	13913	0	0	0	0
Veronica	Plantaginaceae	Forb	100.00	2	Y	Boreal	7	44	4764	0	0	0	0
Agrostidinae	Poaceae	Graminoid	100.00	3	Y	NA	8	39	25474	0	0	0	0
Alopecurinae	Poaceae	Graminoid	100.00	2	Y	NA	3	21	11397	19	1	1	0.00654
BOP clade	Poaceae	Graminoid	100.00		Y	NA	1	5	10639	0	0	0	0
Bromus ciliatus	Poaceae	Graminoid	100.00	3	Y	Intermediate	1	3	225	0	0	0	0

Table B.2 cont.

Taxon name	Family	Functional group	Similarity to reference sequence (%)	Occurrence in present-day vegetation	Occurrence in present day vegetation of Polar Urals according to Pan-Arctic Flora (PAF)	Distribution classification	Max. repeats in sediment samples	Sum repeats in sediment samples	Sum reads in sediment samples	Sum reads in negative controls	Max. repeats in negative controls	Sum repeats in negative controls	Ratio reads negative/ sample
Bromus pumpellianus	Poaceae	Graminoid	100.00	3	Y	Intermediate	1	3	469	0	0	0	0
Festuca	Poaceae	Graminoid	100.00	3	Y	NA	4	102	125194	12568	1	3	0.39383
Hordeum	Poaceae	Graminoid	100.00	0	N	NA	1	5	2271	0	0	0	0
PACMAD clade	Poaceae	Graminoid	98.08		Y	NA	1	3	6866	765	2	2	0.4371
Poaceae	Poaceae	Graminoid	98.08	3	Y	NA	1	5	1129	0	0	0	0
Poeae	Poaceae	Graminoid	98.08		Y	NA	4	121	194258	0	0	0	0
Puccinellia	Poaceae	Graminoid	100.00	2	Y	NA	4	125	283701	0	0	0	0
Polemonium	Polemoniaceae	Forb	100.00	2	Y	Arctic-alpine	2	5	182	0	0	0	0
Bistorta	Polygonaceae	Forb	100.00	3	Y	Boreal	8	196	192252	0	0	0	0
Bistorta vivipara	Polygonaceae	Forb	100.00	3	Y	Intermediate	8	344	441419	5924	2	6	0.05265
Oxyria digyna	Polygonaceae	Forb	100.00	2	Y	Arctic-alpine	8	208	201671	1282	2	4	0.02494
Rumex	Polygonaceae	Forb	100.00	3	Y	NA	4	73	54070	31154	2	7	2.26039
Polytrichaceae	Polytrichaceae	Bryophyte	100.00	NA	NA	NA	8	254	48425	2018	2	3	0.16349
Didymodon icmadophilus	Pottiaceae	Bryophyte	100.00	NA	NA	NA	1	3	111	0	0	0	0

Table B.2 cont.

Taxon name	Family	Functional group	Similarity to reference sequence (%)	Occurrence in present-day vegetation	Occurrence in present day vegetation of Polar Urals according to Pan-Arctic Flora (PAF)	Distribution classification	Max. repeats in sediment samples	Sum repeats in sediment samples	Sum reads in sediment samples	Sum reads in negative controls	Max. repeats in negative controls	Sum repeats in negative controls	Ratio reads negative/ sample
Pottiaceae	Pottiaceae	Bryophyte	100.00	NA	NA	NA	1	15	1741	0	0	0	0
Syntrichia ruralis	Pottiaceae	Bryophyte	100.00	NA	NA	NA	1	3	222	0	0	0	0
Androsace chamaejasme	Primulaceae	Forb	100.00		Y	Arctic-alpine	2	9	1982	0	0	0	0
Douglasia	Primulaceae	Forb	100.00	1	Y	Arctic-alpine	1	4	1840	0	0	0	0
Trientalis	Primulaceae	Forb	100.00	2	Y	Boreal	1	3	324	0	0	0	0
Aconitum lycoctonum	Ranunculaceae	Forb	100.00	0	Y	Boreal	8	45	14185	0	0	0	0
Caltha	Ranunculaceae	Forb	100.00	2	Y	Intermediate	3	46	18710	0	0	0	0
Oxygraphis glacialis	Ranunculaceae	Forb	100.00	1	Y	Arctic-alpine	2	9	4649	0	0	0	0
Ranunculus	Ranunculaceae	Forb	100.00		Y	NA	8	268	122479	7172	2	5	0.22972
Ranunculus arcticus	Ranunculaceae	Forb	100.00	0	Y	Arctic-alpine	2	16	8582	0	0	0	0
Ranunculus pygmaeus	Ranunculaceae	Forb	100.00	1	Y	Arctic-alpine	2	4	194	0	0	0	0
Ranunculus sulphureus	Ranunculaceae	Forb	100.00	1	Y	Arctic-alpine	5	50	22121	0	0	0	0
Thalictroideae	Ranunculaceae	Forb	98.25		Y	NA	2	3	1749	0	0	0	0
Thalictrum	Ranunculaceae	Forb	100.00	1	Y	NA	8	147	95749	0	0	0	0

Table B.2 cont.

Taxon name	Family	Functional group	Similarity to reference sequence (%)	Occurrence in present-day vegetation	Occurrence in present day vegetation of Polar Urals according to Pan-Arctic Flora (PAF)	Distribution classification	Max. repeats in sediment samples	Sum repeats in sediment samples	Sum reads in sediment samples	Sum reads in negative controls	Max. repeats in negative controls	Sum repeats in negative controls	Ratio reads negative/ sample
Trollius	Ranunculaceae	Forb	100.00	2	Y	Boreal	4	14	5909	0	0	0	0
Kiaeria glacialis	Rhabdoweisiaceae	Bryophyte	100.00	NA	NA	NA	1	3	47	0	0	0	0
Rhytidium rugosum	Rhytidiaceae	Bryophyte	100.00	NA	NA	NA	1	3	48	0	0	0	0
Alchemilla	Rosaceae	Forb	100.00	2	Y	Boreal	6	39	14834	0	0	0	0
Dryas octopetala*	Rosaceae	TreesShrubs	100.00	3	Y	Arctic-alpine	8	460	1197046	1733	2	3	0.00568
Filipendula ulmaria	Rosaceae	Forb	98.25	1	Y	Boreal	4	18	4231	4322	1	1	4.00745
Geum	Rosaceae	Forb	100.00	2	Y	NA	8	43	31881	378	1	2	0.04651
Potentilla	Rosaceae	Forb	100.00	2	Y	NA	4	56	21689	0	0	0	0
Spiraea	Rosaceae	Forb	100.00	1	Y	Boreal	7	16	4908	0	0	0	0
Galium	Rubiaceae	Forb	100.00	2	Y	Boreal	5	19	12913	0	0	0	0
Galium boreale	Rubiaceae	Forb	100.00	1	Y	Boreal	6	22	5854	0	0	0	0
Saliceae	Salicaceae	TreesShrubs	100.00	3	Y	NA	8	1034	5754679	107100	3	15	0.07301
Saxifraga	Saxifragaceae	Forb	100.00	2	Y	NA	7	272	236773	0	0	0	0
Saxifraga cespitosa	Saxifragaceae	Forb	100.00	1	Y	Arctic-alpine	4	63	14656	0	0	0	0

Table B.2 cont.

Taxon name	Family	Functional group	Similarity to reference sequence (%)	Occurrence in present-day vegetation	Occurrence in present day vegetation of Polar Urals according to Pan-Arctic Flora (PAF)	Distribution classification	Max. repeats in sediment samples	Sum repeats in sediment samples	Sum reads in sediment samples	Sum reads in negative controls	Max. repeats in negative controls	Sum repeats in negative controls	Ratio reads negative/ sample
Saxifraga hirculus	Saxifragaceae	Forb	100.00	2	Y	Intermediate	7	160	141529	0	0	0	0
Saxifraga oppositifolia	Saxifragaceae	Forb	100.00	2	Y	Arctic-alpine	8	397	481711	0	0	0	0
Saxifragaceae	Saxifragaceae	Forb	100.00		Y	NA	3	81	35802	165	1	1	0.01808
Scapania	Scapaniaceae	Bryophyte	100.00	NA	NA	NA	1	5	116	0	0	0	0
Scapaniaceae	Scapaniaceae	Bryophyte	100.00	NA	NA	NA	1	8	799	0	0	0	0
Sphagnum russowii	Sphagnaceae	Bryophyte	100.00	NA	NA	NA	3	20	11301	0	0	0	0
Thelypteris palustris	Thelypteridaceae	Fern	100.00		N	Boreal	2	6	2436	0	0	0	0
Ulmus glabra	Ulmaceae	TreesShrubs	100.00	0	N	Boreal	2	4	15821	0	0	0	0
Urtica	Urticaceae	Forb	100.00	1	Y	Boreal	2	6	5254	0	0	0	0
Viola	Violaceae	Forb	98.00	2	Y	Boreal	2	6	324	0	0	0	0
Viola epipsila	Violaceae	Forb	98.18	2	Y	Boreal	1	4	380	0	0	0	0
Gymnocarpium dryopteris	Woodsiaceae	Fern	100.00	1	Y	Boreal	2	11	4135	0	0	0	0

Table B.2 cont.

Taxon name	Family	Comment
Amaranthus	Amaranthaceae	Species of this family are encountered only as imported weeds in villages, but they are common in temperate areas of Russia.
Chenopodium capitatum	Amaranthaceae	No arctic-alpine representative in genus
Cratoneuron filicinum	Amblystegiaceae	Grows in forests, mountain forests with pine and birch, and humid forests within PU
Sanionia uncinata	Amblystegiaceae	
Andreaea	Andreaeaceae	
Anthriscus sylvestris	Apiaceae	
Pachypleurum alpinum	Apiaceae	Synonym Ligusticum alpinum. Arctic-alpine taxa but not clearly distinguished from boreal representative based on the trnL marker so cannot be classified (see PAF)
Convallaria majalis	Asparagaceae	Occurs in middle Urals only today
Anthemideae	Asteraceae	No representative in the high-arctic zone. Present in PU, genus Artemisia, Tanacetum, Tripleurospermum, Achillea and grow close to Lake BS, the first in the tundra zone, the second in alder thickets used for reindeer grazing and the third in alder and willow thickets along the shore of the lake.
Arnica	Asteraceae	Sequence sharing within genus. Only the low-arctic Arnica angustifolia subsp. iljinii in region today; other species within genus are sub-arctic-boreal.
Asteraceae	Asteraceae	
Asteroideae	Asteraceae	These are those which are reported from the area of Lake BS - many more are mentioned from the area > 20 km (Antennaria lanata, Tephroseris , Omalotheca, Petasites) or from PU
Carduinae	Asteraceae	In PU genus Cirsium, Saussurea. Cirsium has 2 species, one of which is widespread and found close to BS, in alder thickets
Saussurea	Asteraceae	Saussurea alpina and S. tilisia are most likely taxon.
Aulacomnium	Aulacomniaceae	Occurs in different tundra types, in thickets and open larch forests within PU
Aulacomnium turgidum	Aulacomniaceae	
Alnus	Betulaceae	*syn. Duschekia (=Alnus) fruticosa. It forms dense thickets on the slopes around lake BS today*.
Alnus alnobetula (viridis)	Betulaceae	
Betula	Betulaceae	B. tundrarum occus on River Baidarata in scattered Larix "forest"
Eritrichium	Boraginaceae	Grows within mountain tundra and snow bed sites within PU.
Myosotis	Boraginaceae	Myosotis cespitosa and M. palustris have been reported from a radius of 30 km from Lake BS
Myosotis alpestris	Boraginaceae	
Myosotis arvensis	Boraginaceae	Absent from PU today
Brachytheciaceae	Brachytheciaceae	19 species in PU, 14 in the genus Brachythecium. B cirrosum occurs at altitudes > 500m in grass-moss tundra
Arabidopsis	Brassicaceae	Most likely *Arabidopsis petrea* which is frequent in PU and has identical sequence
Arabis alpina	Brassicaceae	
Braya	Brassicaceae	Only Braya glabella complex in region today. Only one occurrence documented on a calcareous site 80 km south of Lake BS
Cakile (Brassicaceae in NCBI)	Brassicaceae	
Cardamine	Brassicaceae	Two taxa common in region today: C. polemonioides, and C. bellidifolia. BLAST check reveals 100 % similarity to C. Bellidifolia. Three boreal taxa are very rare in the region today are lacking in reference library (C. amara, C. dentata and C. macrophylla). C. amara, C. dentata and C. macrophylla were recorded in the area of Lake BS up to 20 km
Cochlearia	Brassicaceae	
Draba	Brassicaceae	Many species within this genus which are very common in arctic-alpine areas although there are also some boreal representatives. 10 species in the flora of the PU.
Draba pauciflora	Brassicaceae	syn. D. adamsii, D. micropetala, D. oblongata. D.oblongata scateered in the Polar urals region.
Parrya	Brassicaceae	Only Parrya nudicaulis complex in region
Bryaceae	Bryaceae	
Bryum	Bryaceae	
Ptychostomum pallens	Bryaceae	syn. Bryum pallens. Quite common in PU but occurs further south from Lake BS
Linnaea borealis	Caprifoliaceae	
Lonicera	Caprifoliaceae	2 species of genus Lonicera in PU, but not recorded in the area of Lake BS
Valeriana	Caprifoliaceae	2 species in the PU
Arenaria serpyllifolia	Caryophyllaceae	
Cerastium	Caryophyllaceae	Both arctic and boreal taxa representatives within this genus. 8 species in PU, with two occuring in the area of Lake BS
Minuartia	Caryophyllaceae	All six Minuartia species in region are predominantly arctic-alpine taxa. 6 species in PU, of which 5 were recorded in a radius of 30 km around BS
Silene	Caryophyllaceae	Both arctic and boreal represenatives within this genus. 5 species in PU.
Stellaria	Caryophyllaceae	Both arctic and boreal representatives within this genus. 15 species in PU. 5 species in the area of Lake BS
Stellaria holostea	Caryophyllaceae	
Crassula	Crassulaceae	
Rhodiola rosea	Crassulaceae	Grows in humid sites, on rocks, along creeks in the area of Lake BS.
Carex	Cyperaceae	Both arctic and boreal representatives within this genus. The genus comprises 51 species in PU, and is the most species rich genus in the area.
Carex aquatilis	Cyperaceae	

Table B.2 cont.

Taxon name	Family	Comment
Carex lachenalii	Cyperaceae	
Eriophorum	Cyperaceae	
Kobresia	Cyperaceae	3 species of this genus grow in PU, but considerably further south than Lake BS. The northernmost occurrence was recorded for simplisciuscula 68 km to the south
Dicranaceae	Dicranaceae	Largest family of mosses in PU with 41 species, with several species encountered constantly in the upper reaches of the river Baidarata
Distichium	Ditrichaceae	
Distichium inclinatum	Ditrichaceae	
Ditrichum	Ditrichaceae	
Dryopteris	Dryopteridaceae	2 species of this genus occur in PU, Dryopteris expansa goes north only to Malaya Khadata, 50-60 km south of Lake BS
Dryopteris fragrans	Dryopteridaceae	
Shepherdia canadensis /syn. Hippophae canadensis	Elaeagnaceae	BLAST check revealed a 100% match to Hippophae which is not in arctic or boreal database but is present in NCBI database.
Ephedra	Ephedraceae	Many records find Ephedra in LGM sediments, and thought to be indicative of Pleistocene steppe tundra. There are still remants of steppe tundra on south facing slopes. It is not found at Lake BS and in this part of the PU today
Andromeda polifolia	Ericaceae	
Arctostaphylos uva-ursi	Ericaceae	
Empetrum nigrum*	Ericaceae	E. hermaphroditum is found on the shore of lake, BS, whereas E. nigrum is found at an altitude of around 400 m above lake.
Pyrola 1	Ericaceae	
Pyrola 2 alpina (lump into Pyrola)	Ericaceae	There are 20 taxa of Pyrola in Hulten; the majority boreal but P. minor goes into the arctic so this taxa canot be classified.
Pyrola grandiflora	Ericaceae	Poorly distinguished based on trnL marker. Related to the boreal taxon Pyrola rotundifolia
Pyrola minor	Ericaceae	
Pyrola rotundifolia	Ericaceae	
Rhododendron (Ledum palustre)	Ericaceae	Rhododendron tomentosum (syn. Ledum palustre)
Vaccinium uliginosum	Ericaceae	
Vaccinium vitis-idaea	Ericaceae	
Astragalus	Fabaceae	Astragalus frgidus, A. subpolaris or A. alpinus complex are all arctic-alpines. 7 species occur in PU, 4 in the area of Lake BS. A. subpolaris is the most common in the area.
Galegeae (Astragalus umbellatus, A. Alpinus, Oxytropis deflexa)	Fabaceae	Most likely Astragalus of Oxytropis; none of them are common in the high Arctic. No other genera of this tribe occur in PU
Hedysarum hedysaroides	Fabaceae	syn. Hedysarum arcticum. Grows in mountain tundra and high altitude plateaus
Medicago	Fabaceae	Only one species (M. lupulina) reported from Vorkuta settlement, otherwise absent from PU
Oxytropis	Fabaceae	Both Oxytropis campestris subsp. sordida and O. mertensiana are found in the PU today and occupy an arctic-alpine distribution. O. mertensiana is found in the region of Lake BS but is very rare.
Trifolium	Fabaceae	
Grimmiaceae	Grimmiaceae	20 speices in PU. Grimmia ovalis is the most wide-spread species in the region.
Ribes	Grossulariaceae	
Hypnaceae	Hypnaceae	22 species in PU. Two are widespread and have a large ecological amplitude. H. recurvatum o
Hypnum procerrimum	Hypnaceae	not recorded in PU
Juncus biglumis	Juncaceae	Grows on wet rocks, alpipne tundra and alpine desert in PU
Luzula parviflora	Juncaceae	Occurs in many areas of PU and recorded in adjacent valley near on Maloe Shchuchye lake
Mesoptychia badensis	Jungermanniaceae	
Lamiaceae	Lamiaceae	No Lamiaceae are found in bioclimatic zone A-C, only a few scattered in D and E. 15 species of 9 genera in PU today
Linum	Linaceae	
Lycopodiaceae	Lycopodiaceae	BLAST check shows a 100% match to Diphasiastrum alpinum (intermediate), Lucopodium clavatum (boreal), L. Annotinum (boreal). Excluding Huperziaceae and Selaginellaceae, this family includes two genera in PU: Diphasiastrum, Lycopodium; and 6 speices. D alpinum occurs close to lake BS and Lycopodium annotinum in the upper reaches of Malaya Usa and in the upper reaches of the Baidarata river.
Lythrum salicaria	Lythraceae	Absent from PU today
Veratrum	Melanthiaceae	Grows in meadows and thickets of willow and alder in PU.
Mniaceae	Mniaceae	37 species in PU, second most species rich family. 6 species in genus Mnium.
Chamerion angustifolium	Onagraceae	
Castilleja	Orobanchaceae	Matches to C. hyparctica which is rare in larger region today; other intermediate representatives (C lapponica, C artica) are missing in reference library.
Euphrasia	Orobanchaceae	
Pedicularis	Orobanchaceae	Both arctic and boreal representatives within this genus. 11 species in PU, 8 species in Lake BS area
Pedicularis oederi	Orobanchaceae	
Pedicularis pacifica (syn: P. sudetica)	Orobanchaceae	Synonym: P. sudetica ssp. pacifica, an arctic-alpine species complex.
Papaver	Papaveraceae	Papaver dahlianum and P. Lapponicum complex are in the region; no boreal representatives in the region. P. polare is distributed between B. Khadata river and the Baidaratskaya Bay.
Abies	Pinaceae	Not recorded at Lake BS and in this part of the PU
Larix sibirica	Pinaceae	

Table B.2 cont.

Taxon name	Family	Comment
Picea	Pinaceae	Not in catchment today, does not go as far north at present. Occurs in the Valley of Bolshaya Khadata, 40-50 km south of the Lake
Plagiochila	Plagiochilaceae	
Lagotis glauca	Plantaginaceae	Lagotis glauca ssp. minor is found growing in the region withinin snow beds and close to glaciers, along rivers and in mountain tundra.
Plantago	Plantaginaceae	Plantago major is only likely representative in the region.
Veronica	Plantaginaceae	
Agrostidinae	Poaceae	Both arctic and boreal representatives within this genus. Agrostis has sequence sharing within the genus and thus individuals cannot be distinguished based on the trnL marker. 6 species in this genus occur in PU. A. borealis was found at Lake BS, and grows in the moutain tundra belt. Agrostis stolonifera is also typical in PU.
Alopecurinae	Poaceae	Both arctic and boreal represenatives within this genus. 4 species of Alopecurus grow in PU. A. alpinus and A. glaucus were found 12-15 km to north east of Lake BS, close to lake Pedarata
BOP clade	Poaceae	
Bromus ciliatus	Poaceae	
Bromus pumpellianus	Poaceae	
Festuca	Poaceae	Festuca ovina is found at Lake BS, F. rubra was found in the upper reaches of Baidarata, 12-15 km from the lake
Hordeum	Poaceae	Barley so could be contamination, but it is completely absent in the negative controls. Two Hordeum species in boreal zone, see H. jubatum and H. secalinum. BLAST check revealed 100 % match to H. jubatum, and H. secalinum. As matches to many other Hordeum sp., assigned intermediate category
PACMAD clade	Poaceae	
Poaceae	Poaceae	Both arctic and boreal representatives within this genus. 65 indigenous species in this family are growing in PU. This is the largest family in the flora of PU. 12 species of the genus Poa
Poeae	Poaceae	Both arctic and boreal representatives within this genus
Puccinellia	Poaceae	Both arctic and boreal representatives within this genus.
Polemonium	Polemoniaceae	3 species in PU, of which two grow in the area of Lake BS
Bistorta	Polygonaceae	BLAST check revealed 100 % sequence similarity to Bistorta major (Syn P. bistorta) which is common in region today
Bistorta vivipara	Polygonaceae	
Oxyria digyna	Polygonaceae	Grows in meadows, snow beds, close to small rivers
Rumex	Polygonaceae	Both intermediate and boreal representatives but cannot be distinguished based on trnL marker. 12 species in PU, most of them are introduced and occur close to settlements. 3 species occur in the area of Lake BS.
Polytrichaceae	Polytrichaceae	Species of this family are widespread in PUSpecies of this family are widespread in PU
Didymodon icmadophilus	Pottiaceae	
Pottiaceae	Pottiaceae	14 species in PU14 species in PU14 species in PU14 species in PU14 species in PU
Syntrichia ruralis	Pottiaceae	syn. *Tortula ruralis*. In PU it occurs rarely in the tundra, forest tundra and forest zones, but further south than Lake BS
Androsace chamaejasme	Primulaceae	*syn. Androsace lehmanniana*
Douglasia	Primulaceae	syn. Androsace are in general arctic-alpines and A. triflora is frequent in the arctic and rare in bordering boreal zone.
Trientalis	Primulaceae	Grows in thickets close to Lake BS.
Aconitum lycoctonum	Ranunculaceae	
Caltha	Ranunculaceae	Grows in grass rich marshes. Caltha palustris occurs further south in PU
Oxygraphis glacialis	Ranunculaceae	Grows in snow beds, along small creeks and humid sites between rocks.
Ranunculus	Ranunculaceae	Both arctic-alpine and boreal representatives within this genus. 17 speices in PU, 7 species in the area of Lake BS
Ranunculus arcticus	Ranunculaceae	
Ranunculus pygmaeus	Ranunculaceae	Occurs in PU, also at the latitude of Lake BS, but is not reported from L. Morozova's research s
Ranunculus sulphureus	Ranunculaceae	Occurs sporadically across the PU
Thalictroideae	Ranunculaceae	
Thalictrum	Ranunculaceae	Both arctic and boreal representatives within this genus. 3 species in PU, all three grow in the area of Lake BS
Trollius	Ranunculaceae	The only species present in PU, endemic to the Ural mountains. Grows in thickets along rivers
Kiaeria glacialis	Rhabdoweisiaceae	
Rhytidium rugosum	Rhytidiaceae	Common in this region of the PU
Alchemilla	Rosaceae	No arctic-alpine representatives in the genus (but some subarctic). 10 species in the flora of PU, 5 species in the area of BS, at a distance of between 10 and 30 km
Dryas octopetala*	Rosaceae	
Filipendula ulmaria	Rosaceae	Occurs in PU, south of B. Khadyta river. Does not grow as far north as Lake BS today
Geum	Rosaceae	G. rivale (boreal) and G. glaciale (arctic-alpine) rare or scattered in region today. Grows in meadows close to rivers or lakes. Distribution stops around 70-80 km south of Lake BS
Potentilla	Rosaceae	Both arctic and boreal representatives within this genus. 14 species in PU, of which 5 occur in the area of Lake BS
Spiraea	Rosaceae	Occurs in PU, observed in the valleys of the rivers Kara, Khadata, baidarata, but not mentioned whether in the upper reaches of Lake BS.
Galium	Rubiaceae	6 species in PU, 3 species in the area of BS6 species in PU, 3 species in the area of BS
Galium boreale	Rubiaceae	
Saliceae	Salicaceae	Intermediate and boreal representatives within this group which cannot be distinguished based on the trnL marker. The erect forms grow in wet places in the mountain tundra as low thickets (20-30 cm), but can grow up to 60-80 cm in sheltered places. The prostrate forms grow together with moss, lichen and herbs to a height of 2-5(6) cm.
Saxifraga	Saxifragaceae	14 species of this genus in PU, 10 of them occur in the area of Lake BS.

Table B.2 cont.

Taxon name	Family	Comment
Saxifraga cespitosa	Saxifragaceae	
Saxifraga hirculus	Saxifragaceae	
Saxifraga oppositifolia	Saxifragaceae	
Saxifragaceae	Saxifragaceae	In addition to Saxifraga, this family includes the genus Chrysosplenium in the PU.
Scapania	Scapaniaceae	
Scapaniaceae	Scapaniaceae	24 species in PU. B. pseudotricetrum occurs in moutain tundra belt in grass- moss tundra
Sphagnum russowii	Sphagnaceae	
Thelypteris palustris	Thelypteridaceae	
Ulmus glabra	Ulmaceae	not found at BS lake and in this part of the PUnot found at BS lake and in this part of the PU
Urtica	Urticaceae	Reported from the upper reaches of the river Usa
Viola	Violaceae	8 species in PU, 2 of them occur in the area of Lake BS
Viola epipsila	Violaceae	
Gymnocarpium dryopteris	Woodsiaceae	

Appendix C Supporting Information: Paper III

Here we include three supplemental figures containing *sed*aDNA and pollen data from Lake Bolshoye Shchuchye to augment those in the main text. We also include two supplemental tables, one containing summary data and the other containing a description of the key characteristics of the biostratigraphic zones identified in the *sed*aDNA and pollen records.

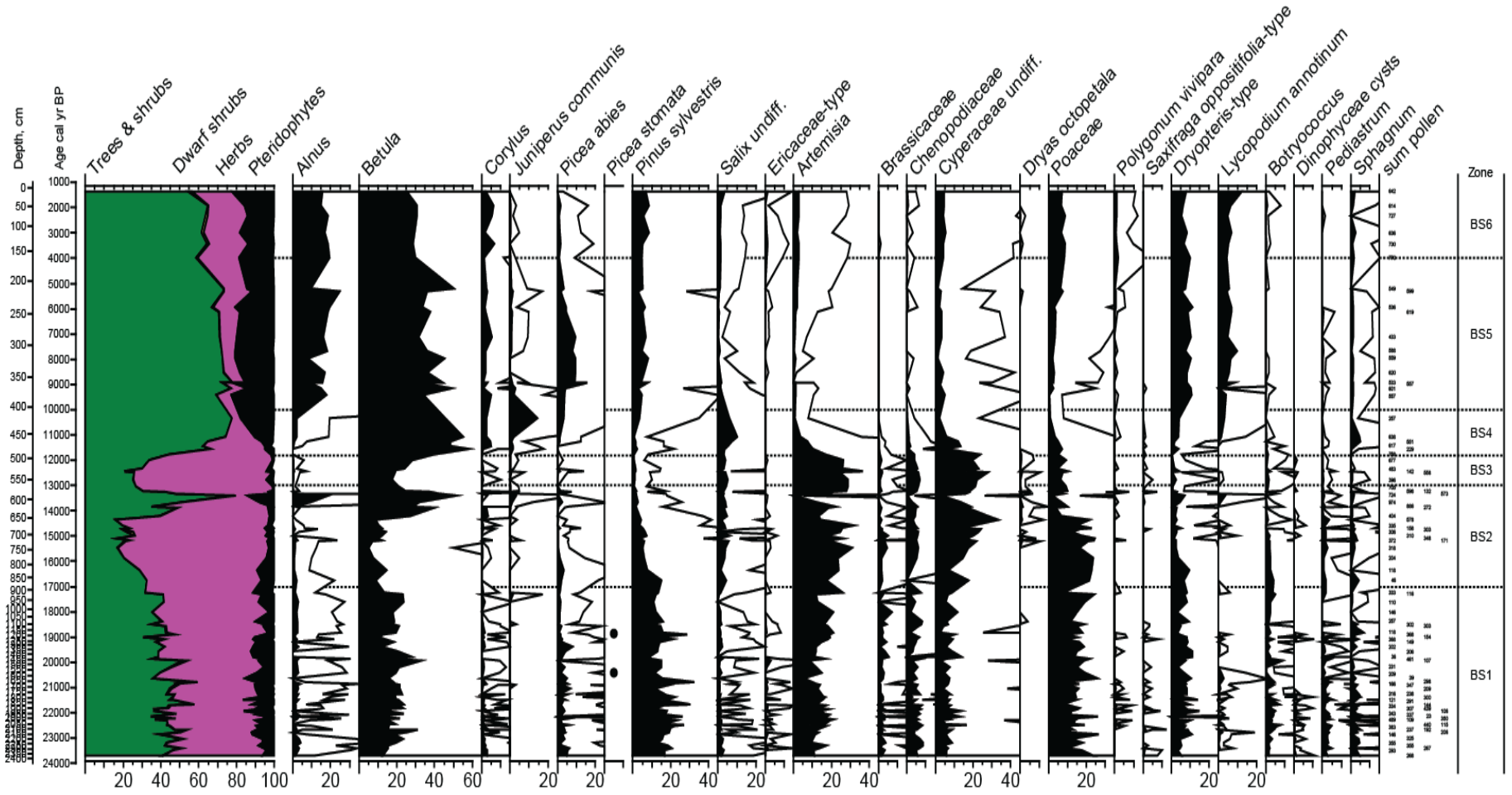

Figure C.1 Percentage pollen diagram of selected taxa for Lake Bolshoye Shchuchye with local vegetation assemblage zones BS1 to BS6 indicated. Pollen percentages are based on the sum of terrestrial pollen and spores (ΣP). Pollen data courtesy of Anne Bjune at the University of Bergen.

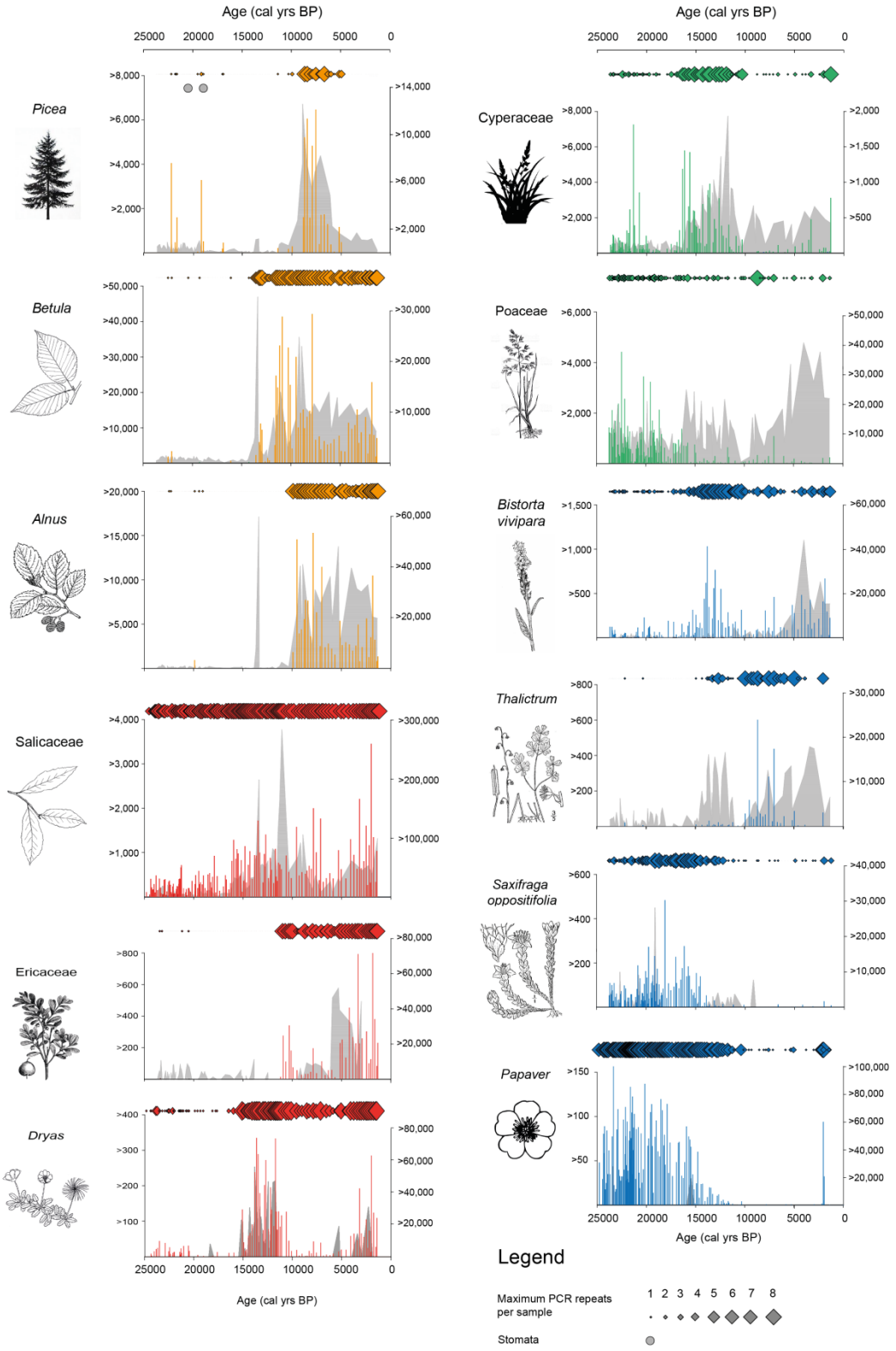

Figure C.2 Selected woody and herbaceous taxa presented as total DNA reads per sample (histogram; right-hand y-axis) and maximum number of PCR replicates (diamond symbols) for the Lake Bolshoye Shchuchye record. Grey shaded area depicts pollen concentration (left-hand y-axis). Grey closed circle indicates presence of *Picea abies* stomata. Note that the height of the y-axis varies among panels.

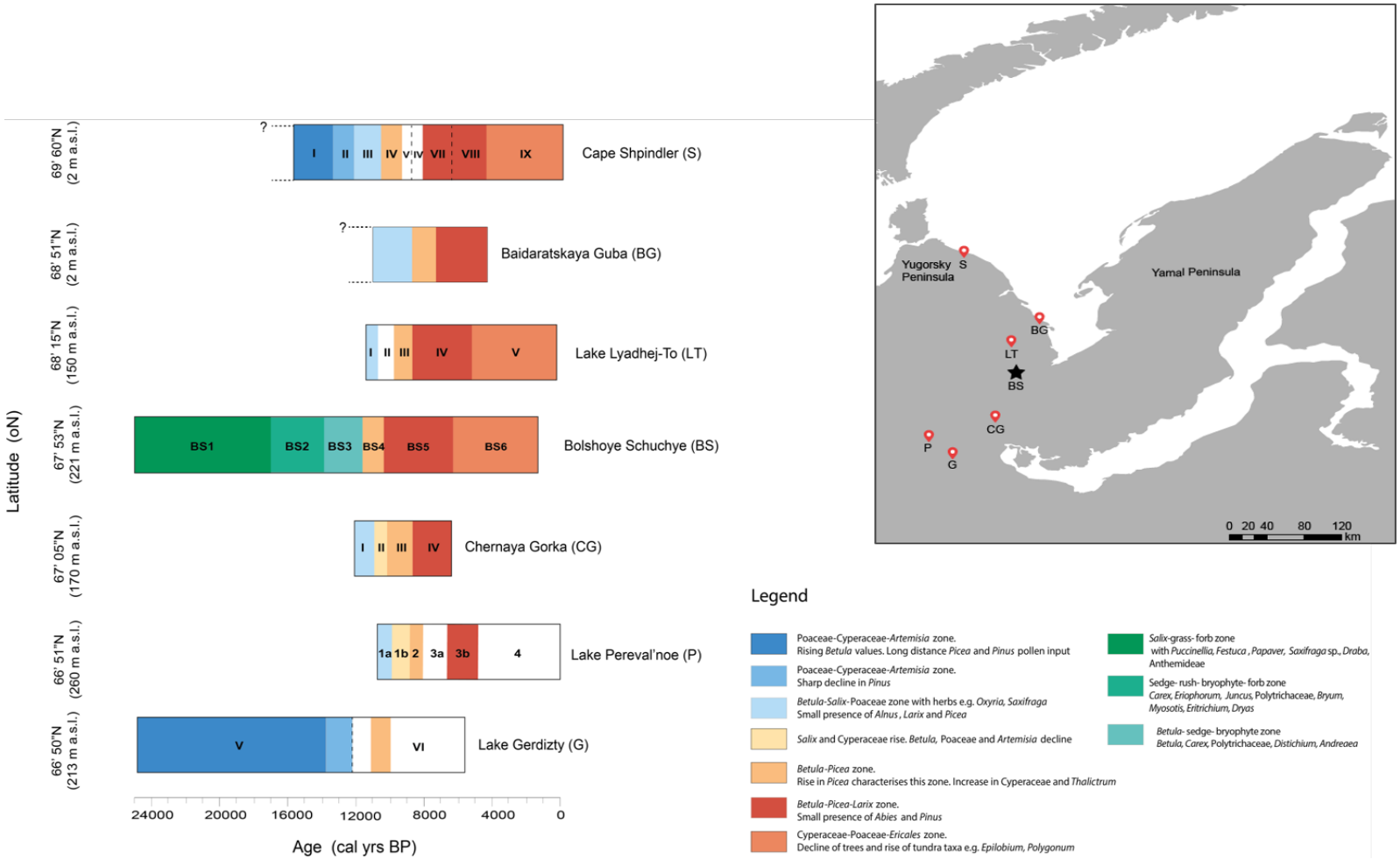

Figure C.3 Compilation of published local pollen assemblage zones (LPAZ's) from nearby sites in the Polar Ural Mountains with a description of the dominant plant taxa present within each zone. Map inset shows location of the sites in relation to Lake Bolshoye Schuchye (BS, this paper). Cape Shpindler (S, from Andreev *et*

al., 2001), Baidaratskaya Guba (BG, from Andreev *et al*., 1998), Lake Lyadhej-To (LT, from Andreev *et al*., 2005), Chernaya Gorka (CG, Janovska *et al*., 2006), Lake Pereval'noe (P, Panova *et al*., 2003) and Lake Gerdizty (G, Svendsen *et al*., 2014). Question mark indicates uncertainty associated with the basal radiocarbon age of the record.

Table C.1 Description of the key characteristics of biostratigraphic zones identified in the *sed*aDNA and pollen records from Lake Bolshoye Shchuchye.

Zone	**Depth (cm)**	**Age (cal. yrs BP)**	***sed*aDNA**	**Pollen**
BS1 Graminoid-*Papaver* zone	2400 - 880	24,000 - 17,000	• Dominance of *Papaver* and grasses such as *Puccinellia*, *Festuca* and *Juncus biglumis* • Presence of forbs such as *Draba*, *Bistorta vivipara*, *Saxifraga* sp. and Anthemideae (likely *Artemisia*) • Presence of shrubs such as *Ephedera* and the mat-forming *Dryas* • Scattered occurrences of *Picea* between *ca.* 21,000 - 18,000 cal. yrs BP	• Dominance of woody taxa such as *Betula*, *Pinus sylvestris* and *Artemisia* with herbs such as Poaceae and Chenopodiaceae • *Picea abies* (up to 10 %) pollen coincident with two finds of *Picea abies* stomata at depths 1580 cm (20,400 cal. yrs BP) and 1190 cm (18,800 cal. yrs BP).
BS2 Cyperaceae-bryophyte zone	880 - 560	17,000 - 13,000	• Distinctive increase in sedges such as *Carex* sp. and *Eriophorum* and increasing values for Anthemideae • Bryophytes reach highest abundance in this zone with increases in taxa such as *Bryum*, *Distichium*, Polytrichaceae, Dicranaceae and Grimmiaceae. • First appearance of forbs such as *Pedicularis oederi*, *Lagotis glauca*, *Astragalus*, *Rumex* and *Caltha* appear. • *Dryas* and *Betula* increase a little later in this zone *ca.* 15,000 cal. yrs BP but the rise in *Betula* is short-lived, declining to low values at the end of this zone.	• Distinctive increase in Cyperaceae (up to 35 %) and *Artemisia* (up to 30 %). • Decline in *Pinus sylvestris*

BS3 *Salix*-Cyperaceae-*Dryas* zone	560 - 490	13,000 - 11,800	• Short-lived but distinct decline in *Betula* • *Dryas*, Salicaceae and *Carex* maintain high values • Presence of forbs such as *Geum*, *Cardamine*, *Valeriana*, *Hedysarum hedysarioides*, *Thalictrum* and *Castilleja*	• Short-lived but distinct decline in *Betula* from up to >50 % in zone BS2 to <20 % in this zone. • Short-lived disappearance of *Alnus* • *Dryas*, *Salix* and Cyperaceae maintain high values
BS4 *Salix-Betula-Picea* zone	490 - 400	11,800 - 10,000	• Recovery of *Betula* to high abundance (up to 8 PCR repeats) • Main rise in *Picea* and *Alnus* • Decline in *Dryas* and *Carex* • First appearance of dwarf shrubs such as *Vaccinium uligonosum*, *Vaccinium vitis-idaea*, *Empetrum*, *Arctostaphylos uva-ursi* • Increase in forbs such as *Bistorta vivipara, Rumex, Rannunculus, Pedicularis oederi* and *Lagotis glauca*	• Recovery of *Betula* to high values (up to 60 %) • Increase in woody taxa such as *Picea*, *Alnus, Pinus sylvestris* and *Artemisia* and ferns such as *Dryopteris* • Short-lived peak in *Juniperus communis* (up to 15 %) and increasing values (5 - 10 %) of *Lycopodium annotinum*
BS5 Salix-*Betula-Picea-Larix-Alnus*- fern zone	400 - 160	10,000 - 4000	• First appearance of *Larix* and rise in *Alnus* • Betula and Pinus continue to rise • Short-lived presence of ferns e.g. *Dryopteris fragans*, *Gymnocarpium dryopteris*, *Thelypteris palustris* • Increase in herbs such as Agrostidinae, *Myosotis arvensis*, *Filipendula ulmaria* and *Galium boreale* • *Thalictrum* reaches highest values	• *Betula* and *Picea* reach highest values in this zone up to 58 % and 17 %, respectively. • *Thalictrum* increases
BS6 *Salix-Betula-Alnus-Dryas*- Ericaceae zone	160 - 0	4000 - 1300	• Disappearance of tree taxa *Picea* and *Larix* and ferns • Recovery of *Dryas* to high abundance • *Salix, Betula* and *Alnus* maintain high values • *Dwarf shrubs e.g. Vaccinium sp. remain abundant*	• Woody taxa e.g. *Alnus*, *Corylus*, *Pinus sylvestris* maintain high percentages whilst *Picea abies* and *Betula* decline to low values • Percentages for *Artemisia* increase slightly in this zone (up to 5 %)

Table C.2 All taxa detected from *sed*aDNA and pollen analyses at Lake Bolshoye Shchuchye, sorted according to family. All sedaDNA data is based on the final dataset after post-identification filtering steps.

			Sediment samples					Extraction negative controls			PCR negative controls			Sampling (water) negative controls		
Family	Taxon in sedaDNA	Taxon in pollen	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads	Max pollen concentration	Total sum of pollen grains identified	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads
Amaranthaceae	Amaranthus		1	8	1306			0	0	0	0	0	0	0	0	0
	Chenopodium capitatum		1	5	3040			1	1	404	1	2	6	0	0	0
Amblystegiaceae	Cratoneuron filicinum		1	4	661			0	0	0	0	0	0	0	0	0
	Sanionia uncinata		2	3	85			0	0	0	0	0	0	0	0	0
Andreaeaceae	Andreaea		3	24	8504			0	0	0	0	0	0	0	0	0
Apiaceae		Angelica-type				35	1									
	Anthriscus sylvestris		7	36	10501			0	0	0	0	0	0	0	0	0
		Apiaceae undiff.				294	36									
	Pachypleurum alpinum		4	43	23330			0	0	0	0	0	0	1	1	1
Aquifoliaceae		Ilex				105	29									
Asparagaceae	Convallaria majalis		3	14	11068			0	0	0	0	0	0	0	0	0
Asteraceae		Achillea-type				11	171									
	Anthemideae		8	538	397197			2	3	3	2	6	1056	3	7	1490
	Arnica		1	5	127			0	0	0	0	0	0	0	0	0
		Artemisia				9872	4807									
	Asteraceae		1	8	345			0	0	0	0	0	0	0	0	0
	Asteroideae		8	68	32295			0	0	0	1	1	590	1	2	35
	Carduinae		8	170	207445			2	6	8	1	3	842	2	5	5983
	Saussurea		7	41	24411			0	0	0	0	0	0	1	1	2
		Solidago-type				441	113									

Table C.2 cont.

			Sediment samples					Extraction negative controls			PCR negative controls			Sampling (water) negative controls		
Family	Taxon in sedaDNA	Taxon in pollen	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads	Max pollen concentration	Total sum of pollen grains identified	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads
Aulacomniaceae	Aulacomnium		3	22	4369			0	0	0	0	0	0	0	0	0
	Aulacomnium turgidum		5	25	11670			0	0	0	0	0	0	0	0	0
Betulaceae	Alnus	Alnus	4	22	3819	17106	2340	0	0	0	0	0	0	0	0	0
	Alnus alnobetula (viridis)		8	248	508214			1	4	5	1	2	2	2	3	3
		Alnus viridis				1024	67									
	Betula	Betula	8	353	388941	46916	10301	2	8	3189	1	1	2	1	4	706
		Betula/Corylus/Myrica				59	4									
		Corylus				3020	587									
Boraginaceae	Eritrichium		7	145	125989			1	1	1	0	0	0	1	2	2
	Myosotis		8	66	18838			1	1	1	0	0	0	0	0	0
	Myosotis alpestris		8	278	345359			2	6	7	0	0	0	1	3	5
	Myosotis arvensis		8	32	7227			0	0	0	0	0	0	0	0	0
Brachytheciaceae	Brachytheciaceae		2	17	5278			0	0	0	0	0	0	1	1	24
Brassicaceae	Arabidopsis		3	29	19567			0	0	0	0	0	0	0	0	0
	Arabis alpina		1	3	433			0	0	0	0	0	0	0	0	0
	Cakile (Brassicaceae in NCBI)	Brassicaceae	2	9	15896	1346	438	2	3	36	1	1	1	1	1	1
	Braya		1	8	13273			1	1	1	0	0	0	0	0	0
	Cardamine		2	16	7127			0	0	0	0	0	0	1	1	1
	Cochlearia		1	9	8140			0	0	0	0	0	0	0	0	0
	Draba		7	302	659189			1	3	3	1	3	524	1	1	1

Table C.2 cont.

			Sediment samples					Extraction negative controls			PCR negative controls			Sampling (water) negative controls		
Family	Taxon in sedaDNA	Taxon in pollen	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads	Max pollen concentration	Total sum of pollen grains identified	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads
	Draba pauciflora		1	3	805			0	0	0	0	0	0	0	0	0
	Parrya		6	75	75272			1	1	1	0	0	0	0	0	0
Bryaceae	Bryaceae		2	16	1984			0	0	0	0	0	0	0	0	0
	Bryum		4	55	15685			0	0	0	0	0	0	0	0	0
	Ptychostomum pallens		1	4	99			0	0	0	0	0	0	0	0	0
Campanulaceae		Campanula-type				41	1									
Cannabaceae		Hummulus/Cannabis-type				104	8									
Caprifoliaceae	Linnaea borealis	Linnaea	7	11	1684	24	1	0	0	0	0	0	0	0	0	0
	Lonicera	Lonicera pericy	7	11	1011	22	1	0	0	0	0	0	0	0	0	0
	Valeriana	Valeriana sambucifolia-type	8	91	41071	55	3	0	0	0	0	0	0	1	1	1
Caryophyllaceae	Arenaria serpyllifolia	Arenaria-type	1	4	19399	30	1	0	0	0	1	1	973	0	0	0
		Caryophyllaceae undiff.				192	110									
	Cerastium	Cerastium-type	5	59	45845	299	82	0	0	0	0	0	0	0	0	0
		Dianthus-type				123	19									
	Minuartia		2	24	18200			0	0	0	0	0	0	0	0	0
	Silene	Silene dioica-type	2	6	2125	35	3	0	0	0	0	0	0	0	0	0
		Silene vulgaris-type				20	1									
	Stellaria		2	33	26210			1	1	1	1	2	559	0	0	0
	Stellaria holostea		1	4	2655			0	0	0	0	0	0	0	0	0
Chenopodiaceae		Chenopodiaceae				2991	1293									

Table C.2 cont.

			Sediment samples					Extraction negative controls			PCR negative controls			Sampling (water) negative controls		
Family	Taxon in sedaDNA	Taxon in pollen	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads	Max pollen concentration	Total sum of pollen grains identified	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads
Crassulaceae	Crassula		1	9	5241			0	0	0	0	0	0	0	0	0
	Rhodiola rosea		5	49	52335			1	1	1	0	0	0	0	0	0
		Sedum				126	10									
		Juniperus communis				3019	207									
Cyperaceae	Carex	Carex-type	8	280	24369	210	15	0	0	0	0	0	0	0	0	0
	Carex aquatilis		2	11	320			0	0	0	0	0	0	0	0	0
	Carex lachenalii		1	7	1735			0	0	0	0	0	0	0	0	0
		Cyperaceae undiff.				7728	3808									
	Eriophorum		3	15	553			0	0	0	0	0	0	0	0	0
	Kobresia		1	15	877			0	0	0	0	0	0	0	0	0
Dennstaedtiaceae		Pteridium aquilinum				121	4									
Dicranaceae	Dicranaceae		5	72	30384			1	1	1	1	1	215	0	0	0
Ditrichaceae	Distichium		3	23	3587			0	0	0	0	0	0	0	0	0
	Distichium inclinatum		1	4	97			0	0	0	0	0	0	0	0	0
	Ditrichum		1	4	186			0	0	0	0	0	0	0	0	0
Dryopteridaceae	Dryopteris	Dryopteris-type	6	13	12283	7652	2071	0	0	0	0	0	0	0	0	0
	Dryopteris fragrans		3	14	2283			0	0	0	0	0	0	0	0	0
		Dryopteris filix-mas				285	13									
Elaeagnaceae	Shepherdia canadensis		1	5	8256			1	1	1100	0	0	0	0	0	0
		Hippophaë rhamnoides				29	1									

Table C.2 cont.

			Sediment samples					Extraction negative controls			PCR negative controls			Sampling (water) negative controls		
Family	Taxon in sedaDNA	Taxon in pollen	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads	Max pollen concentration	Total sum of pollen grains identified	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads
Ephedraceae	Ephedra	Ephedra	2	3	7899	46	3	0	0	0	0	0	0	0	0	0
Ericaceae	Andromeda polifolia		8	18	4685			0	0	0	0	0	0	0	0	0
	Arctostaphylos uva-ursi		2	9	1595			0	0	0	0	0	0	0	0	0
	Empetrum nigrum *	Empetrum nigrum *	8	100	36881	146	11	1	1	1	1	1	1	1	1	1
		Ericaceae-type				578	113									
	Pyrola 1		2	13	953			0	0	0	0	0	0	0	0	0
	Pyrola alpina 2 lump into Pyrola		1	3	173			0	0	0	0	0	0	0	0	0
	Pyrola grandiflora		1	5	442			0	0	0	0	0	0	0	0	0
	Pyrola minor		1	3	177			0	0	0	0	0	0	0	0	0
	Pyrola rotundifolia		4	13	1102			0	0	0	0	0	0	0	0	0
	Rhododendron (Ledum palustre)		8	206	269342			2	4	4	1	1	1	1	2	2
	Vaccinium uliginosum	Vaccinium-type	8	210	233974	146	12	2	5	5	1	2	173	2	3	5
	Vaccinium vitis-idaea		8	184	36368			0	0	0	0	0	0	0	0	0
Equisetaceae		Equisetum				1734	184									
Fabaceae	Astragalus		8	148	76621			0	0	0	0	0	0	0	0	0
		Astragalus cf. A. alpinus				43	4									
		Fabaceae undiff.				55	14									
	Galegeae		1	3	1606			0	0	0	0	0	0	0	0	0
	Hedysarum hedysaroides	Hedysarum	6	76	11396	30	2	0	0	0	0	0	0	0	0	0
	Medicago		1	3	1778			0	0	0	0	0	0	0	0	0

Table C.2 cont.

			Sediment samples					Extraction negative controls			PCR negative controls			Sampling (water) negative controls		
Family	Taxon in sedaDNA	Taxon in pollen	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads	Max pollen concentration	Total sum of pollen grains identified	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads
	Oxytropis		4	58	31456			0	0	0	0	0	0	0	0	0
	Trifolium		2	3	7265			0	0	0	0	0	0	0	0	0
Fagaceae		Fagus sylvatica				34	1									
		Quercus				158	13									
Gentianaceae		Gentiana-type				21	1									
		Gentianella-type				73	2									
Geraniaceae		Geranium sylvaticum-type				86	5									
Grimmiaceae	Grimmiaceae		4	74	31814			0	0	0	0	0	0	1	1	1
Grossulariaceae	Ribes		8	31	5398			0	0	0	0	0	0	0	0	0
Hydrodictyaceae		Pediastrum				1197	417									
Hymenophyllaceae		Hymenophyllum				46	8									
Hypnaceae	Hypnaceae		2	10	1091			0	0	0	0	0	0	0	0	0
	Hypnum procerrimum		1	3	78			0	0	0	0	0	0	0	0	0
Juglandaceae		Pterocarya				50	4									
Juncaceae	Juncus biglumis		3	52	26699			0	0	0	0	0	0	0	0	0
	Luzula parviflora		1	4	1578			0	0	0	0	0	0	0	0	0
Jungermanniaceae	Mesoptychia badensis		2	15	614			0	0	0	0	0	0	0	0	0
Lamiaceae	Lamiaceae		1	12	1969			1	1	4	0	0	0	0	0	0
Liliaceae		Gagea-type				280	4									
		Liliaceae undiff.				86	4									

Table C.2 cont.

			Sediment samples					Extraction negative controls			PCR negative controls			Sampling (water) negative controls		
Family	Taxon in sedaDNA	Taxon in pollen	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads	Max pollen concentration	Total sum of pollen grains identified	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads
Linaceae	Linum		1	6	519			0	0	0	0	0	0	0	0	0
Lycopodiaceae		Diphasiastrum				121	12									
		Huperzia selago				256	20									
	Lycopodiaceae	Lycopodium undiff.	6	147	137186	35	4	2	14	41280	1	3	543	3	8	13205
		Lycopodium annotinum				4596	753									
		Lycopodium clavatum				71	9									
Lythraceae	Lythrum salicaria		1	4	540			0	0	0	0	0	0	0	0	0
Malvaceae		Tilia cordata				70	3									
Melanthiaceae	Veratrum		5	41	7381			0	0	0	0	0	0	0	0	0
Menyanthaceae		Menyanthes trifoliata				42	1									
Mniaceae	Mniaceae		2	7	221			0	0	0	0	0	0	0	0	0
Myricaceae		Myrica gale				45	2									
Nelumbonaceae		Lotus-type				24	2									
Nymphaeaceae		Nuphar				24	1									
Ophioglossaceae		Botrychium				26	1									
Oleaceae		Fraxinus excelsior				257	13									
Onagraceae	Chamerion angustifolium		7	22	4794			0	0	0	0	0	0	0	0	0
		Epilobium				140	10									
Orobanchaceae	Castilleja		6	18	3608			0	0	0	0	0	0	0	0	0
	Euphrasia		1	3	368			0	0	0	0	0	0	0	0	0

Table C.2 cont.

			Sediment samples					Extraction negative controls			PCR negative controls			Sampling (water) negative controls		
Family	Taxon in sedaDNA	Taxon in pollen	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads	Max pollen concentration	Total sum of pollen grains identified	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads
	Pedicularis	Pedicularis	4	29	42082	35	4	1	2	2	0	0	0	0	0	0
	Pedicularis oederi		8	219	79530			0	0	0	0	0	0	1	1	1
	Pedicularis pacifica (syn. P. sudetica)		7	82	30169			0	0	0	1	1	50	0	0	0
		Rhinantus-type				70	8									
Papaveraceae	Papaver	Papaver	8	919	4017519	43	3	5	30	43	4	16	28	3	10	24
Pinaceae	Abies	Abies	2	5	83463	70	1	0	0	0	0	0	0	0	0	0
	Larix sibirica	Larix	8	99	110499	121	1	0	0	0	1	1	1	1	1	2
	Picea	Picea abies*	8	84	83039	6707	1003	2	4	3796	1	2	1534	1	3	17195
		Picea abies stomata					2									
		Pinus sylvestris*				11517	3175									
Plagiochilaceae	Plagiochila		1	3	86			0	0	0	0	0	0	0	0	0
Plantaginaceae	Lagotis glauca		8	224	268435			2	4	5	0	0	0	1	1	1
	Plantago	Plantago undiff.	1	4	13913	64	3	0	0	0	0	0	0	0	0	0
		Plantago lanceolata				70	1									
		Plantago major/P. media				26	1									
		Plantago maritima				20	1									
	Veronica		7	44	4772			0	0	0	0	0	0	0	0	0
Poaceae	Agrostidinae		8	39	25479			1	1	1	0	0	0	0	0	0
	Alopecurinae		3	21	11408			1	1	7	1	1	19	0	0	0
	BOP clade		1	5	10639			0	0	0	0	0	0	0	0	0

Table C.2 cont.

			Sediment samples					Extraction negative controls			PCR negative controls			Sampling (water) negative controls		
Family	Taxon in sedaDNA	Taxon in pollen	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads	Max pollen concentration	Total sum of pollen grains identified	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads
	Bromus ciliatus		1	3	232			0	0	0	0	0	0	0	0	0
	Bromus pumpellianus		1	3	476			0	0	0	0	0	0	0	0	0
	Festuca		4	102	125403			1	3	9488	2	3	14	1	3	3083
	Hordeum		1	5	2271			0	0	0	0	0	0	0	0	0
	PACMAD clade		1	3	6258			2	2	764	1	1	1	0	0	0
	Poaceae	Poaceae	1	5	1139	4779	3773	0	0	0	1	1	2	0	0	0
	Poeae		4	121	194341			0	0	0	1	1	2	0	0	0
	Puccinellia		4	125	283766			0	0	0	1	1	1	0	0	0
Polemoniaceae	Polemonium		2	5	188			0	0	0	0	0	0	0	0	0
Polygonaceae	Bistorta		8	196	192430			1	3	3	0	0	0	0	0	0
	Bistorta vivipara	Bistorta vivipara	8	344	441520	1103	86	2	11	2964	2	5	2716	3	7	7
	Oxyria digyna	Oxyria digyna	8	208	201736	70	13	0	0	0	2	3	1214	2	4	5
		Polygonum aviculare-type				27	2									
	Rumex	Rumex acetosa-type	4	73	54088	662	184	1	2	74	2	4	11146	0	0	0
		Rumex acetosella-type				110	13									
		Rumex longifolius-type				32	3									
Polypodiaceae		Polypodium vulgare				46	3									
Polytrichaceae	Polytrichaceae		8	254	48686			0	0	0	2	2	2007	0	0	0
Potamogetonaceae		Potamogeton (Eupotamogeton)				140	4									
Pottiaceae	Didymodon icmadophilus		1	3	135			0	0	0	0	0	0	0	0	0

Table C.2 cont.

			Sediment samples					Extraction negative controls			PCR negative controls			Sampling (water) negative controls		
Family	Taxon in sedaDNA	Taxon in pollen	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads	Max pollen concentration	Total sum of pollen grains identified	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads
	Pottiaceae		1	15	1778			0	0	0	0	0	0	0	0	0
	Syntrichia ruralis		1	3	262			0	0	0	0	0	0	0	0	0
Primulaceae	Androsace chamaejasme		2	9	1984			0	0	0	0	0	0	0	0	0
	Douglasia		1	4	1840			0	0	0	0	0	0	0	0	0
	Trientalis	Trientalis europea	1	3	324	211	6	0	0	0	0	0	0	0	0	0
Pteridaceae		Cryptogramma crispa				121	20									
Ranunculaceae	Aconitum lycoctonum		8	45	14192			0	0	0	0	0	0	0	0	0
	Caltha	Caltha-type	3	46	18724	186	40	0	0	0	0	0	0	0	0	0
	Oxygraphis glacialis		2	9	4650			1	1	1	0	0	0	0	0	0
	Ranunculus	Ranunculus undiff.	8	268	122556	150	40	1	3	26	2	2	6776	0	0	0
	Ranunculus arcticus		2	16	8584			0	0	0	0	0	0	0	0	0
		Ranunculus acris-type				318	58									
		Ranunculus flammula-type				110	3									
	Ranunculus pygmaeus		2	4	194			0	0	0	0	0	0	0	0	0
	Ranunculus sulphureus		5	50	22132			0	0	0	0	0	0	0	0	0
	Thalictroideae		2	3	1749			0	0	0	0	0	0	0	0	0
	Thalictrum	Thalictrum	8	147	95776	450	252	1	2	2	1	1	1	0	0	0
	Trollius	Trollius europaeus	4	14	5916	55	1	0	0	0	0	0	0	0	0	0
Rhabdoweisiaceae	Kiaeria glacialis		1	3	47			0	0	0	0	0	0	0	0	0
Rhytidiaceae	Rhytidium rugosum		1	3	79			0	0	0	0	0	0	0	0	0

Table C.2 cont.

			Sediment samples					Extraction negative controls			PCR negative controls			Sampling (water) negative controls		
Family	Taxon in sedaDNA	Taxon in pollen	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads	Max pollen concentration	Total sum of pollen grains identified	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads
	Filipendula ulmaria	Filipendula	4	18	4240	147	5	1	1	3	0	0	0	0	0	0
	Geum		8	43	31899			1	2	259	1	1	120	0	0	0
	Potentilla	Potentilla-type	4	56	21765	158	28	1	1	1	0	0	0	0	0	0
		Prunus padus				43	1									
		Rosaceae undiff.				171	26									
		Rubus chamaemorus				64	7									
		Sorbus-type				127	9									
	Spiraea		7	16	4909			0	0	0	0	0	0	0	0	0
Rubiaceae		Rubiaceae				86	23									
	Galium		5	19	12917			0	0	0	0	0	0	0	0	0
	Galium boreale		6	22	5862			1	1	1	0	0	0	0	0	0
Salicaceae	Saliceae	Salix undiff.	8	1034	5754845	3771	900	6	42	20441	4	16	65381	4	14	4186
		Salix herbaceae-type				252	18									
		Populus tremula				279	10									
Sapindaceae		Acer				22	1									
Saxifragaceae	Saxifraga		7	272	236913			1	1	1	1	2	2	1	1	1
		Saxifraga cernua-type				150	12									
	Saxifraga cespitosa	Saxifraga cespitosa-type	4	63	14707	49	1	0	0	0	0	0	0	0	0	0
	Saxifraga hirculus	Saxifraga hirculus-type	7	160	141610	85	2	1	1	1	0	0	0	0	0	0
	Saxifraga oppositifolia	Saxifraga oppositifolia-type	8	397	481851	449	33	1	2	3	1	2	2	1	1	1
	Saxifragaceae		3	81	35867			1	3	3	1	1	165	0	0	0
Scapaniaceae	Scapania		1	5	155			0	0	0	0	0	0	0	0	0
	Scapaniaceae		1	8	823			0	0	0	0	0	0	0	0	0

Table C.2 cont.

			Sediment samples					Extraction negative controls			PCR negative controls			Sampling (water) negative controls		
Family	Taxon in sedaDNA	Taxon in pollen	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads	Max pollen concentration	Total sum of pollen grains identified	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads
Scrophulariaceae		Melampyrum				150	8									
		Scrophulariaceae				85	5									
Selaginellaceae		Selaginella selaginoides				147	6									
Sphagnaceae	Sphagnum russowii	Sphagnum	3	20	11319	2094	635	0	0	0	0	0	0	0	0	0
Thelypteridaceae	Thelypteris palustris		2	6	2442			0	0	0	0	0	0	0	0	0
Typhaceae		Sparganium-type				77	12									
		Typha latifolia				44	1									
Ulmaceae	Ulmus glabra	Ulmus	2	4	15821	546	56	1	1	1	0	0	0	0	0	0
Urticaceae	Urtica	Urtica	2	6	5267	377	7	0	0	0	0	0	0	0	0	0
Violaceae	Viola		2	6	327			0	0	0	0	0	0	0	0	0
	Viola epipsila		1	4	380			0	0	0	0	0	0	0	0	0
Woodsiaceae	Gymnocarpium dryopteris	Gymnocarpium dryopteris	2	11	4137	2012	436	0	0	0	0	0	0	0	0	0

Appendix D Supporting Information: Paper IV

Here we include three supplemental figures containing the full taxon list identified by *sed*aDNA analyses at Chisholm Lake to augment those in the main text. We also include one supplemental table containing summary *sed*aDNA data.

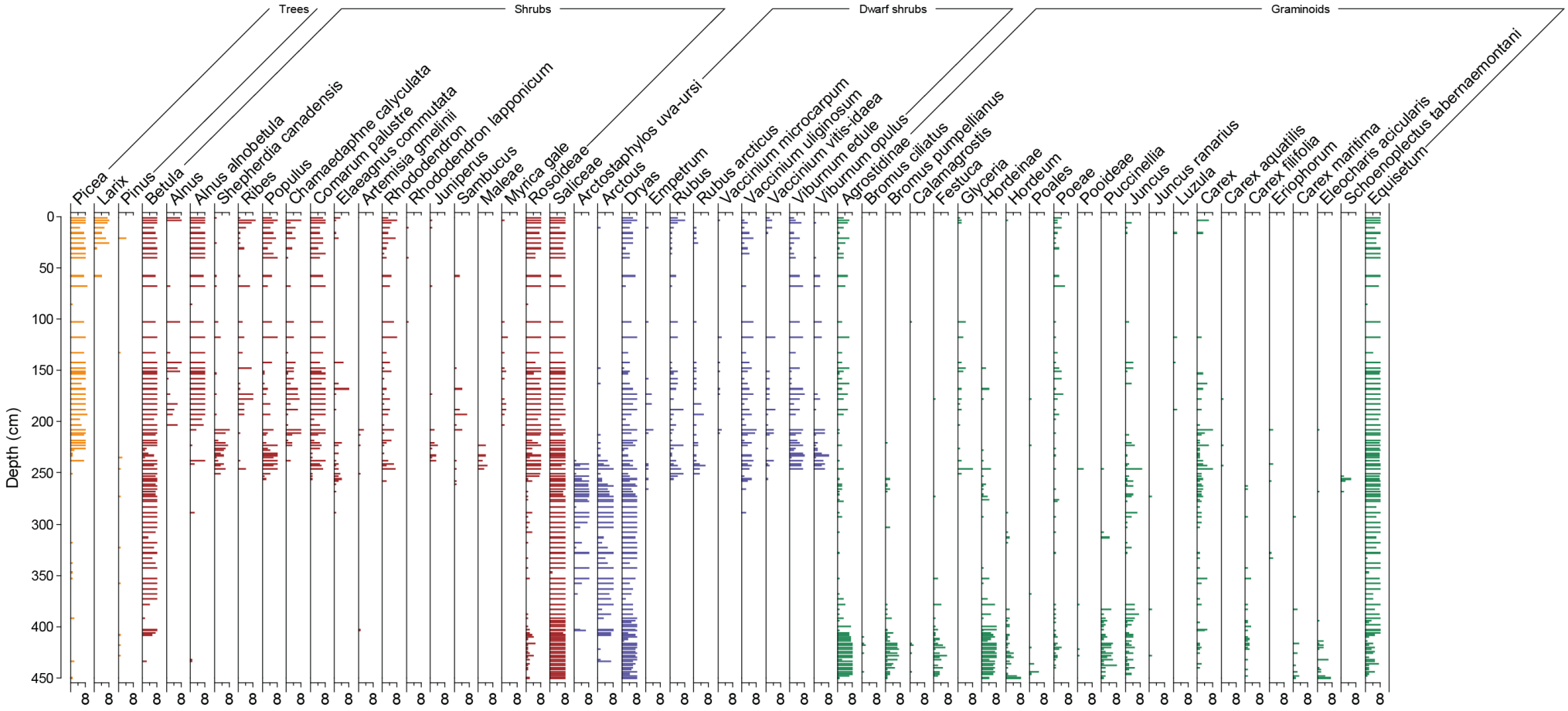

Figure D.1 Woody plant taxa and graminoids detected by *sed*aDNA at Chisholm Lake. The x-axis refers to number of PCR replicates (out of eight) a taxon was detected within per sample.

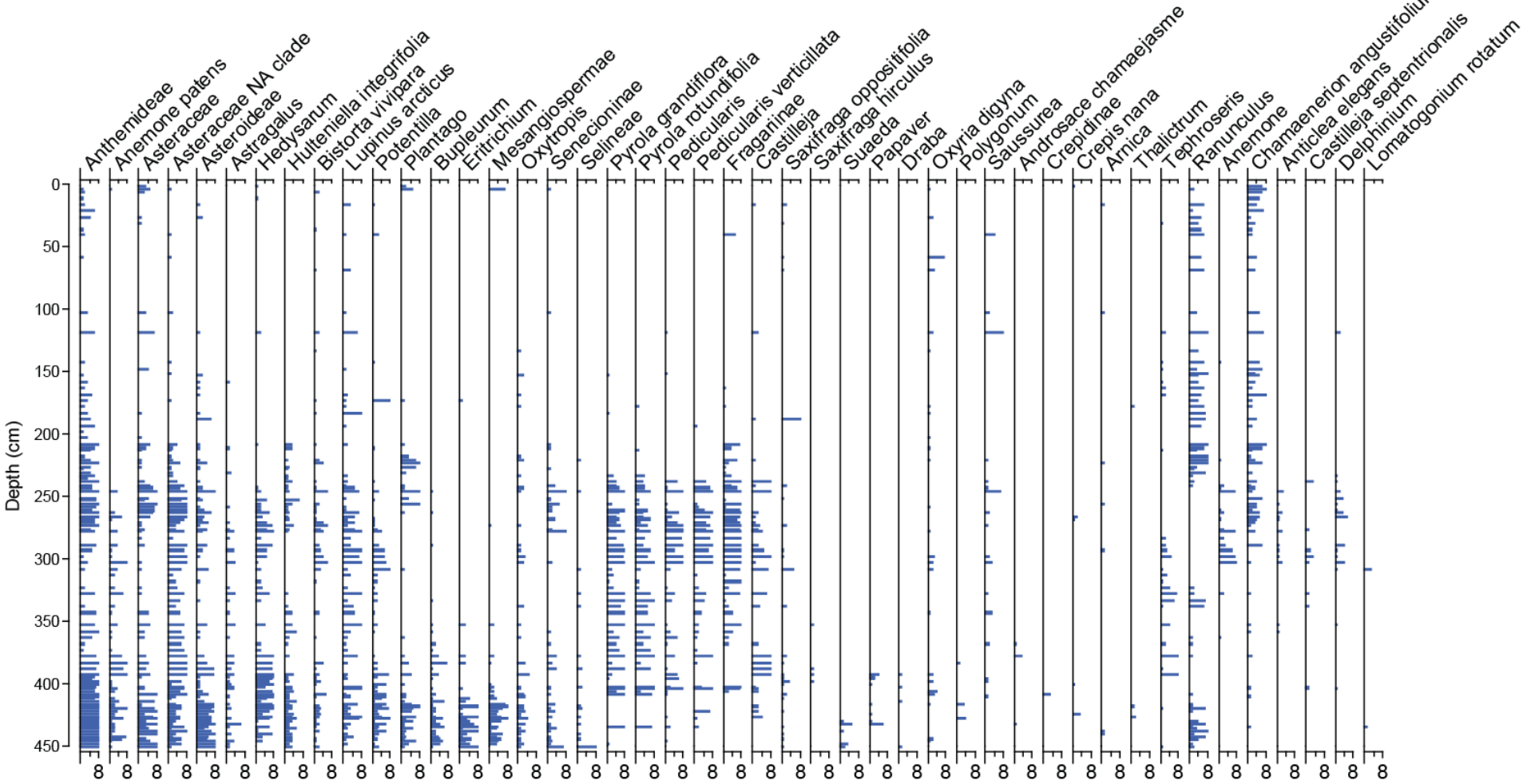

Figure D.2 Forb taxa detected by *sed*aDNA at Chisholm Lake. The x-axis refers to number of PCR replicates (out of eight) a taxon was detected within per sample.

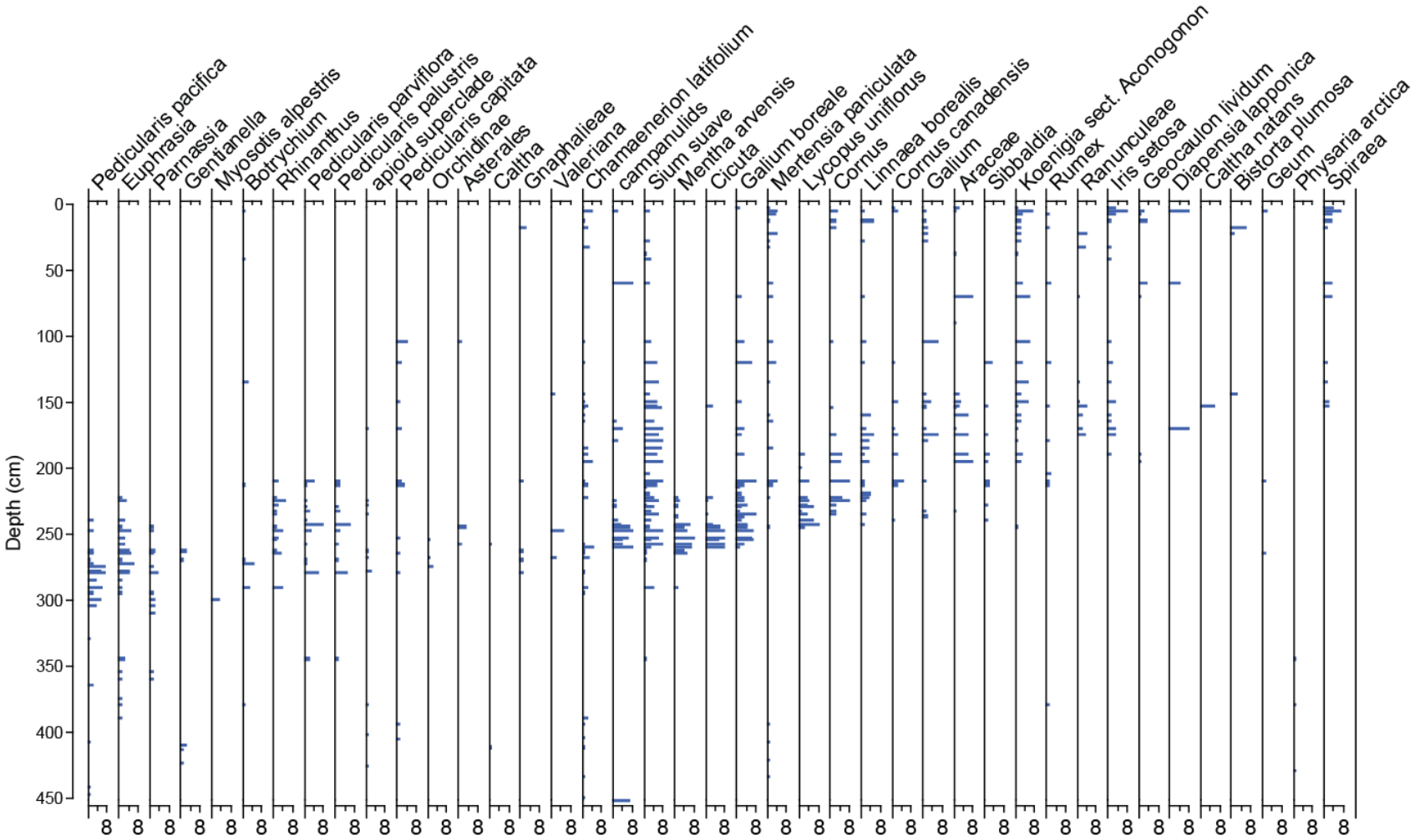

Figure D.2 cont.

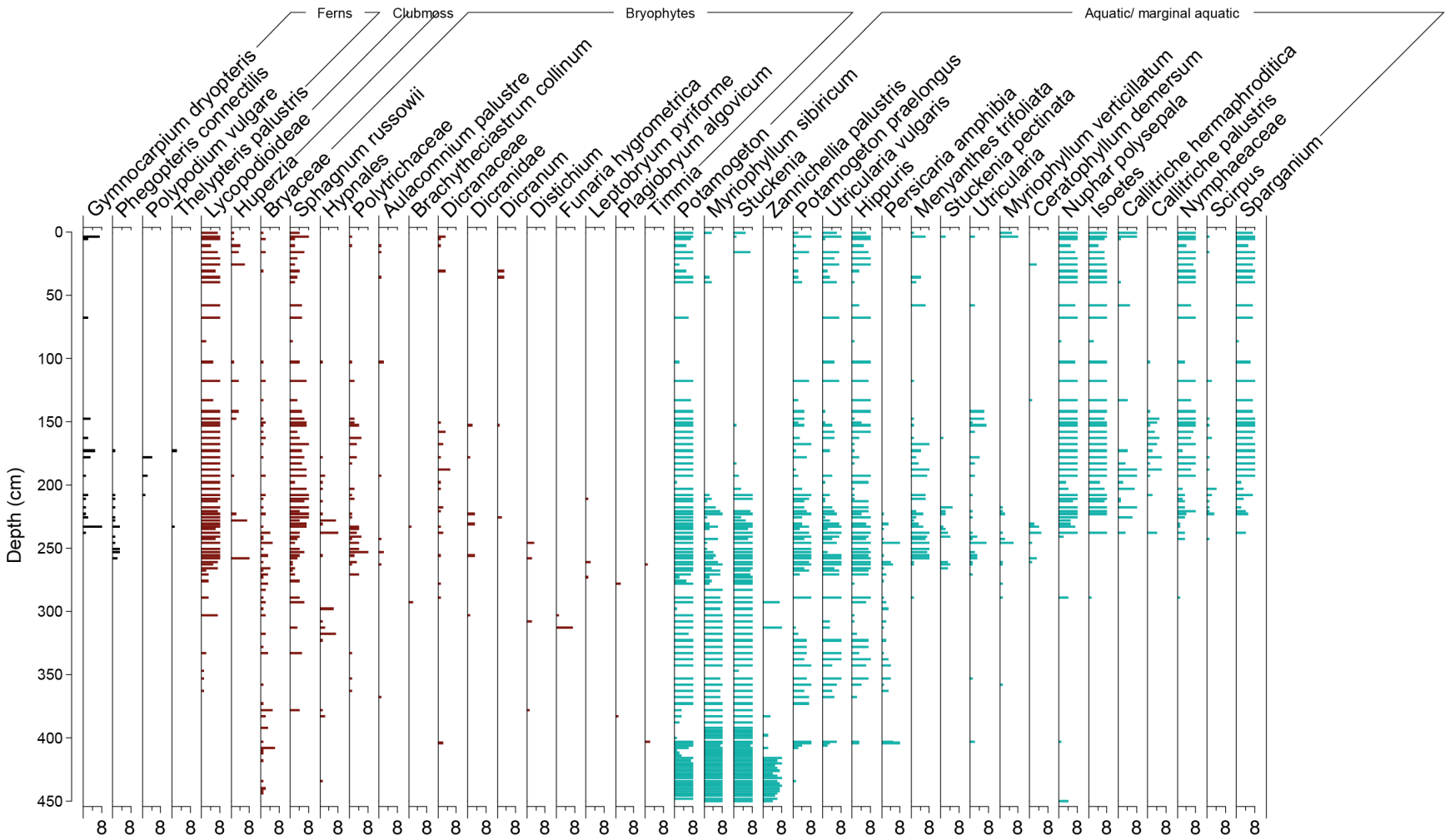

Figure D.3 Fern, bryophyte and aquatic plant taxa detected by *sed*aDNA at Chisholm Lake. The x-axis refers to number of PCR replicates (out of eight) a taxon was detected within per sample.

Table D.1 All taxa detected by sedaDNA analyses from Chisholm Lake with a comment on their biogeographic distribution and presence within the present-day regional flora.

		Sediment samples			Extraction negative controls			PCR negative controls				
Family	Taxon in sedaDNA	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads	Present in Hulten (1968) flora	Comment
Adoxaceae	Sambucus	7	27	13507	0	0	0	0	0	0		
	Viburnum edule	8	170	338	0	0	0	0	0	0	Y	
	Viburnum opulus	8	76	1341	0	0	0	0	0	0		
Apiaceae	Bupleurum	7	80	2071	0	0	0	0	0	10	Y	Likely species B. triradiatum, low to high meadows to tundra, often present on dry slopes
	Cicuta	8	54	9653	0	0	0	0	0	0	Y	C. douglasii and C. mackenzii likely species. Present at Chena Hot Springs
	Selineae	8	17	639	0	0	0	0	0	374	Y	
	Sium suave	8	172	14233	0	0	0	0	0	0	Y	Present in Alaska but not currently in local catchment
Araceae	Araceae	8	49	3051	0	0	0	0	0	0	Y	Calla palustris is only species within this family- occurs in interior to west Alaska
Asteraceae	Anthemideae	8	519	125728	0	0	0	1	2	37512	Y	
	Arnica	1	7	152	0	0	0	1	2	5	Y	
	Artemisia gmelinii	3	8	14847	0	0	0	0	0	35035		synonym Artemisia sacrorum and A. vestita
	Asteraceae	15	264	10618	0	0	0	2	4	7842	Y	
	Asteraceae North American clac	8	375	1717	0	0	0	0	0	21	Y	
	Asteroideae	9	229	1856	0	0	0	1	2	123	Y	
	Crepidinae	3	3	3097	0	0	0	0	0	463	Y	

Table D.1 cont.

		Sediment samples			Extraction negative controls			PCR negative controls				
Family	Taxon in sedaDNA	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads	Present in Hulten (1968) flora	Comment
	Crepis nana	3	8	4259	0	0	0	0	0	5	Y	Widespread on disturbed soils
Asteraceae	Gnaphalieae	2	6	57	0	0	0	0	0	0	Y	
	Hulteniella integrifolia	6	138	387	0	0	0	1	2	0		synonym Arctanthemum integrifolium
	Saussurea	8	41	11324	0	0	0	1	2	0	Y	
	Senecioninae	8	78	661721	0	0	0	0	0	3061	Y	
	Tephroseris	8	70	838933	0	0	0	0	0	474		
Aulacomniaceae	Aulacomnium palustre	2	11	5359	0	0	0	0	0	1077	Y	Moss taxon of moist habitata inc. lake edge
Betulaceae	Alnus	8	69	898	0	0	0	0	0	0	Y	
	Alnus alnobetula	8	243	69	0	0	0	4	8	0	Y	
	Betula	8	572	2413	0	0	0	2	4	0	Y	
Boraginaceae	Eritrichium	8	77	1418	0	0	0	0	0	0	Y	E. splendens present within the area
	Mertensia paniculata	4	42	368	0	0	0	0	0	0	Y	
	Myosotis alpestris	3	3	1115	0	0	0	0	0	0	Y	
Brachytheciaceae	Brachytheciastrum collinum	2	3	127	0	0	0	0	0	0		Moss species- unknown distribution in Alaska
Brassicaceae	Draba	1	3	1557	0	0	0	0	0	0	Y	Several species within this genus present in Alaska

Table D.1 cont.

		Sediment samples			Extraction negative controls			PCR negative controls				
Family	Taxon in sedaDNA	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads	Present in Hulten (1968) flora	Comment
	Physaria arctica	1	3	2011	0	0	0	0	0	0	Y	Brassica. Synonym Lesquerella
Bryaceae	Bryaceae	6	97	117	0	0	0	0	0	0	Y	
	Plagiobryum algovicum	2	3	1336	0	0	0	0	0	0	Y	
Caprifoliaceae	Linnaea borealis	5	46	15	0	0	0	0	0	0	Y	
	Valeriana	5	8	158	0	0	0	0	0	0	Y	
Celastraceae	Parnassia	3	18	93	0	0	0	0	0	7	Y	
Ceratophyllaceae	Ceratophyllum demersum	5	20	168	0	0	0	0	0	267	Y	
Chenopodiaceae	Suaeda	5	10	53	0	0	0	0	0	0		Likely species is S. occudentalis. Reported from the interior, not currently near site
Comandraceae	Geocaulon lividum	3	12	1786	0	0	0	0	0	0	Y	Widespread distribution. Occurs in dry forests
Cornaceae	Cornus	8	47	1104	0	0	0	0	0	0	Y	
	Cornus canadensis	5	19	640	0	0	0	0	0	0	Y	Widespread distribution
Cupressaceae	Juniperus	4	20	557	0	0	0	0	0	0	Y	J. communis is most likely species
Cyperaceae	Carex	12	143	1558	0	0	0	0	0	4803	Y	
	Carex aquatilis	1	3	2623	0	0	0	0	0	0	Y	Widespread aquatic
	Carex filifolia	3	29	7920	0	0	0	0	0	0	Y	Present on dry ridges in St Elias

Table D.1 cont.

		Sediment samples			Extraction negative controls			PCR negative controls				
Family	Taxon in sedaDNA	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads	Present in Hulten (1968) flora	Comment
	Carex maritima	3	14	113175	0	0	0	0	0	0	Y	Present in the mountains and shortes within interior Alaska and St Elias
	Eleocharis acicularis	7	29	10406	0	0	0	0	0	0	Y	Widespread distribution
	Eriophorum	2	8	36255	0	0	0	0	0	0	Y	
	Schoenoplectus tabernaemonta	5	9	593	0	0	0	0	0	1378	Y	Multiple synonyms inc. Schoenoplectus lacustris
	Scirpus	4	23	7704	0	0	0	0	0	0	Y	
Cystopteridaceae	Gymnocarpium dryopteris	8	41	498	0	0	0	0	0	0	Y	Widespread with N Alaska and the interior
Diapensiaceae	Diapensia lapponica	8	20	14677	0	0	0	0	0	0	Y	Widespread on the heaths
Dicranaceae	Dicranaceae	5	33	7507	0	0	0	0	0	0	Y	
	Dicranum	3	9	3981	0	0	0	0	0	0	Y	
	Distichium	3	8	3977	0	0	0	0	0	0	Y	
Dryopteridaceae	Dryopteris	7	67	14	0	0	0	1	2	0	Y	Several species within this genus present in Alaska
Elaeagnaceae	Elaeagnus commutata	8	52	14685	0	0	0	0	0	0	Y	Present within interior Alaska
	Shepherdia canadensis	8	80	16551	0	0	0	0	0	0	Y	
Equisetaceae	Equisetum	24	996	1385	0	0	0	0	0	21	Y	
Ericaceae	Arctostaphylos uva-ursi	23	282	195	0	0	0	0	0	0	Y	Widespread, circumpolar species.

Table D.1 cont.

		Sediment samples			Extraction negative controls			PCR negative controls				
Family	Taxon in sedaDNA	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads	Present in Hulten (1968) flora	Comment
	Arctous	10	262	1446	0	0	0	0	0	0	Y	
	Chamaedaphne calyculata	8	115	372	0	0	0	0	0	0	Y	Widespread- present in moist areas
	Empetrum	4	19	1740515	0	0	0	1	2	148	Y	Likely species is E. nigrum
	Pyrola grandiflora	16	260	241	0	0	0	0	0	0	Y	
	Pyrola rotundifolia	8	184	180492	0	0	0	0	0	0	Y	
	Rhododendron	8	126	4486	0	0	0	0	0	0	Y	
	Rhododendron lapponicum	1	3	4717	0	0	0	0	0	4292	Y	
	Vaccinium microcarpum	2	10	5517	0	0	0	0	0	0	Y	
	Vaccinium uliginosum	8	144	3617	0	0	0	0	0	0	Y	
	Vaccinium vitis-idaea	5	50	38597	0	0	0	0	0	0	Y	
Fabaceae	Astragalus	6	70	6537	0	0	0	0	0	0	Y	
	Hedysarum	8	256	295304	0	0	0	0	0	1036	Y	H. alpinum is widespread in the region
	Lupinus arcticus	11	244	299	0	0	0	0	0	0	Y	
	Oxytropis	5	64	13918	0	0	0	0	0	399	Y	
Funariaceae	Funaria hygrometrica	7	8	237	0	0	0	0	0	0	Y	
Gentianaceae	Gentianella	2	7	282	0	0	0	0	0	0		
	Lomatogonium rotatum	3	4	1109	0	0	0	0	0	0	Y	
Grossulariaceae	Ribes	9	81	44	0	0	0	0	0	0	Y	
Haloragaceae	Myriophyllum sibiricum	8	498	3628	0	0	0	0	0	130	Y	Synonym Myriophyllum spicatum
	Myriophyllum verticillatum	8	31	3305	0	0	9769	0	0	0	Y	

Table D.1 cont.

		Sediment samples			Extraction negative controls			PCR negative controls				
Family	Taxon in sedaDNA	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads	Present in Hulten (1968) flora	Comment
Iridaceae	Iris setosa	8	33	264	0	0	0	0	0	0	Y	
Isoetaceae	Isoetes	8	278	1399704	0	0	0	0	0	58	Y	Isoetes muricata is most likely species
Juncaceae	Juncus	9	158	100061	0	0	0	0	0	0	Y	
	Juncus ranarius	1	3	5233	0	0	0	0	0	0	Y	Multiple synonyms inc. Juncus bufonius
	Luzula	2	7	1020	0	0	0	0	0	0	Y	
Lamiaceae	Lycopus uniflorus	8	43	430	0	0	0	0	0	0		In the interior but shows a disjunct distribution in Canada. Possibly introduced.
	Mentha arvensis	8	53	3212	0	0	0	1	2	0	Y	
Lentibulariaceae	Utricularia	7	63	90	0	0	0	0	0	0	Y	
	Utricularia vulgaris	8	283	2032	0	0	0	0	0	0	Y	
Lycopodiaceae	Huperzia	8	42	4538	0	0	0	0	0	0	Y	Widespread in region
	Lycopodioideae	8	408	673	0	0	0	2	4	0	y	
Meesiaceae	Leptobryum pyriforme	2	4	545	0	0	0	0	0	0	Y	
Melanthiaceae	Anticlea elegans	3	17	17837	0	0	0	0	0	0		Synonym Zigadenus. Present in Alaska on warm, dry Populus slopes and bluff sites
Menyanthaceae	Menyanthes trifoliata	8	148	724	0	0	0	1	2	0	Y	
Myricaceae	Myrica gale	3	17	383	0	0	0	0	0	0	Y	
Nymphaeaceae	Nuphar polysepala	8	304	109309	0	0	0	0	0	0	Y	
	Nymphaeaceae	13	248	9159	0	0	0	0	0	24	Y	
Onagraceae	Chamaenerion angustifolium	8	196	1334	0	0	0	0	0	0	Y	Widespread- common name fireweed
	Chamaenerion latifolium	5	55	64385	0	0	0	0	0	30	Y	Widespread-- wide leaved fireweed

Table D.1 cont.

		Sediment samples			Extraction negative controls			PCR negative controls				
Family	Taxon in sedaDNA	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads	Present in Hulten (1968) flora	Comment
Ophioglossaceae	Botrychium	5	15	49	0	0	0	0	0	0	Y	
Orchidaceae	Orchidinae	2	4	2659	0	0	0	0	0	8	Y	
Orobanchaceae	Castilleja	20	166	206	0	0	0	0	0	0	Y	
	Castilleja septentrionalis	3	14	2040	0	0	0	0	0	0	Y	
	Euphrasia	6	50	2263	0	0	0	0	0	0	Y	E. disjuncta present within the area
	Pedicularis	20	176	28733	0	0	0	0	0	37194	Y	
	Pedicularis capitata	4	19	2451	0	0	0	0	0	1783	Y	
	Pedicularis pacifica	7	55	2142	0	0	0	0	0	120	Y	Synonym Pediularis sudetica subsp. pacific
	Pedicularis palustris	6	23	3062	0	0	0	0	0	9		
	Pedicularis parviflora	8	32	3812	0	0	0	0	0	1434	Y	
	Pedicularis verticillata	12	168	1944	0	0	0	0	0	0	Y	
	Rhinanthus	5	28	201	0	0	0	0	0	0		Occurs S of the Alaska range today.
Papaveraceae	Papaver	6	18	263	0	0	0	1	2	0	Y	
Pinaceae	Larix	8	50	6717	0	0	0	1	2	0	Y	Most likely species is L. laricina
	Picea	9	295	1622	2	2	0	5	12	0	Y	
	Pinus	4	14	164	1	3	0	3	7	0		Not in Alaska, except SE.
Plantaginaceae	Callitriche hermaphroditica	8	78	3288	0	0	0	9	18	0	Y	Present in N and C Alaska and has a circumpolar distribution. Aquatic taxon
	Callitriche palustris	6	46	410123	0	0	0	0	0	69	Y	Multiple synonyms inc. Callitriche alpina, C. cespitosa
	Hippuris	8	358	23463	0	0	0	0	0	482	Y	H. vulgaris is widespread in the region
	Plantago	8	128	13088	0	0	0	0	0	36	Y	

Table D.1 cont.

		Sediment samples			Extraction negative controls			PCR negative controls				
Family	Taxon in sedaDNA	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads	Present in Hulten (1968) flora	Comment
Poaceae	Agrostidinae	8	302	5155098	0	0	0	0	0	75274	Y	
	Bromus ciliatus	2	6	2134	0	0	0	0	0	0	Y	Present on rocky slopes and thickets, present in upper tanana and S Alaska to Canada. Beringian taxon
	Bromus pumpellianus	7	94	2248	0	0	0	0	0	0	Y	Present on dry, grassy slopes, meadows to tundra, Beringian taxon
	Calamagrostis	2	6	5222	0	0	0	0	0	5	Y	
	Festuca	7	64	52	0	0	0	0	0	985	Y	
	Glyceria	8	42	800	0	0	0	0	0	0	Y	
	Hordeinae	8	206	410	0	0	0	2	4	0	Y	
	Hordeum	8	49	14026	0	0	0	0	0	20	Y	H. jubatum is most likely species
	Poeae	6	97	65	0	0	0	1	2	0	Y	
	Pooideae	3	6	135020	0	0	0	0	0	1833	Y	
Polygonaceae	Bistorta plumosa	6	9	861	0	0	0	0	0	0	Y	Synonym Polygonum bistorta
	Bistorta vivipara	6	109	29315	0	0	0	1	2	0	Y	Circumpolar dry meadows, heath, widespread in Alaska up to 2000m
	Koenigia islandica	7	63	6480	0	0	0	0	0	32	Y	
	Oxyria digyna	7	46	246	0	0	0	0	0	0	Y	
	Persicaria amphibia	9	69	63134	0	0	0	0	0	97	Y	Synonym Polygonum amphibium
	Polygonum	4	8	81660	0	0	0	0	0	36762	Y	
	Rumex	2	13	36941	0	0	0	0	0	0	Y	
Polypodiaceae	Polypodium vulgare	4	7	780	0	0	0	0	0	108	Y	
Polytrichaceae	Polytrichaceae	8	78	429	0	0	0	0	0	0	Y	

Table D.1 cont.

		Sediment samples			Extraction negative controls			PCR negative controls				
Family	Taxon in sedaDNA	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads	Present in Hulten (1968) flora	Comment
Potamogetonaceae	Potamogeton	26	1188	14664	0	0	0	1	2	0	Y	
	Potamogeton praelongus	8	324	40062	0	0	0	2	4	0	Y	Patchy distribution. Not present near the site today but seems like it been recorded in the Tanana valley
	Stuckenia	8	606	2843419	0	0	0	1	2	73472		
	Stuckenia pectinata	5	32	939	0	0	0	0	0	1559		
	Zannichellia palustris	8	136	41	0	0	0	0	0	0	Y	Only a few records but circumpolar in distribution- Hulten suggests more common than is recorded.
Primulaceae	Androsace chamaejasme	3	5	21379	0	0	0	0	0	7206	Y	Present on rocky slopes in the tundra to >1700 m/ Mean range in Alaska in western uplands
Ranunculaceae	Anemone	8	54	1937	0	0	0	0	0	0	Y	
	Anemone patens	8	139	59	0	0	0	0	0	0	Y	Present in central interior and east on bluffs and dry tundra
	Caltha	1	2	21	0	0	0	1	2	0	Y	
	Caltha natans	6	6	21	0	0	0	0	0	0	Y	Present in interior Alaska within moist areas
	Delphinium	5	32	158	0	0	0	0	0	0	Y	Widespread
	Ranunculeae	4	21	393	0	0	0	0	0	0	Y	
	Ranunculus	8	245	47	0	0	0	0	0	0	Y	
	Thalictrum	2	4	0	0	0	0	0	0	0	Y	
Rosaceae	Comarum palustre	8	266	2829	0	0	0	0	0	6	Y	Synonym Potentilla palustris
	Dryas	8	613	291	0	0	0	0	0	0	Y	Several species within this genus present in Alaska
	Fragariinae	8	228	591	0	0	0	0	0	0	Y	
	Geum	2	4	74	0	0	0	0	0	0	Y	
	Maleae	5	18	286	0	0	0	2	4	0		Amelanchier- present on bluff sites
	Potentilla	8	169	208	0	0	0	0	0	5	Y	
	Rosoideae	21	540	133	0	0	0	2	4	0	Y	
	Rubus	8	112	110	0	0	0	0	0	0	Y	

Table D.1 cont.

		Sediment samples			Extraction negative controls			PCR negative controls				
Family	Taxon in sedaDNA	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads	Max PCR replicates	Sum of PCR replicates	Sum of DNA reads	Present in Hulten (1968) flora	Comment
	Rubus arcticus	6	44	84	0	0	0	0	0	0	Y	
	Sibbaldia	3	14	100	0	0	0	0	0	0	Y	Likely species is S. procumbens
	Spiraea	7	30	94	0	0	0	0	0	0	Y	Likely species S. beauverdiana
Rubiaceae	Galium	6	33	297	0	0	0	0	0	0	Y	
	Galium boreale	8	94	135	0	0	0	0	0	0	Y	Grows in open forest, widespread distribution
Salicaceae	Populus	8	187	76	0	0	0	0	0	0	Y	
	Saliceae	8	827	38	0	0	0	13	26	0	Y	
Saxifragaceae	Saxifraga hirculus	1	4	197	0	0	0	0	0	0	Y	
	Saxifraga oppositifolia	11	47	29	0	0	0	2	4	0	Y	
Sphagnaceae	Sphagnum russowii	8	271	12	0	0	0	2	4	0	Y	
Thelypteridaceae	Phegopteris connectilis	3	19	78	0	0	0	0	0	0		Multiple synonyms inc. Dryopteris phegoteris, Polypodium connectile, P. phegoteris
	Thelypteris palustris	2	3	117	0	0	0	0	0	0		Synonym Dryopteris thelypteris and Thelypteris thelypteroides
Timmiaceae	Timmia	2	3	210	0	0	0	0	0	0	Y	
Typhaceae	Sparganium	8	234	232	1	1	0	1	1	37	Y	
Higher taxonomic group	Asterales	3	5	1229	0	0	0	0	0	0	Y	
Higher taxonomic group	campanulids	10	64	332	0	0	0	0	0	15	Y	
Higher taxonomic group	Dicranidae	3	13	4385	0	0	0	0	0	1863	Y	
Higher taxonomic group	Hypnales	8	46	145155	0	0	37717	0	0	61632	Y	
Higher taxonomic group	Mesangiospermae	11	104	8860	3	3	57784	3	3	207	Y	
Higher taxonomic group	Poales	5	12	4350	0	0	0	0	0	7820	Y	

Appendix E Ecological Indicator Values

Here I present preliminary analyses on the *sed*aDNA record from Lake Bolshoye Shchuchye (Chapters 4 and 5) using classifications such as Landolt (1977) ecological indicator values and minimum July temperature requirements of plant taxa identified by *sed*aDNA, where possible. I present four supplemental figures which show preliminary analyses using Landolt (1977) indicator values and one showing a preliminary analysis of the northernmost bioclimatic zone (from Walker *et al*., 2005) of taxa identified by *sed*aDNA. I also present a supplementary table of the compiled data for these analyses.

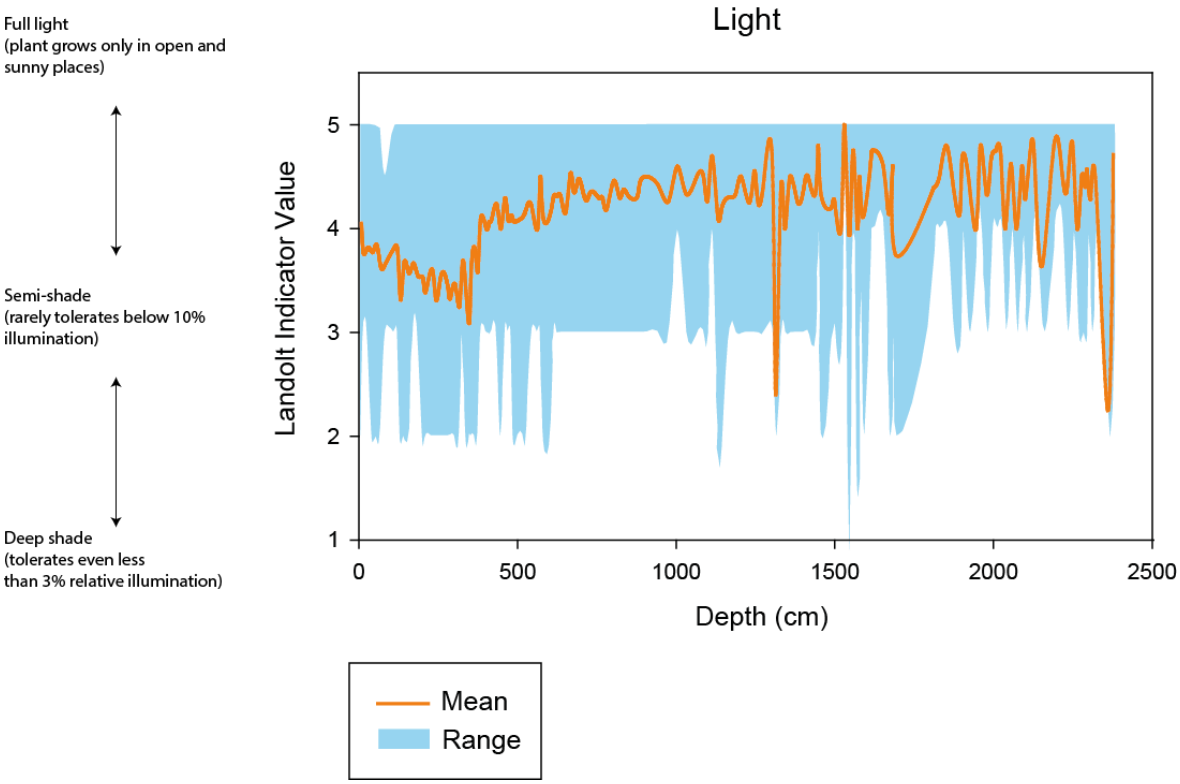

Figure E.1 Mean and range of values for Landolt (1977) light parameter for plant taxa detected by *sed*aDNA per sample at Lake Bolshoye Shchuchye.

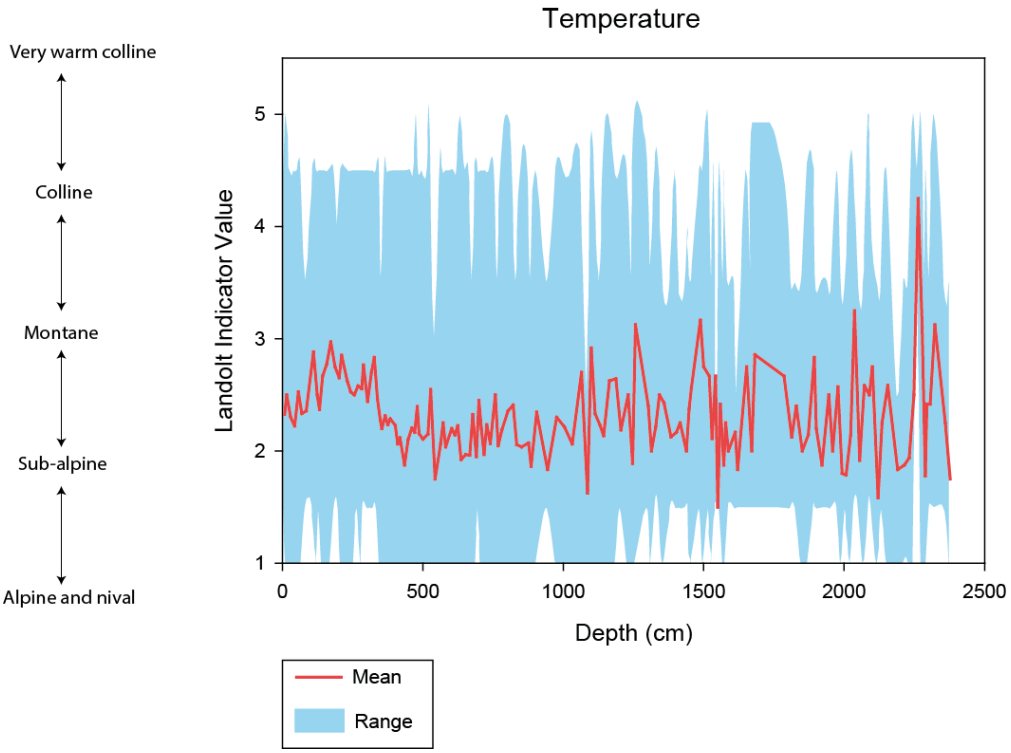

Figure E.2 Mean and range of values for Landolt (1977) temperature parameter for plant taxa detected by *sed*aDNA per sample at Lake Bolshoye Shchuchye.

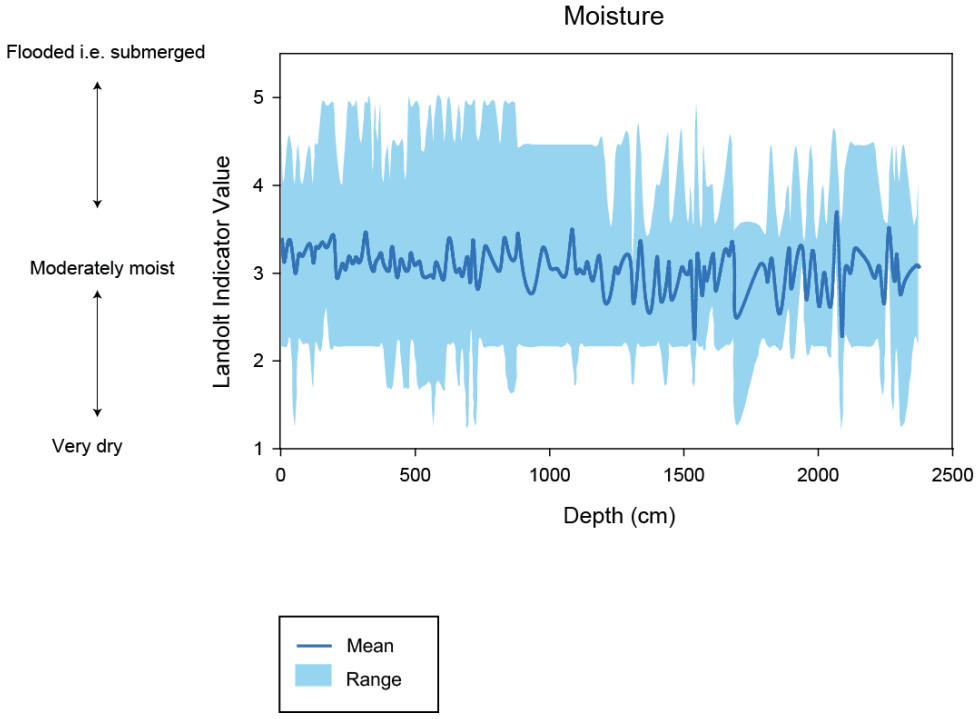

Figure E.3 Mean and range of values for Landolt (1977) moisture parameter for plant taxa detected by *sed*aDNA per sample at Lake Bolshoye Shchuchye.

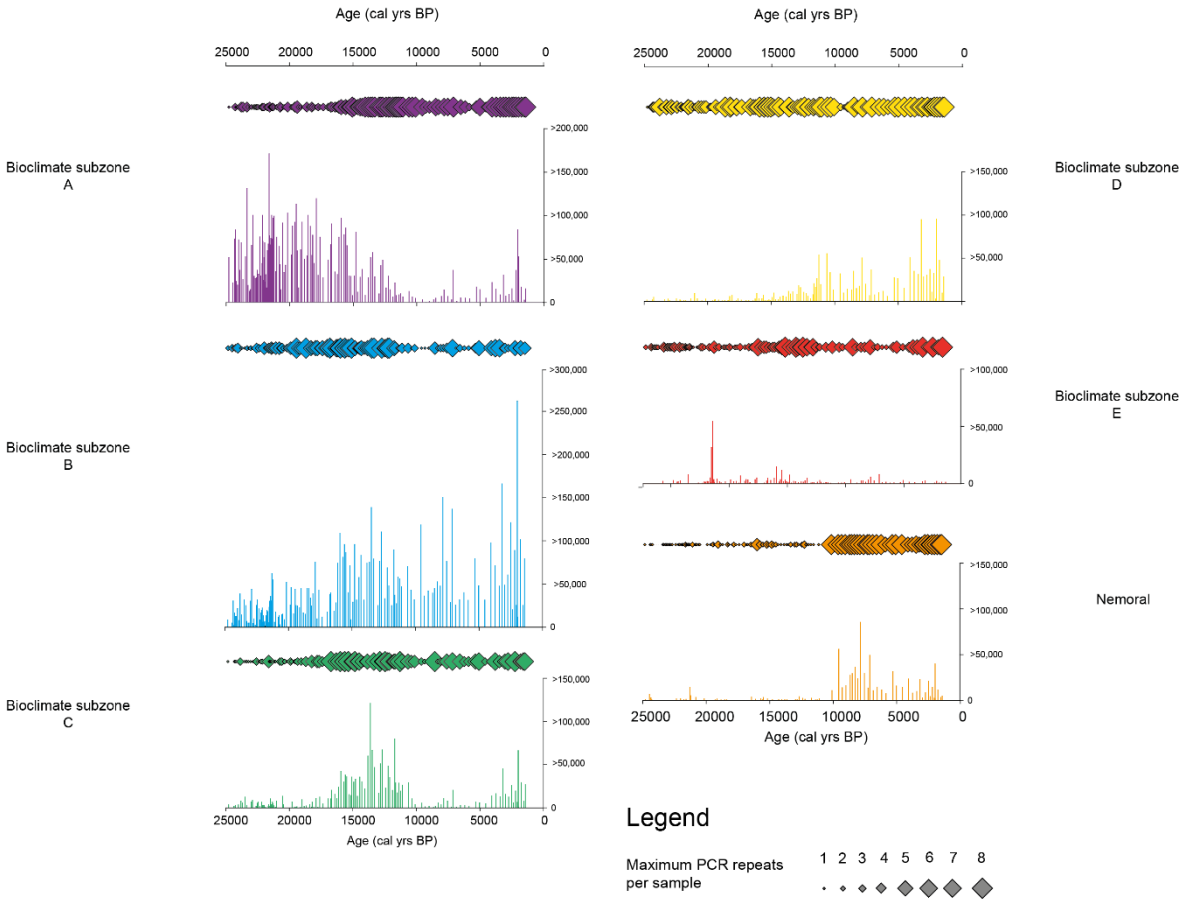

Figure E.4 Total DNA reads (histogram; y-axis) and maximum PCR replicates (diamond symbols) per sample for taxa identified by *sed*aDNA at Lake Bolshoye Shchuchye grouped according to their northernmost bioclimatic zone (defined by Walker *et al.*, 2005). Note that the height of the y-axis varies amongst panels.

Table E.1 All taxa detected by *sed*aDNA at Lake Bolshoye Shchuchye with published or inferred (based on ecology and biogeographic distribution) ecological indicator values and minimum July temperature inferred from biogeographic distribution of taxa with the circumpolar bioclimatic zones A-E (Walker *et al*., 2005).

	Landolt (1977) indicator value										Minimum July Temp based on Arctic bioclimatic zones (Walker *et al*., 2005)					
Taxon identified by sedaDNA	Temp	Continent.	Light	Moisture	Reaction	Nutrients	Growth forms	Leaf duration	Root depth	Comment	Taxon used to infer climatic conditions	Comment	Min. July Temp (°C)	Northernmost zone where frequent	Northernmost zone where present	Zone A-E and abundance (f=frequent, s= scattered, r= rare)
Abies	3	2	1	4	3	3	p	i	5	Published	A. balsamea	Present only in borderline Arctic	>12	E	Ef	E
Aconitum lycoctonum	2.5	2	3	3.5	3	4	h	s	2	Published						
Agrostidinae																
Alchemilla	1.5	3	4	2.5	1	2	c	w		Published	A. glabra, A. glabriformis, A. glomerulans, A. murbeckiana, A. obtusa, A.	Uncertain presence in zone C, one species glomerulans scattered in C. Temp requirements based on zone D as mostly frequent and scattered.	8	E	Ef	Cs
Allium sativum	4.5	4	3	3	4	3	g	t		Published		A. strictum, A. schoenoprasum				
Alnus	2	2	3	4	2	4	n	s	3.5	Inferred	A. incana s. lat	A. viridis Es	11	N	Er	E
Alnus alnobetula (viridis)	2	2	3	4	2	4	n	s	3.5	Published	A. incana s. lat	A. viridis Es	11	N	Er	E
Alopecurinae							h	s								
Amaranthus	4.5	2	4	2	3	4	t	s		Published						
Andreaea	1.5	4	3	2	x	x	h	i	1	Inferred						
Andromeda polifolia	3	2	4	4.5	1	1	z	i	2	Published	A. polifolia			D	Df	D
Androsace chamaejasme	1.5	4	4	2	5	2	h	s		Published	A. chamaejasme		8	D	Df	C
Anthemideae																
Anthriscus sylvestris	2	3	x	3.5	4	4	h	t		Published	A. sylvestris			N	Nf	E
Arabidopsis	3.5	4	4	2	3	3	t	sw	1.5	Published						
Arabis alpina	3	3	4	2.5	5	2	c	i		Published	A. alpina		6	C	Br	B
Arctostaphylos uva-ursi	2	4	3	2	3	2	z	i	3	Published	A. uva-ursi			N	Nf	
Arenaria serpyllifolia	4	x		4	x	4	4	t	ws		Published					

Table A.E. cont.

	Landolt (1977) indicator value										Minimum July Temp based on Arctic bioclimatic zones (Walker *et al*., 2005)					
Taxon identified by sedaDNA	Temp	Continent.	Light	Moisture	Reaction	Nutrients	Growth forms	Leaf duration	Root depth	Comment	Taxon used to infer climatic conditions	Comment	Min. July Temp (°C)	Northernmost zone where frequent	Northernmost zone where present	Zone A-E and abundance (f=frequent, s= scattered, r= rare)
Arnica	2	3	4	3.5	2	4	h	s		Published		A. iljinii in Urals. Not in PAF				
Asteraceae																
Asteroideae																
Astragalus	1.5	4	5	2.5	4	2	h	s		Published		A. frigidus, gorodkovii, igoschinae, norvegicus, uligonosus, subpolaris	8	D	Df	C
Aulacomnium	1.5	5	4	3.5	x	x	h	i	1	Inferred						
Aulacomnium turgidum	1.5	5	4	3.5	x	x	h	i	1	Inferred						
Betula	3	3	4	4	x	2	p	s	2.5	Published	B. nana		8	D	Cr	C
Bistorta	1	4	4	3	3	x	h	s		Inferred	Bistorta vivipara (syn. Polygonum viviparum)		1	A	Af	A
Bistorta vivipara	1	4	4	3	3	x	h	s		Inferred	Bistorta vivipara (syn. Polygonum viviparum)		1	A	Af	A
BOP clade																
Brachytheciaceae																
Braya	1	3	5	2	4	1	h	t?		Published				B	Bs	A
Bromus ciliatus	3	x	3	3.5	x	x	h	s		Inferred	Bromopsis pumpelliana s.lat	Syn. Bromopsis				
Bromus pumpellianus	1.5	4	4	2	x	x	h	s		Inferred						
Bryaceae																
Bryum	1.5	5	4	4	x	x	h	i	1	Inferred			12	N	Nf	C
Cakile (Brassicaceae in NCBI)	2	2	5	2	x	x	h	s		Inferred	C. maritima, C. edentula		12	N	Nf	C
Caltha	3	3	3	5	3	3	h	t	1.5	Published	PAF only has C. palustris	C. palustris, C. arctica	10	E	Ef	C

Table E.1 cont.

	Landolt (1977) indicator value										Minimum July Temp based on Arctic bioclimatic zones (Walker *et al*., 2005)					
Taxon identified by sedaDNA	Temp	Continent.	Light	Moisture	Reaction	Nutrients	Growth forms	Leaf duration	Root depth	Comment	Taxon used to infer climatic conditions	Comment	Min. July Temp (°C)	Northernmost zone where frequent	Northernmost zone where present	Zone A-E and abundance (f=frequent, s= scattered, r= rare)
Cardamine	3	3	2	3.5	3	4	g	w	1	Published	C. amara, bellidifolia, dentata, macrophylla	C. amara, bellidifolia, dentata, macrophylla, nymanii, pratensis		N	Nf	E
Carduinae																
Carex	2	2	4	4	3	2	h	s		Inferred	Carex aquatilis		10	E	Ef	D
Carex aquatilis	3	2	4	4	3	2	h	t	3	Published	Carex aquatilis		10	E	Ef	D
Carex lachenalii	1.5	2	5	4	2	1	h	t		Published	Carex lachenalii		8	D	Df	C
Castilleja	3	x	3	1.5	x	x	h	s		Inferred	C. arctica, hyparctica, lapponica	C. arctica, hyparctica, lapponica	12	N	Ns	D
Cerastium	1.5	x	5	2	x	2	c-h	w		Published	C. davuricum, arvense, regelii	C. arvense, dahuricum, holesteoidea, jenissejense, igoscjomae, pauciflorum, porphyrii, regelii	10	E	Af	A
Chamerion angustifolium	2.5	4	4	3.5	2	x	h	s		Inferred						
Chenopodium capitatum																
Cochlearia	1	4	5	3.5	x	x	h	s		Inferred						
Convallaria majalis	3.5	3	3	2.5	3	2	g	s	2	Published						
Crassula	2.5	x	5	5	x	x	a	?		Inferred	C. aquatica		12	N	Ns	n
Cratoneuron filicinum	2.5	3	4	4.5	2	x	h	i	1	Inferred						
Dicranaceae																
Didymodon icmadophilus	1.5	4	5	1.5	x	x	h	i	1	Inferred						
Distichium	2.5	x	5	3.5	x	x	h	i	1	Inferred						
Distichium inclinatum	2.5	x	5	3.5	x	x	h	i	1	Inferred						

Table E.1 cont.

	Landolt (1977) indicator value										Minimum July Temp based on Arctic bioclimatic zones (Walker *et al*., 2005)					
Taxon identified by sedaDNA	Temp	Continent.	Light	Moisture	Reaction	Nutrients	Growth forms	Leaf duration	Root depth	Comment	Taxon used to infer climatic conditions	Comment	Min. July Temp (°C)	Northernmost zone where frequent	Northernmost zone where present	Zone A-E and abundance (f=frequent, s= scattered, r= rare)
Ditrichum	1	3	4	3	x	x	h	i	1	Inferred						
Douglasia	2	x	5	2	x	x				Inferred	D. ochotensis		8	D	Df	C
Draba	1.5	4	5	2	5	1	c	w		Published	D. pauciflora		1	A	Af	A
Draba pauciflora	1	5	4	4	3	x	h	s		Inferred	D. pauciflora		1	A	Af	A
Dryas octopetala*	1.5	3	5	2.5	5	2	z	i	2	Published	Dryas octopetala		6	C	Br	B
Dryopteris	3	2	2	3.5	3	3	h	s		Published	D. fragrans		6	C	Cf	B
Dryopteris fragrans	2.5	2	3	1.5	x	x		s		Inferred	D. fragrans		6	C	Cf	B
Empetrum nigrum*	x	2	4	3.5	1	2	n-z	i		Published	E. nigrum s. lat.		6	D	Br	B
Ephedra	4.5	5	5	1	5	2	c	i	4	Published						
Eriophorum	3	3	4	4.5	x	2	g	t	3	Published	E. gracile, russeolum, medium, vaginatum, brachyantherum	E. brachyantherum, gracile, medium, russeolum, scheuchzeri, vaginatum,	10	E	Ef	D
Eritrichium	1	3	5	3	2	2	c	s	1	Published	E. villosum, and villosum subsp. pulvinatum	E. villosum, and villosum subsp. pulvinatum		C	Cf	A
Euphrasia	3	4	5	1.5	5	2	thp	s	1	Published	E. frigida	E. frigida, E. wettsteinii	6	D	Df	B
Festuca	1.5	3	5	2	5	2	h	t		Published	Festuca hyperborea	Few Festuca speceis reach this far north	2	B	As	A
Filipendula ulmaria	3	3	3	4	3	4	h	t	2	Published	F. ulmaria		10	E	Dr	D
Galegeae																
Galium	3	4	3	3	4	2		s		Inferred	G. boreale		10	E	Ef	D
Galium boreale	3	4	3	3	4	2	g	s	2.5	Published	G. boreale		10	E	Ef	D
Geum	3	3	3	4	3	4	h	w	1.5	Published						

Table E.1 cont.

	Landolt (1977) indicator value										Minimum July Temp based on Arctic bioclimatic zones (Walker *et al*., 2005)					
Taxon identified by sedaDNA	Temp	Continent.	Light	Moisture	Reaction	Nutrients	Growth forms	Leaf duration	Root depth	Comment	Taxon used to infer climatic conditions	Comment	Min. July Temp (°C)	Northernmost zone where frequent	Northernmost zone where present	Zone A-E and abundance (f=frequent, s= scattered, r= rare)
Gymnocarpium dryopteris	2.5	2	2	3	2	3	g	s		Published	Gymnocarpium dryopteris		10	E	Dr	D
Hedysarum hedysaroides	1.5	3	4	3	4	3	h	s	4	Published						
Hordeum	4.5	2	4	3.5	3	4	thp	s		Published	H. jubatum		12	N	Nf	E
Hypnaceae																
Hypnum procerrimum	2	4	4	1.5	x	x	h	i	1	Inferred						
Juncus biglumis	1	2	5	4.5	3	2	h	t		Published	J. biglumis		1	A	Af	A
Kiaeria glacialis	1	5	4	2.5	x	x	h	i	1	Inferred						
Kobresia	1.5	3	5	4	4	1	h	s	1	Published	Kobresia sihirica, myosuroides m simpliciuscul a	Kobresia sihirica	8	D	Df	B
Lagotis glauca	1	5	5	3.5	x	x	h	s		Inferred	L. glauca		6	C	Cf	B
Lamiaceae																
Larix sibirica	1.5	5	5	2	2	x	p	s		Inferred	L. sibirica		12	N	Es	E
Linnaea borealis	2	4	2	3	2	1	z	i	1	Published						
Linum	5	4	5	1.5	4	2	h	s		Published		Rare in zone E. 7 species in this genus in PAF	10			
Lonicera	2.5	3	4	2.5	x	x	n	s		Inferred	Species present in Urals flora not in PAF	Lonicera altaica, pallasii, xylosteum,				
Luzula parviflora	3	2	2	3	x	x	h	w		Inferred	Luzula parviflora		10	E	Ef	D
Lycopodiaceae																
Lythrum salicaria	4	3	3	4	3	3	h	s	2.5	Published						

Table E.1 cont.

	Landolt (1977) indicator value										Minimum July Temp based on Arctic bioclimatic zones (Walker *et al*., 2005)					
Taxon identified by sedaDNA	Temp	Continent.	Light	Moisture	Reaction	Nutrients	Growth forms	Leaf duration	Root depth	Comment	Taxon used to infer climatic conditions	Comment	Min. July Temp (°C)	Northernmost zone where frequent	Northernmost zone where present	Zone A-E and abundance (f=frequent, s= scattered, r= rare)
Mesoptychia badensis																
Minuartia	2	4	4	2.5	2	3	c	w		Published		M. uralensis, not in PAF				
Mniaceae																
Myosotis	2.5	3	3	2	5	3	t	w		Published	Myosotis alpestris		6	C	Cf	B
Myosotis alpestris	1.5	3	4	3	4	3	h	w		Published	Myosotis alpestris		6	C	Cf	B
Myosotis arvensis	3.5	4	4	2	3	3	k-t	w s		Published	Myosotis arvensis		12	N	Nf	N
Oxygraphis glacialis											Oxygraphis glacialis		6	C	Cs	B
Oxyria digyna	1	2	5	3.5	4	2	h	s	1	Published	Oxyria digyna		1	A	Af	A
Oxytropis	1.5	3	5	1.5	5	2	h	s		Published		O. mertensiana, O. sordida	8	D	Df	C
Pachypleurum alpinum																
PACMAD clade																
Papaver	1	x	4	1.5	x	x	h	s		Inferred	Papaver dahlianum possible according to Alm		1	A	Af	A
Parrya	2	x	4	3.5	x	x	h	s		Inferred		5 species in PAF	4	B	Bf	A
Pedicularis	1.5	3	4	4	4	2	h.hp	s		Published	P. pacifica		10	E	Ef	D
Pedicularis oederi	1.5	4	4	3	5	2	h.hp	s		Published	P. oederi		8	D	Df	C
Pedicularis pacifica (syn. P. sudetica subsp. pacifica)	1.5	3	4	4	4	2	h.hp	s		Inferred	P. pacifica		10	E	Ef	D
Picea													12	N	Es	E

Table E.1 cont.

	Landolt (1977) indicator value										Minimum July Temp based on Arctic bioclimatic zones (Walker *et al*., 2005)					
Taxon identified by sedaDNA	Temp	Continent.	Light	Moisture	Reaction	Nutrients	Growth forms	Leaf duration	Root depth	Comment	Taxon used to infer climatic conditions	Comment	Min. July Temp (°C)	Northernmost zone where frequent	Northernmost zone where present	Zone A-E and abundance (f=frequent, s= scattered, r= rare)
Plantago	2	4	4	3.5	3	3	h	t		Published	P. maritima		8	D	Df	C
Poaceae																
Poeae																
Polemonium	2.5	4	4	3	3	4	h	t		Published	P. acutiflorum, boreale	P. acutiflorum, P. borealis	8	D	Df	C
Polytrichaceae																
Potentilla	3	3	4	3	3	4	h	t		Published	Potentilla pulchella	Most Potentilla frequent in D or E	2	B	As	A
Pottiaceae																
Ptychostomum pallens																
Puccinellia	3.5	4	4	3.5	3	4	h	w		Published	Puccinellia valiana	Most Puccinellia frequent in C, D, E and N	1	A	Af	A
Pyrola 1	2	4	3	3	3	x	h.hp	w		Inferred	P. minor		10	E	Ef	C
Pyrola 2	1	5	4	3	x	x	h.hp	w		Inferred						
Pyrola grandiflora	1.5	x	4	3	4	x	h.hp	w		Inferred	P. graniflora		8	D	Df	C
Pyrola minor	2	3	3	2	2		h.hp	w		Published	P. minor		10	E	Ef	C
Pyrola rotundifolia	2.5	4	2	3.5	4		h.hp	w		Published	P. rotundifolia					
Ranunculus	4.5	1	3	3.5	3	3	h.hp	w		Inferred	Ranunculus pygmaeus		1	A	Af	A
Ranunculus arcticus	2	4	4	2	4	x	h.hp	s		Inferred	Ranunculus arcticus		6	C	Cf	B
Ranunculus pygmaeus	1	1	4	4	2	2	h.hp	w		Published	Ranunculus pygmaeus		1	A	Af	A
Ranunculus sulphureus	1.5	5	4	4	4	x	h.hp	s		Inferred						

Table E.1 cont.

	Landolt (1977) indicator value										Minimum July Temp based on Arctic bioclimatic zones (Walker *et al*., 2005)					
Taxon identified by sedaDNA	Temp	Continent.	Light	Moisture	Reaction	Nutrients	Growth forms	Leaf duration	Root depth	Comment	Taxon used to infer climatic conditions	Comment	Min. July Temp (°C)	Northernmost zone where frequent	Northernmost zone where present	Zone A-E and abundance (f=frequent, s= scattered, r= rare)
Rhodiola rosea	1.5	3	3	3.5	3	2	h.hp	w		Published						
Rhododendron	2	2	3	3	4	2	z	i		Published	R. lapponicum		8	D	Df	C
Rhytidium rugosum	2.5	3	5	1.5	5	x	h	i	1	Inferred						
Ribes	3.5	2	2	3.5	3	4	n	s		Published		R. glabrum, not in PAF				
Rumex	2	2	4	3.5	3	5	h	t		Published	Rumex acetosa		12	N	Es	N
Saliceae	2	x	3	3.5	x	x	n	s		Inferred	S. polaris		3	B	Ar	A
Sanionia uncinata	2	x	3	2	x	x	h	i	1	Inferred						
Saussurea	1.5	3	5	2.5	3	2	h	w		Published	S. angustifolia		8	D	Df	C
Saxifraga	2	2	4	4	3.5	2.5				Inferred	Saxifraga cespitosa		1	A	Af	A
Saxifraga cespitosa	2		4	3.5	4		h	t		Inferred	Saxifraga cespitosa		1	A	Af	A
Saxifraga hirculus	2.5	2	4	4.5	3	3	c	s	1	Published	Saxifraga hirculus		3	B	Bf	A
Saxifraga oppositifolia			5	3.5		2	c	i		Published	Saxifraga_oppositifolia-type	Whic taxa are included in this type?	1	A	Af	A
Saxifragaceae	2	2	4	4	3.5	2.5				Inferred	Saxifraga hirculus		3	B	Bf	A
Scapania	2	x	4	4	2	3	h	i	1	Inferred						
Scapaniaceae	2	x	4	4	2	3	h	i	1	Inferred						
Shepherdia canadensis syn. Hippophae canadensis																
Silene	5	3	4	2	3	3	h	t		Published	Silene acualis		2	B	As	A
Sphagnum russowii	3	3	3	4	2	3	h	i	1	Inferred						

Table E.1 cont.

	Landolt (1977) indicator value										Minimum July Temp based on Arctic bioclimatic zones (Walker *et al*., 2005)					
Taxon identified by sedaDNA	Temp	Continent.	Light	Moisture	Reaction	Nutrients	Growth forms	Leaf duration	Root depth	Comment	Taxon used to infer climatic conditions	Comment	Min. July Temp (°C)	Northernmost zone where frequent	Northernmost zone where present	Zone A-E and abundance (f=frequent, s= scattered, r= rare)
Stellaria	3	3	2	3	2	3		s		Inferred	S.penduncularis	S. penduncularis	6	D	Df	C
Stellaria holostea	4.5	3	2	3	2	3	h	w		Published	S. holostea		>12	N	Ns	E
Syntrichia ruralis	2.5	3	4	1.5	5	2	h	i	1	Inferred						
Thalictroideae																
Thalictrum	4.5	4	3	3	3	3	h	s		Published	Thalictrum alpinum		6	D	Br	B
Thelypteris palustris	3.5	3	3	4.5	3	3	g	s		Published						
Trientalis	2.5	3	3	3.5	2	2	t	s		Published	T. europaea		10	E	Ef	D
Trifolium	5	3	4	1	2	2	h	w		Published	T. lupinaster, repens		12	N	Nf	E
Trollius	2.5	3	4	4	3	3	h	s	1.5	Published	T. apertus, T. europaeus	T. apertus, europaeus	10	E	Ef	D
Ulmus glabra	4	2	2	3.5	3	3	p	s		Published	Ulmus glabra	Northern limit Nordland, Norway	>12	Nemoral	Not PAF	Nemoral
Urtica	2.5	2	2	3.5	3	4	h	s		Published	Urtica dioica		12	N	Nf	E
Vaccinium uliginosum	2.5	3	3	4	1	2	z	s	1	Published	V. uligonosum		6	D	Df	C
Vaccinium vitis-idaea	2.5	3	3	2.5	1	2	z	i	1.5	Published	V. vitis-idaea	V.. capitata, v. wolgensis	6	D	Df	C
Valeriana	1	3	4	2	2	2	h	w		Published	Valeriana capitata		6	D	Df	C
Veratrum	4.5	4	3	3.5	3	4	h	s		Published		V. lobelianum- not in PAF				
Veronica	3.5	3	3	2	4	2	t	w		Published	V. alpina, v.longifolia	V. alpina, v. Longifolia, v. serpyllifolia	6	D	Df	C
Viola	1.5	3	2	3	3	x		h	s	Inferred	V. epipsila		10	E	Ef	D
Viola epipsila	1.5	3	2	3	3	x		h	s	Inferred	V. epipsila		10	E	Ef	D

Appendix F Related Paper: Clitellate worms (Annelida) in Lateglacial and Holocene sedimentary DNA records from the Polar Urals and northern Norway

In this last Appendix, I present an additional published paper, of which I am second author, which tests the use of a universal mammal marker (Giguet-Covex *et al*., 2014) on the same sediment samples from Uhca Rohči' Lake and Lake Bolshoye Shchuchye as the plant *sed*aDNA records presented in this thesis (Chapters 3 to 6) are based on.

BOREAS An international journal of Quaternary research

Clitellate worms (Annelida) in lateglacial and Holocene sedimentary DNA records from the Polar Urals and northern Norway

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BOREAS

Lammers, Y., Clarke, C. L., Erséus, C., Brown, A. G., Edwards, M. E., Gielly, L., Haflidason, H., Mangerud, J., Rota, E., Svendsen, J. I. & Alsos, I. G. 2019 (April): Clitellate worms (Annelida) in lateglacial and Holocene sedimentary DNA records from the Polar Urals and northern Norway. *Boreas*, Vol. 48, pp. 317–329. https://doi.org/10.1111/bor.12363. ISSN 0300-9483.

While there are extensive macro- and microfossil records of a range of plants and animals from the Quaternary, earthworms and their close relatives amongst annelids are not preserved as fossils and therefore the knowledge of their past distributions is limited. This lack of fossils means that clitellate worms (Annelida) are currently underused in palaeoecological research, even though they can provide valuable information about terrestrial and aquatic environmental conditions. Their DNA might be preserved in sediments, which offers an alternative method for detection. Here we analyse lacustrine sediments from lakes in the Polar Urals, Arctic Russia, covering the period 24 000–1300 cal. a BP, and NE Norway, covering 10 700–3300 cal. a BP, using a universal mammal 16S rDNA marker. While mammals were recorded using the marker (reindeer was detected twice in the Polar Urals core at 23 000 and 14 000 cal. a BP, and four times in the Norwegian core at 11 000 cal. a BP and between 3600–3300 cal. a BP), worm extracellular DNA 'bycatch' was rather high. In this paper we present the first reported worm detection from ancient DNA. Our results demonstrate that both aquatic and terrestrial clitellates can be identified in late-Quaternary lacustrine sediments, and the ecological information retrievable from this group warrants further research with a more targeted approach.

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The fact that earthworms (Clitellata: Megadrili) have an important function in cycling nutrients and structuring soils was famously recognized by Darwin (Darwin 1881). Both earthworms as well as potworms (Clitellata: Enchytraeidae) are used as indicator species in various environmental studies of modern soils and aquatic systems as some are very tolerant of pollution while others are very sensitive (Karaca *et al.* 2010). In theory, they have high potential as indicators due to their known sensitivity to soil conditions including temperature, moisture status, soil texture and pH range (Edwards & Lofty 1977; Beylich & Graefe 2009). However, as soft-bodied organisms, worms rarely get preserved in sediments except as trace fossils and earthworm calcite granules (which can be radiocarbon dated; Canti 2003). Their limited preservation means that worms are currently underused in palaeoecology, even though they can provide valuable ecological information.

DNA barcoding has proven to be an important tool for the identification of species through the amplification and sequencing of small, yet informative, parts of the genome (Hebert *et al.* 2003). The barcoding process was revolutionized with the advent of next-generation sequencing, allowing complex samples such as environmental DNA to be barcoded (metabarcoding; Taberlet *et al.* 2012). Since then metabarcoding has been applied to a wide range of organisms, such as nematodes (Porazinska *et al.* 2009), plants (Taberlet *et al.* 2007; Parducci *et al.* 2017; Zimmermann *et al.* 2017), clitellate worms (Bienert *et al.* 2012; Epp *et al.* 2012; Pansu *et al.* 2015), amphibians and bony fishes (Valentini *et al.* 2015), fungi (Buée *et al.* 2009; Epp *et al.* 2012) and a range of other organisms (Thomsen & Willerslev 2015; Domaizon *et al.* 2017).

After being released into the environment by organisms, extracellular DNA degrades over time, but stabilized smaller fragments can persist over longer periods bound to fine-grained sediment particles or due to low temperatures (Pääbo *et al.* 2004; Willerslev *et al.* 2004; Barnes & Turner 2016). Thus, lake sediments in arctic or mountainous regions are prime locations for the recovery of ancient DNA (Parducci *et al.* 2012; Giguet-Covex *et al.* 2014; Pedersen *et al.* 2016). Metabarcoding of sedimentary ancient DNA (*seda*DNA; Haile *et al.* 2009) can provide valuable information about past environments and augment traditional methods such as pollen or macrofossils (Pedersen *et al.* 2013; Parducci *et al.* 2015;

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Zimmermann *et al.* 2017) and is of particular interest for taxa that leave limited traces in the fossil record such as worms (Domaizon *et al.* 2017). Metabarcoding efforts targeting enchytraeid worms in ancient permafrost have been attempted before, but unlike those in modern soils, they yielded no results (Epp *et al.* 2012), suggesting that detection of worms in *sed*aDNA is not as straightforward as for other taxa that have been explored.

In this study, we set out to analyse mammalian DNA from lateglacial and Holocene lake sediments for faunal reconstruction, but because of the low retrieval of mammalian DNA and the unexpected clitellate DNA barcoding 'bycatch', we explore the potential for DNA-based clitellate palaeoecology.

Study sites

Polar Urals

Lake Bolshoye Shchuchye is located in the northernmost Polar Ural Mountains of Arctic Russia (latitude 67°53′24″N, longitude 66°18′53″E, altitude 221 m a.s.l.; Fig. 1). Bolshoye Shchuchye is an elongated lake (12 km long, 1 km wide) located in a NW–SE orientated valley with a maximum water depth of 136 m in its central part (Svendsen *et al.* 2019) and up to 160 m of lacustrine sediments in the central and northern parts (Haflidason *et al.* 2019). The lake is flanked by steep rock faces but the terrain is more open towards its north side, resulting in a total catchment area of 215 km^2 (Svendsen *et al.* 2019). The bedrock consists of Proterozoic-Cambrian basaltic and andesitic rocks in the eastern and northwestern parts of the catchment and Ordovician quartzite and phyllitic rocks in the southwestern catchment (Dushin *et al.* 2009). The Polar Urals remained mostly ice-free during the Last Glacial Maximum (LGM), except for cirque glaciers or minor valley glaciers (Svendsen *et al.* 2004). Current climate conditions are cold and continental with mean summer temperatures of 7 °C (Solomina *et al.* 2010).

Varanger Peninsula

The Uhca Rohči lake (70°19′07″N, 30°01′44″E; Fig. 1) is unnamed on the 1:50 000 Norwegian Topographic Map (Norgeskart; https://www.norgeskart.no), but we use this name as the lake is located close to a river site with the local Sami name 'Uhca Rohči'. Uhca Rohči is a small lake (<1 ha) in a depression situated at 138 m a.s.l. within the river valley of Komagdalen on the Varanger Peninsula, northeast Finnmark, Norway. The Varanger Peninsula is a low-relief plateau (200–600 m a.s.l.) moulded from the Proterozoic paleic surface (pre-Quaternary erosion surface) by marine and glacial processes (Siedlecka & Roberts 1992). Relief is strongly controlled by rock type and structure, with the ridges being formed of Cambrian quartzites and sandstones, whilst valleys are eroded into shales and mudstones. There is evidence that, having undergone uplift during the Pliocene, the area was subject to processes of erosion related to former sea levels, and glacial erosion (Fjellanger & Sørbel 2007). The lake's bedrock is composed of sandstone and mudstone (The Geological survey of Norway; www.ngu.no) and the Komagdalen valley was probably deglaciated by 15.4–14.2 ka BP; the peninsula was certainly free of glacial ice by 13 000–12 000 cal. a BP (Stokes *et al.* 2014; Hughes *et al.* 2016; Stroeven *et al.* 2016). The current climate is low Arctic with a mean summer temperature of 8.7 °C (Norwegian Meteorological Institute; www.met.no).

Material and methods

Polar Urals lake sediment

Lake Bolshoye Shchuchye was cored during several expeditions between 2007 and 2009. The 24-m-long core 506-48 that was sampled for metabarcoding was obtained in July 2009 from the southern part of the lake (67°51′22.2″N, 66°21′30.1″E). Coring was conducted with a UWITEC Piston Corer (http://www.uwitec.at) using 2-m-long by 10-cm-diameter PVC or 2-m-long by 9-cm-diameter steel tubes. The full core was obtained by taking consecutive segments from the same hole. All sections were stored and transported at above 0 °C to avoid freezing of the material. The core was subsampled within the Centre for Geobiology and Microbiology (University of Bergen) in a laminar flow cabinet and in the presence of subsampling controls (open water samples) in order to detect laboratory contamination. Due to deformation near the top of each core segment, the samples form a non-continuous record and a second core was taken parallel to the first core at a 35 cm offset to account for the deformations (Svendsen *et al.* 2019) but was not sampled for this study. Age determination was based on 26 AMS radiocarbon dates from plant macrofossils provided by the Poznań Radiocarbon Laboratory. Dates were calibrated using INTCAL13 (Reimer *et al.* 2013) and the online Calib program (Stuiver *et al.* 2018). A full chronology and sedimentology of this core is described by Svendsen *et al.* (2019).

Varanger Peninsula lake sediment

The Uhca Rohči lake was cored in February 2016 with a modified Nesje piston-corer (Nesje 1992), using a 4-m-long and 10-cm-diameter ABS polymer pipe. A 2.5-m-core was retrieved and cut in the field into 1-m sections, which were sealed to reduce the risk of contaminating the sediments. The core sections were kept at above 0 °C conditions in the field and during transport to avoid freezing of the sediments and were stored in a 4 °C cold room at the Tromsø University Museum (TMU). Sampling of the core took place in a dedicated ancient DNA laboratory. The age of the core was determined based on seven AMS radiocarbon

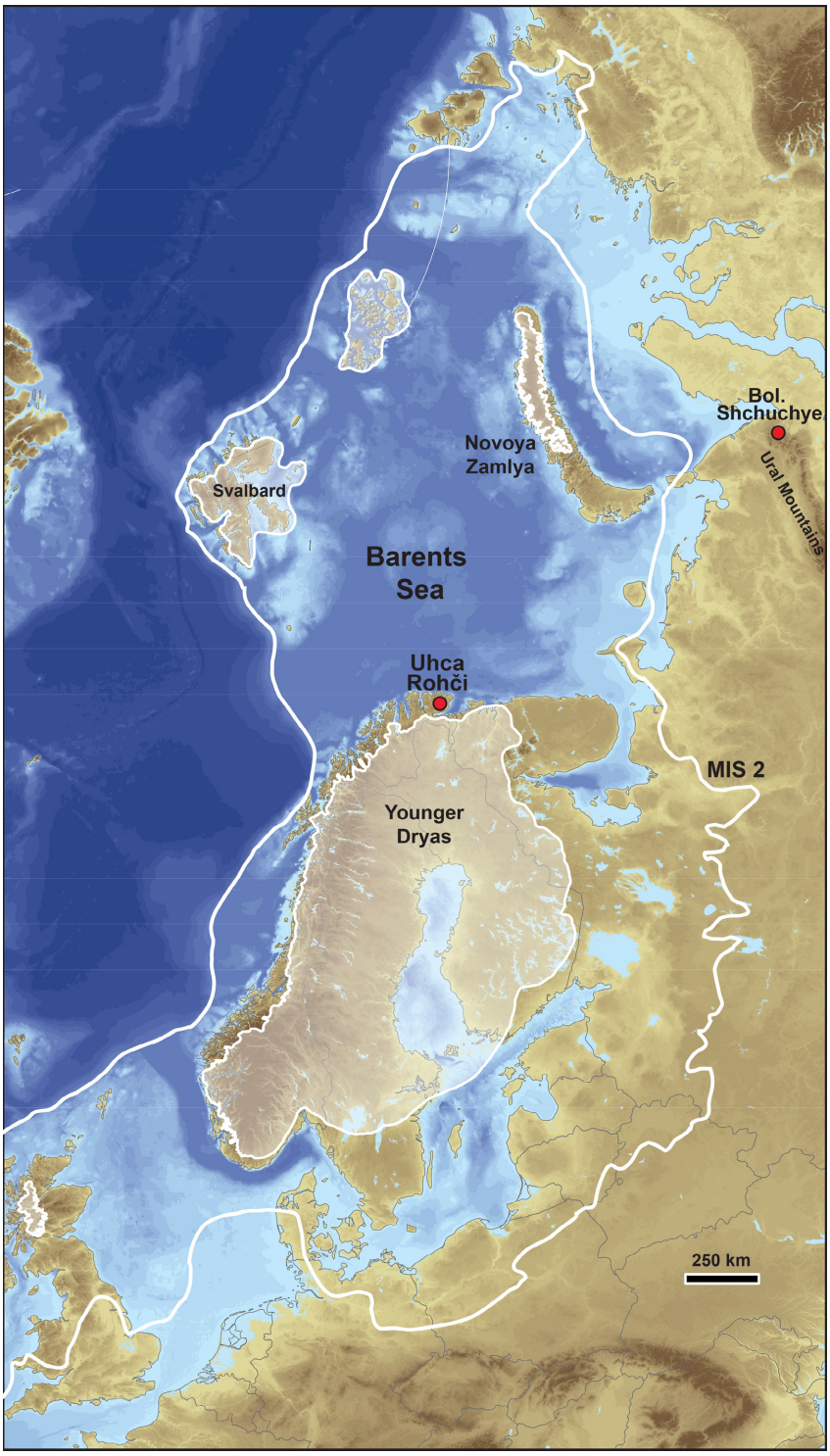

Fig. 1. The location of Lake Bolshoye Shchuchye in the Polar Urals of Arctic Russia and Lake Uhca Rohči on the Varanger Peninsula, northeast Finnmark, Norway. The outer white line represents the extension of the Eurasian ice sheet during Marine Isotope Stage 2 (20 000–15 000 a BP). The inner white shaded area represents the ice sheet during the Younger Dryas (12 800–11 400 a BP). [Colour figure can be viewed at www.boreas.dk]

dates on terrestrial plant macrofossils provided by the Poznań Radiocarbon Laboratory. Dates were calibrated using the terrestrial INTCAL13 curve (Reimer *et al.* 2013), and the age model was constructed using the Bayesian framework calibration software 'Bacon' (v2.2; Blaauw & Christen 2011), which was implemented in R (v3.2.4; R Core Team 2017). A full sedimentology and chronology of this core is described by Clarke *et al.* (2019).

DNA extraction

For the Polar Urals site, 153 lake sediment samples, 17 extraction controls and three subsampling controls underwent DNA extraction. DNA from the Varanger site was extracted from 77 sediment samples and nine extraction controls. All extractions were done at the Tromsø University Museum ancient DNA lab, using PowerMax soil DNA isolation kit (MOBIO Laboratories, Carlsbad, CA, USA), following the manufacturer's protocol with minor modifications by Alsos *et al.* (2016).

PCR amplification and sequencing

PCR reactions were carried out in a dedicated PCR room for ancient DNA at the Laboratoire d'Ecologie Alpine (Université Grenoble Alpes, France), using the MamP007F and MamP007R primers that target a ~70-bp-long part of the mammalian mitochondrial 16S rDNA (Giguet-Covex *et al.* 2014). Both forward and reverse primers had the same unique 8-bp tag on the 5′ end to allow sample multiplexing (Binladen *et al.* 2007; Valentini *et al.* 2009). In addition to the forward and reverse primers, the human blocking primer MamP007_B_Hum1 was added to suppress the amplification of human material (Giguet-Covex *et al.* 2014). The PCR reactions for each lake were carried out at different times to avoid cross-contamination of material. The Polar Urals samples included additional nine PCR negatives (excluding template DNA) and four PCR positives (including the marsupial *Didelphis marsupialis*, not found in Europe). The Varanger samples included six PCR negatives. For each sample, eight PCR repeats were carried out following a previously described PCR protocol (Giguet-Covex *et al.* 2014). PCR products were cleaned and pooled following the methods described by Alsos *et al.* (2016). Libraries (four for the Polar Urals core and two for the Varanger core) were prepared using the PCR free 'MetaFAST' library preparation protocol at Fasteris SA, Switzerland, and sequenced on an Illumina HiSeq 2500 at 2 × 125 bp paired-end sequencing.

DNA sequence analysis

The sequence data were analysed with the OBITOOLS software package (Boyer *et al.* 2016), using default settings unless otherwise specified. Paired-end data were merged with the *illuminapairedend* function and alignments with a score lower than 40 were removed. Data were demultiplexed with *ngsfilter* based on the known PCR tags. Identical sequences were merged with *obiuniq* and singleton sequences and those shorter than 10 bp were removed. Sequences were corrected for PCR and sequencing errors with *obiclean* with a 'head' to 'internal' ratio of 0.05. The remaining sequences were identified by comparing them to the EMBL nucleotide database (r133) with *ecotag*.

The identified sequences were further filtered in R (v3.4.2; R Core Team 2017) with a custom R script. Sequence occurrences that had fewer than 10 reads for a repeat were removed, to account for low-level sequence errors that survived the *obiclean* step and tag switching (Schnell *et al.* 2015). Only sequences that had a 100% match to reference data were kept. Furthermore, sequences had to be present in at least one sediment sample with two or more repeats. If that condition was met, single occurrences for other sediment samples were kept in. Finally, a sequence could only be present in the control samples with at most one repeat; if a sequence was found in a control sample with two or more repeats it was removed from the total data set. Common laboratory contaminants, such as human, *Homo sapiens*, pig, *Sus scrofa*, and chicken, *Gallus gallus* (Leonard *et al.* 2007), were manually removed from the list of sequences that survived filtering.

In silico primer analysis

The *ecoPCR* program (Ficetola *et al.* 2010) was used to calculate the mismatches between clitellate (=oligochaete) worms and the MamP007F – MamP007R primers. The target taxonomic group was set to NCBI TAXID 6381 (referred to as subclass Oligochaeta), the maximum number of mismatches in the primer to five, the amplicon size range to 10–100 bp and the EMBL r133 nucleotide release as database. For each clitellate family and species with available data in the EMBL release, the following were calculated: mean length of the amplicon, mean number of mismatches in each primer and the presence of mismatches in the last three bases of the primer 3′ end, which can hinder amplification (Kwok *et al.* 1990; Wu *et al.* 2009).

The same procedure was repeated for the following families that could be observed in the metabarcoding results: Cervidae (TAXID 9850), Hominidae (TAXID 9604), Phasianidae (TAXID 9005), Suidae (TAXID 9821) and Cercopagididae (TAXID 77756), with the exception that an amplicon size range of 25–150 bp was used to account for the longer expected fragment length.

Results

Polar Urals samples

A total of 80 983 160 raw reads was obtained for the four Polar Urals sequence libraries, which could be assigned to 68 521 unique sequences. Post-identification filtering reduced the number of sequences to 17, representing 1 123 241 reads. The sequences belonged to reindeer (*Rangifer tarandus* – two occurrences in the core at 23 000 and 14 000 cal. a BP, with a total of 27 133 reads) and eight clitellate taxa: two Enchytraeidae (*Enchytraeus norvegicus*, *Henlea perpusilla*), one Glossoscolecidae (*Pontoscolex corethrurus*) and five Lumbricidae (*Aporrectodea rosea*, *Dendrobaena octaedra*, *Bimastos norvegicus*, *Octolasion cyaneum* and *Octolasion tyrtaeum*) (Fig. 2, Table S2). The results also included seven

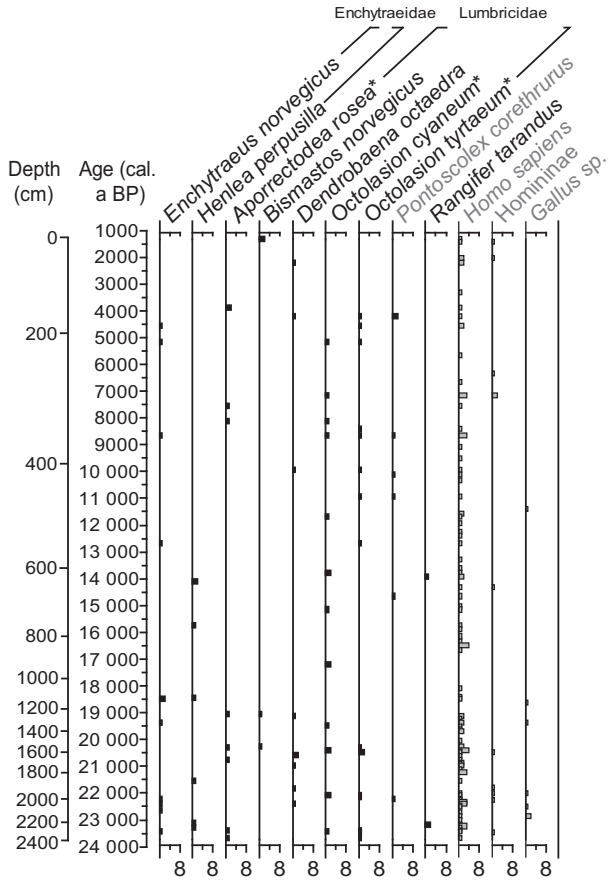

Fig. 2. Metabarcoding results for the Polar Urals core. The width of the bars indicates the number of PCR repeats. The grey taxa were assumed to be laboratory contaminants and were manually removed from the results. Non-cold tolerant taxa or taxa not found in the Polar Urals are indicated with an asterisk. The result for *Homo sapiens* is a combination of seven different *H. sapiens* sequences for which the maximum number of repeats is plotted.

Hominidae sequences (six assigned to *Homo sapiens* and one to Hominidae) and one *Gallus* sequence that survived the filtering criteria and were manually removed.

Species that did not survive filtering include steppe bison (*Bison priscus*, 100% match), Arctic lemming (*Dicrostonyx torquatus,* 98% match), rock ptarmigan (*Lagopus muta*, 99% match) and mountain hare (*Lepus timidus*, 98% match); these species are expected in the region, but none of them occurred in more than one sample and one repeat and thus did not survive our filtering criteria.

Varanger Peninsula samples

We obtained 52 562 858 raw reads for the two Varanger Peninsula (Uhca Rohči) libraries that represented 22 461 unique sequences. After R filtering, 18 sequences remained, representing 877 555 reads, which belong to: *Rangifer tarandus* (four occurrences at 10 800 cal. a BP and three between 3300 and 3600 cal. a BP, with 44 979 reads), the spiny water flea (cercopagidid cladoceran) *Bythotrephes longimanus* (six occurrences at 4900, 5600, 5700, 6300, 6500 and 9100 cal. a BP, sum 38 085 reads) and the oligochaete *Lumbriculus variegatus* (one occurrence at 10 800 cal. a BP with 227 reads) (Fig. 3, Table S3). A total of 12 *Homo sapiens*, one *Sus* and one *Gallus* sequences survived filtering and were manually removed.

Several worm taxa did not survive filtering, including *Dendrobaena octaedra*, *Tubifex tubifex* and a *Limnodrilus* sequence that could not be identified to species level. None of these taxa were detected in multiple repeats for a sample, but they are taxa that can be expected to occur in the Varanger area today.

In silico primer analysis

Primer matches between the mammal primer and annelid sequences could be calculated for 22 clitellate families and 1756 species (mean 175 sequences per family, SD = 317.7) out of the 28 families listed in the NCBI taxonomy database. The weighted average numbers of mismatches in the forward and reverse primer were 2.07 (SD = 0.05) and 2.04 (SD = 0.24), respectively, with an average estimated amplicon length of 35.7 bp (SD = 0.65; excluding primers).

The results for the clitellate families and the species that were detected in the metabarcoding results are displayed in Table 1, along with the mammalian and avian results. A full account of all clitellate families and species is provided in Table S1. The mismatch overview here is limited by the available clitellate data on EMBL, and some mismatch numbers might be over- or underestimated for some families depending on sampling and sequencing biases or depth.

Discussion

Mammal records

Rangifer tarandus was the only mammal in the Polar Urals and Varanger lake sediments that was detected in several PCR replicates (one Polar Urals sample with two repeats, Fig. 2, and three Varanger samples with two, three and four repeats, Fig. 3). *R. tarandus* was detected in a limited number of samples, furthermore, replicability was poor, with at most four out of eight PCR repeats. The limited presence is surprising, as *R. tarandus* has a circumpolar Eurasian distribution. It is known from western Norway at 13 500 cal. a BP from the village Blomvåg 30 km northwest of Bergen (Lie 1986; Mangerud *et al.* 2017) and it would be expected that *R. tarandus* was one of the first species immigrating north and west into Varanger after the ice receded after the LGM. Likewise, it is not surprising that *R. tarandus* was present in the Urals to the northeast of the Eurasian–Fennoscandian ice sheet during the Late Weichselian (24 000–15 000 a BP) as this area was probably its main glacial refugium based on genetic data (Flagstad & Roed 2003; Yannic *et al.* 2014; Kvie *et al.* 2016).

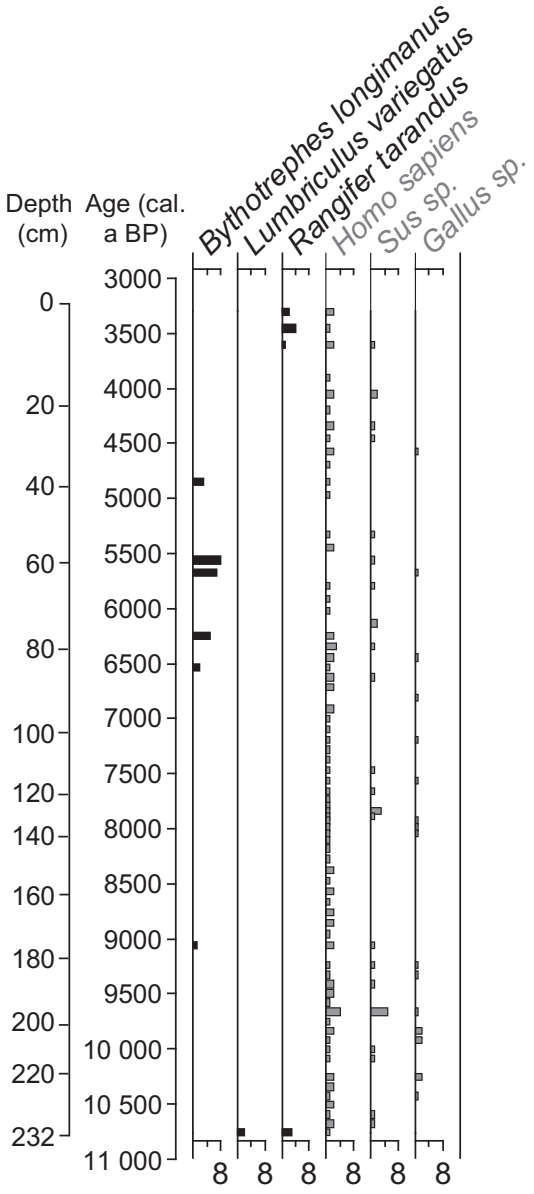

Fig. 3. Metabarcoding results for the Varanger core. The width of the bars indicates the number of PCR repeats. The grey taxa were assumed to be laboratory contaminants and were manually removed from the results. The result for *Homo sapiens* is a combination of 12 different *H. sapiens* sequences for which the maximum number of repeats is plotted.

The other mammals detected, *Bison priscus*, *Dicrostonyx torquatus* and *Lepus timidus*, are all likely for the sites in the period studied but were filtered out because they could only be observed in one sample and with one PCR repeat out of eight. There is always a trade-off between losing assumed true positives and keeping false negatives when setting a cut-off level for filtering (Ficetola *et al.* 2015). Lowering the cut-off level to include these taxa would increase our data set with many records that we suspected to be false positives. While probability statistics may be used to inform the likelihood of a record to represent a true positive, they require an independent record for calibration (Alsos *et al.* 2018). Thus, without records of bones, detection when there are low read numbers and few PCR repeats should be interpreted with caution. Furthermore, even if the filtered taxa were included, the limited occurrences in the records (only a single sample) suggest that the approach used here lacks the capability to reliably detect taxon presence, and thus is not appropriate for palaeoecological reconstructions.

The poor detection of mammals may either be explained by low DNA concentrations in extracts due to lack of template material, potentially caused by the low amounts of mammalian DNA deposited in the lakes, the age of the sediments or the size of the target amplicon. The amount of DNA deposited in the lake might be limited by accessibility for mammals, such as the steep slopes surrounding Lake Bolshoye Shchuchye. Alternatively, the plentiful water sources in the Komagdalen valley on Varanger Peninsula could have resulted in deposition of mammalian DNA over a large region, effectively diluting it in the process. Ancient DNA fragments found in lake sediments are of a relatively small length (Pedersen *et al.* 2015) and it is possible that the longer fragment required for the amplification of mammal material (*R. tarandus* requires a fragment of 111 bp, including primers) is too restricted in older sediments, especially considering the low biomass of mammals compared to other groups such as plants or invertebrates. Metabarcoding studies that successfully targeted ancient mammal DNA either worked with frozen material from localities affected by permafrost (Willerslev *et al.* 2003; Haile *et al.* 2009; Boessenkool *et al.* 2012) where conditions possibly preserved longer fragments (Pääbo *et al.* 2004; Willerslev *et al.* 2004), or with lake sediments from locations that had high mammalian concentrations, either due to migration routes (Pedersen *et al.* 2016), due to a waterhole (Graham *et al.* 2016) or due to human influence (Giguet-Covex *et al.* 2014). Thus, a combination of low mammal DNA concentration and long target fragment length may have caused the poor detection of mammals.

It is unlikely that failed DNA extractions are responsible for the poor mammal results, as the same DNA extracts were used for the metabarcoding of plants with the *g-h* universal plant primers (Taberlet *et al.* 2007) and produced successful results for both the Varanger (Clarke *et al.* 2019) and Polar Urals sites (C. L. Clarke, pers. comm. 2018). The success for plants could be explained by the obvious higher biomass and thus DNA contribution to the sediments and a potentially lower average fragment length required for amplification; for example the plant data from the Varanger site had an average length of 44.3 bp (±15.6) (Clarke *et al.* 2019) compared to the 73 bp of *Rangifer tarandus*.

The limited amount of mammal template material in the sediment extracts may have led to the amplification of laboratory contaminants and off-target species. *Homo sapiens* was by far the most dominant species in the filtered results for both the Polar Urals and Varanger

Table 1. The amplicon lengths and mismatches between the taxa and families that were detected in the metabarcoding results and the MamP007F–MamP007R mammal primers. * = this taxon is in reality a species complex.

Family	Species	Number of sequences	Average amplicon length (bp)	Forward primer		Reverse primer	
				Average mismatches	% 3′-end mismatches	Average mismatches	% 3′-end mismatches
Clitellate							
Acanthodrilidae		71	35.52 (±1.06)	2.06 (±0.29)	0	1.89 (±0.36)	0
Almidae		36	36.94 (±0.74)	2	0	2	0
Enchytraeidae		239	33.61 (±0.97)	2.05 (±0.25)	1.67	1.82 (±0.96)	1.67
	Enchytraeus norvegicus	1	34	2	0	1	0
	Henlea perpusilla	1	34	2	0	2	0
Eudrilidae		6	34.67 (±1.7)	2	0	2.67 (±0.94)	33.33
Glossoscolecidae		16	34.00 (±1.8)	2.38 (±0.48)	0	2.75 (±1.03)	12.5
	*Pontoscolex corethrurus**	7	33.86 (±1.64)	2	0	3.29 (±0.7)	14.29
Hormogastridae		585	35.38 (±1.34)	2.03 (±0.2)	0	1.98 (±0.19)	0.17
Lumbricidae		1037	35.74 (±1.22)	2.07 (±0.26)	2.41	1.97 (±0.2)	0.19
	*Aporrectodea rosea**	143	35.76 (±0.56)	2.23 (±0.42)	0.7	2.01 (±0.08)	0
	*Bimastos norvegicus**	9	37	2.22 (±0.63)	0	2	0
	*Dendrobaena octaedra**	15	35.73 (±0.77)	2	0	2	0
	Octolasion cyaneum	1	36	2	0	2	0
	*Octolasion tyrtaeum**	3	36.66 (±0.94)	2	0	2	0
Lumbriculidae		73	36.55 (±1.15)	2.03 (±0.16)	0	1.67 (±0.52)	0
	*Lumbriculus variegatus**	30	36.87 (±0.34)	2	0	2	0
Megascolecidae		763	35.86 (±1.21)	2.14 (±0.59)	0.66	1.86 (±0.4)	0.26
Moniligastridae		77	35.78 (±1.3)	2.13 (±0.41)	0	1.84 (±0.58)	2.6
Sparganophilidae		21	34.86 (±0.35)	2	0	2	0
Tubificidae		907	36.21 (±1.98)	2.06 (±0.29)	1.87	2.42 (±0.96)	20.84
Mammalia							
Cervidae		302	73.07 (±0.51)	0.03 (±0.27)	0.7	0.03 (±0.21)	0.7
	Rangifer tarandus	9	73	0	0	0	0
Hominidae		39 080	72.13 (±4.61)	0.07 (±0.58)	0.8	0.07 (±0.57)	0.9
	Homo sapiens	38 492	72.1 (±4.26)	0.06 (±0.53)	0.6	0.6 (±0.53)	0.8
Suidae		428	76.93 (±12.95)	0.66 (±1.65)	0.72	0.65 (±1.64)	10
	Sus sp.	400	76.99 (±13.4)	0.7 (±1.7)	0.78	0.69 (±1.68)	10.8
Aves							
Phasianidae		236	76.09 (±10.08)	2.23 (±0.81)	9.75	1.34 (±1.06)	5.5
	Gallus gallus	126	77.59 (±11.23)	2.33 (±0.98)	9.52	1.44 (±1.24)	8.7

samples before human DNA sequences were manually removed (767 186 out of 1 123 241 reads and 706 027 out of 877 555 reads for the Polar Urals and Varanger cores, respectively). Chicken, *Gallus gallus* (both sites), and pig, *Sus scrofa* (Varanger only), made up the remaining contaminants. Chicken could be amplified in both samples due to the limited differences between the mammalian primers used and the binding sites for chicken (Table 1). The amplification of *H. sapiens* was possible even in the presence of a human blocking primer, which is further indication that there was a limited amount of non-human template material available in the DNA extracts (Boessenkool *et al.* 2012).

The problems with the mammal primer presented here support the case for the exploration of alternative primers or methods for the detection of mammals in ancient sediments, especially where template material is probably low. Several metabarcoding primer sets have been suggested for mammals, with the shortest sets amplifying a mitochondrial 16S fragment of 68–71 bp (Rasmussen *et al.* 2009) or 60–84 bp (Giguet-Covex *et al.* 2014), both of which might be too long for reliable amplification of low concentration mammal material in ancient lake sediments. Alternative primer sets might yield better results if they target a shorter fragment or do not amplify common laboratory contaminants by targeting a narrower taxonomic group. Other alternatives are to bypass the usage of primers altogether by either shotgun sequencing sediment extracts (Pedersen *et al.* 2016; Seersholm *et al.* 2016) or by using DNA target capture methods (Slon *et al.* 2017).

Presence of worms

Off-target amplification of earthworms and other clitellates was observed in both the Polar Urals and the Varanger samples. Such amplification can be expected when there is limited target template available in the DNA extracts (Sipos *et al.* 2007; Schloss *et al.* 2011; Brown *et al.* 2015). The *in silico* amplification of clitellates with MamP007F and MamP007R primers revealed that 17 families and 849 species have a low number of mismatches (two or fewer outside the primer 3′ end) and that these

could potentially be amplified if there is limited competing mammal template available.

Metabarcoding potential of the mitochondrial 16S region targeted in this study has previously been demonstrated for earthworms with specific primers (Bienert *et al.* 2012). A comparison between the mammalian primers used in this study and the earthworm primers developed by Bienert *et al.* (2012) is given in Table 2. The forward primers are highly similar, with only a two base pair difference to account for the mismatches between the mammalian and earthworm primer binding sites; the reverse primer is shifted by four bases, but is otherwise comparable, once more indicating that the used mammal primers can amplify worms.

Another factor is the potential amount of DNA present in the sediment for various groups of organisms. Enchytraeidae biomass in Svalbard is estimated to be 1160 kg km^{-2} (Byzova *et al.* 1995) and Lumbricidae biomass in the northern Ural mountains is calculated to be 24 000 kg km^{-2} (Ermakov & Golovanova 2010). Thus, the clitellate numbers are far higher than common herbivorous mammals such as the North American brown lemming (*Lemmus trimucronatus*) at 30 kg km^{-2} in the Canadian Arctic (Fauteux *et al.* 2015) or *R. tarandus* in central Norway at 165 kg km^{-2} (Finstad & Prichard 2000; Vistnes *et al.* 2001). These rough biomass numbers give an indication that worms can produce vastly more DNA than the relatively sparse mammals, meaning that the clitellate DNA has a higher chance of being captured in the sediments. The difference in DNA production and contribution to the sediments, along with the additional problems of mammalian DNA described above, make worms more likely to be detected via metabarcoding.

Additionally, the clitellate amplicon length is considerably shorter than that of the mammalian taxa. The amplicon (excluding primer binding sites) for the mammals detected in the Polar Urals and Varanger cores is 74 bp on average (Table 1) and the average amplicon length for all clitellate families is 35 bp (Table S1). The shorter clitellate amplicon length increases the potential amount of template material in highly fragmented *seda*DNA compared to the longer, and thus rarer mammalian target material. The downside of a shorter amplicon is the potential loss of taxonomic resolution, a problem that is difficult to estimate given the limited reference material available for clitellates.

Four worm species that are reported to be cold tolerant were recorded in the Polar Urals samples, and these could be expected to survive in the region. The enchytraeid *Henlea perpusilla* (six samples, one sample with two repeats) is found throughout Europe and is capable of surviving in the Arctic (Birkemoe *et al.* 2000). *Enchytraeus norvegicus* (10 samples, one sample with two repeats) is also known to have a broad range, extending from sea level in the Mediterranean (Rota *et al.* 2014) to colder temperate zones (Rota 1995) and at high (>1400 m a.s.l.) elevations in southern Norway (C. Erséus, unpublished data). The cosmopolitan lumbricid *Dendrobaena octaedra* (one sample with two repeats) has frost-tolerant populations in Finland, Greenland and Magadan Oblast, eastern Russia (Rasmussen & Holmstrup 2002). *Bimastos norvegicus* (three samples, one sample with two repeats) is part of the taxonomically difficult *Bimastos rubidus* (syn. *Dendrodrilus rubidus*) species complex, which is abundant in Scandinavia and European Russia, and is reported as freeze resistant. However, the known distribution today does not extend to the Ural region (Berman *et al.* 2010).

The remaining three lumbricid earthworms are less likely to be present in the northern Polar Urals, although they all show wide altitudinal ranges at lower latitudes. *Octolasion cyaneum* (12 samples, one sample with two repeats) is native to central and western Europe, and current records extend up to southern Finland and northern Sweden (Terhivuo & Saura 2006). In Norway it can be found to elevations of around 1000 m a.s.l. in the south, and in lowland localities north of the Arctic Circle (C. Erséus, unpublished data), but it is most often associated with human habitats. *Octolasion tyrtaeum* (also referred to as *Octolasion lacteum* (Shekhovtsov *et al.* 2014); 16 samples, one sample with two repeats) is a species complex with two cryptic lineages (Heethoff *et al.* 2004); it occurs in Europe, with populations extending to central Finland (Terhivuo & Saura 2006) and the taiga forests of European Russia (Perel 1979). *Aporrectodea rosea* (eight samples, one sample with two repeats) is also a species complex with a range that extends northwards from the Mediterranean towards central Finland (Terhivuo & Saura 2006) and the Middle Urals (Perel 1979). Tiunov *et al.* (2006) associate its occurrences in the northern part of the European Russian plain with cultivated soil (e.g. vegetable gardens), secondary deciduous forests and river valleys. The species found in the Urals is the one referred to as *A. rosea* L1 in the BOLD database, and this also occurs north of the Arctic Circle in Norway (C. Erséus, unpublished data). Although none of these lumbricids is recorded in the Polar Urals today, it is not unlikely that they were there during the Holocene Hypsithermal or other warmer periods.

Table 2. Comparison of the mammalian MamP007F – MamP007R primers with the ewB – ewC earthworm primers developed by Bienert *et al.* (2012).

	Forward	Reverse
Mammalian	5′–CGAGAAGACCCTATGGAGCT–3′	5′–CCGAGGTCRCCCCAACC–3′
Earthworm	5′–CAAGAAGACCCTATAGAGCTT–3′	5′–GGTCGCCCCAACCGAAT–3′

The glossoscolecid earthworm *Pontoscolex corethrurus* (six samples, one with two repeats) is a species complex with a circum-tropical distribution, native to South and Central America, but introduced in tropical and subtropical regions worldwide (Taheri *et al.* 2018). The family has no relatives in temperate environments and the genetic distance to other clitellate families rules out misidentification due to amplification or sequencing errors. The closest annelid sequence in GenBank belongs to the Asian *Amynthas glabrus* (Megascolecidae) recorded in China (Sun *et al.* 2017) and Japan (Blakemore 2003), at 77% sequence identity and with an edit distance of 8. The closest species in the results presented here is *Octolasion tyrtaeum* (Lumbricidae) at 63% sequence identity and an edit distance of 15. The most likely explanation for the detection of *Pontoscolex corethrurus* is contamination in the laboratory, possibly due to the reagents used.

The expected clitellate diversity in the Polar Urals is high based on previous lake diversity assessments. Baturina *et al.* (2014) recorded 30 aquatic species in the region. Unfortunately, little is known about the terrestrial clitellate diversity in the Polar Urals, making it difficult to assess how much of the diversity is captured in this study and what potential improvements can be made.

Only one annelid sequence was recorded at the Varanger site, representing a species in the *Lumbriculus variegatus* (one sample with two repeats) species complex. This complex has a current cosmopolitan distribution, but the particular lineage found on Varanger is an unidentified, probably undescribed, species; in its short (36 bp) 16S barcode it is 100% identical to the form of *L. variegatus* referred to as clade III by Gustafsson *et al.* (2009). Elsewhere, it has been recorded from Greenland, high-elevation sites (1000–1400 m a.s.l.) on the Scandinavian Peninsula (S. Martinsson & C. Erséus, unpublished data) and a lake at >3000 m a.s.l. in California (Gustafsson *et al.* 2009). This suggests that the complex is at least partially cold-adapted and could occur in northern Norway. In addition to the *Lumbriculus* species, the cercopagidid cladoceran *Bythotrephes longimanus* (six samples, five with two or more repeats) was detected, a species that is native to northern Europe and previously recorded on the Varanger Peninsula (Hessen *et al.* 2011).

Previous metabarcoding efforts of modern sediments on the Varanger Peninsula with enchytraeid specific primers targeting the mitochondrial 12S region resulted in identifications of *Cognettia sphagnetorum* and *Mesenchytraeus armatus* (Epp *et al.* 2012). Neither of these species could be detected in the results presented here. The discrepancy can be explained by the different primers used (the enchytraeid-specific primers can be expected to perform better than mammal primers used in this study), the age of the sediments (modern sediments compared to 3304–10 759 cal. a BP sediments), the type of sediment and how it retains DNA (heath and meadow plots compared to lake sediments) and local variation in clitellate diversity. In-lake sampling of northern Norwegian lakes (C. Erséus & M. Klinth, unpublished data) indicates a high clitellate diversity (20–30 species). Both the previous metabarcoding study and in-lake sampling indicate that the results obtained here are an underestimation of the true diversity.

Although not reported before, after re-analysing the data presented by Giguet-Covex *et al.* (2014) we noted that clitellate sequences were also recovered. However, the eight species that could be identified (*Aporrectodea caligninosa*, *Chamaedrilus sphagnetorum*, *C. glandulosus*, *Dendrodrilus rubidus*, *Eiseniella tetraedra*, *Henlea perpusilla*, *Lumbricus meliboeus* and *Tubifex tubifex*) neither survived the filtering criteria applied by the authors (amplicon length shorter than 50 bp or identified as non-mammalian) nor the criteria used in this study (each taxon was only detected in a single repeat). Their annelid results are probably worse than the results presented in this study, due to the overall higher quality and success rate for mammalian DNA, but confirm that the annelid bycatch in this study is not a fluke.

The overall scattered detections of worm sequences in the Polar Urals and Varanger samples are most likely due to the non 'worm-specific' primers used, hindering, but not completely preventing, the amplification of the material. Furthermore, the detection of the four unexpected earthworm species warrants an explanation. These species might represent true positives, which have not been recorded in the region and represent past distributions during warmer periods. Alternatively, they could be artefacts of limited DNA reference material and might be misidentified to the wrong species or a consequence of amplification or sequencing errors. Finally, the observed worm sequences could be the results of contamination, either in the field or during sampling, extracting and amplification of the DNA. The laboratory standards used along with the negative controls give some confidence that these results are true detections, but contamination cannot be fully ruled out and is a likely explanation for the tropical *Pontoscolex corethrurus*.

Palaeoenvironmental implications of the worm detections

The sediments of Bolshoye Shchuchye (Polar Urals) are low in organic matter (1–5% LOI, see Fig. 4, based on Svendsen *et al.* 2019) and are essentially silt and clay. Given the thermal sensitivity of worms and the long record at this site (0–24 000 cal. a BP), we might expect a temporal pattern in the worm occurrence. At the species level this is not the case with the two Enchytraeidae (*Enchytraeus norvegicus* and *Henlea perpusilla*) occurring in both warm periods, such as the Holocene, and cold periods, including Heinrich Stadial 2 (24 000–22 000 cal. a BP). This is also true for the lumbricid earthworms (*Aporrectodea rosea*, *Bimastos norvegicus*, *Dendrobaena octaedra*, *Octolasion cyaneum* and *O. tyrtaeum*), which occur in the Holocene and the Lateglacial. When aggregated, the DNA shows distinctly greater and more continuous values for the Lumbricidae in the Holocene but no trend in the Enchy-

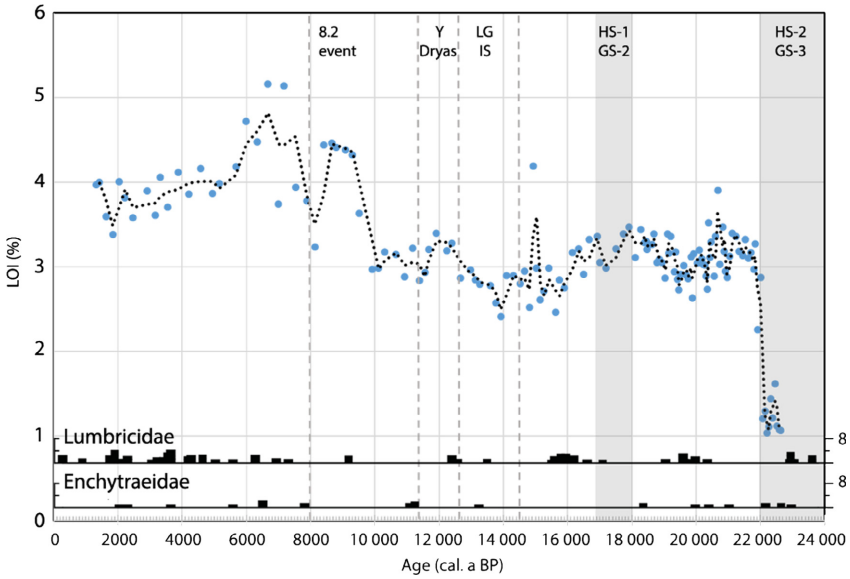

Fig. 4. Aggregated worm data from Lake Bolshoye Shchuchye (Polar Urals) with the core LOI and a two-period moving average (dotted line). Y Dryas is the Younger Dryas and LG IS is the Lateglacial interstadial. HS and GS are the Heinrich Stadial events and the Greenland Stadials from Rasmussen *et al.* (2014), respectively. [Colour figure can be viewed at www.boreas.dk]

traeidae (Fig. 4). These records suggest that both the soils and the lake sediments remained biologically active over the last 24 000 years, and that soils were almost certainly not set to zero biologically during the LGM or the Lateglacial stadials, when cold, dry conditions prevailed and the vegetation was predominantly tundra-steppe (Svendsen *et al.* 2014). However, the results do suggest higher rates of worm activity, and thus more soil formation, during the Holocene than during the Late Weichselian.

Potential for annelids in ancient DNA

Although the results for the worms detected in this study are not optimal, due to mismatched primers, overall poor metabarcoding results, low number of 'bycatch' taxa and perhaps some contamination of the samples, they indicate that earthworms and other clitellates can be identified in ancient sediments up to 24 000 years old. The use of a more optimized primer targeting short barcode regions in annelids, as has been done for the mitochondrial 16S and 12S regions (Bienert *et al.* 2012; Epp *et al.* 2012; Pansu *et al.* 2015), should increase clitellate diversity and detection reliability. Once detection methods have been optimized, tracking clitellate communities through time in ancient sediments may yield valuable information and proxies for various environmental conditions, such as temperature, soil moisture and acidity (Edwards & Lofty 1977; Beylich & Graefe 2009).

Conclusions

The results presented in this study show that the detection of mammalian material in ancient lake sediments in the Sub-Arctic via 16S metabarcoding is possible, but not without problems. The highly fragmented nature of *seda*DNA means that amplification of long fragments of low biomass taxa is problematic and might benefit from alternative identification methods. By contrast, clitellate worms look like a more promising group for metabarcoding in older late-Quaternary sediments. Although a previous attempt to retrieve enchytraeid material from permafrost sediments failed (Epp *et al.* 2012), the combination of suitable primers for targeting short fragments, high biomass (for earthworms in particular) and DNA contribution to the sediments warrants further investigation in the group and the possible effects of age and sediment types on metabarcoding success.

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Data availability

The merged forward and reverse reads for both the Varanger and Polar Urals core, the used primer and tag sequences per sample, R script for the OBITOOLS filtering and the filtered OBITOOLS output are available on Dryad: https://doi.org/10.5061/dryad.g0f4hv0.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at http://www.boreas.dk.

Table S1. Average mismatches between all clitellate families, species and the MamP007F – MamP007R mammal primers.

Table S2. Metabarcoding results for the Polar Urals core, including the repeats, read abundances and sequence information.

Table S3. Metabarcoding results for the Varanger core, including the repeats, read abundances and sequence information.

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