Sedimentary ancient DNA from Lake Skartjørna, Svalbard: Assessing the resilience of arctic flora to Holocene climate change

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Abstract

Reconstructing past vegetation and species diversity from arctic lake sediments can be challenging because of low pollen and plant macrofossil concentrations. Information may be enhanced by metabarcoding of sedimentary ancient DNA (*sed*aDNA). We developed a Holocene record from Lake Skartjørna, Svalbard, using *sed*aDNA, plant macrofossils and sediment properties and compared with published records. All but two genera of vascular plants identified as macrofossils in this or a previous study were identified with *sed*aDNA. Six additional vascular taxa were found, plus two algal and 12 bryophyte taxa by *sed*aDNA analyses, which also detected more species per sample than macrofossil analysis. A shift from *Salix polaris*-dominated vegetation with *Koenigia islandica*, Ranunculaceae spp. and the relatively thermophilic species *Arabis alpina* and *Betula* to *Dryas octopetala*-dominated vegetation ~6600–5500 cal. BP suggests a transition from moist conditions 1–2°C warmer than today to colder/drier conditions.[AQ: 1] This coincides with a decrease in runoff, inferred from core lithology, and an independent record of declining lacustrine productivity. This mid-Holocene change in terrestrial vegetation is broadly coincident with changes in records from marine sediments off the west coast of Svalbard. Over the Holocene *sed*aDNA records little floristic change, and it clearly shows species persisted near the lake during time intervals when they are not detected as macrofossils. The flora has shown resilience in the presence of a changing climate, and, if future warming is limited to 2°C or less, we might expect only minor floristic changes in this region. However, the Holocene record provides no analogues for greater warming.

Keywords

ancient DNA, Arctic, climate change, metabarcoding, plant macrofossils, vegetation reconstruction

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Introduction

Future global warming is expected to be strongest in the Arctic, with summer temperatures likely 2–4°C (or more) higher than today and sea-ice cover drastically reduced (Collins et al., 2013; Xu et al., 2013). Our understanding of likely responses to future environmental change is aided by studies of the past, such as the reconstruction of long-term vegetation dynamics and species diversity patterns in relation to past climate change. While most arctic vegetation reconstructions to date have been based on pollen and macrofossils (e.g. Bennike, 2013; Bigelow, 2013; Kienast, 2013), the potential of a molecular approach has recently been demonstrated (Anderson-Carpenter et al., 2011; Willerslev et al., 2003, 2014). Analysis of sedimentary ancient DNA (*sed*aDNA) augments information on past species composition derived from conventional techniques (Giguet-Covex et al., 2014; Jørgensen et al., 2012; Pansu et al., 2015; Parducci et al., 2012b; Pawlowska et al., 2014). In the Arctic, cold conditions favour good preservation of material, and small floras allow the development of comprehensive molecular reference libraries (Sønstebø et al., 2010), both of which contribute to effective results. However, further exploration of the method is required (Pedersen et al., 2015), particularly in relation to lake sediments, which are important palaeo-archives in the Arctic (e.g. Kaufman et al., 2009; Over-peck et al., 1997).

Pollen analyses of arctic sediments can show compositional changes in herb-dominated tundra vegetation (e.g. Cwynar, 1982; Fredskild, 1973), but palynologists must deal with the low pollen concentrations that result from low pollen productivity (Lamb

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and Edwards, 1988). Indeed, in the High Arctic, pollen concentrations may be too low to provide sufficient material for reliable reconstructions (Birks, 1991; Rozema et al., 2006). Pollen grains are variably resolved taxonomically, particularly in the Arctic, where taxa in several diverse groups are barely distinguishable below family level (e.g. Poaceae, Cyperaceae, Salicaceae). In contrast, macrofossil records can be more floristically informative, as they are often identifiable to genus or species level, and they are also more representative of the local vegetation (Birks, 2003; Birks and Birks, 2000). However, deposition and preservation of identifiable plant remains vary considerably among species and sites.

How vegetation is represented by *sed*aDNA is less well understood. Yoccoz et al. (2012) demonstrated that the amount of DNA (i.e. sequence abundance) in modern soil samples was related to local above-ground biomass. Noisy but significant linear relationships showed an approximate 1:1 relationship for graminoids, whereas woody taxa were under-represented and forbs over-represented in the DNA. These patterns may reflect the absolute amount of DNA in the soil, as determined by litter turnover rate, lignin content and root:shoot ratio (Yoccoz et al., 2012). To date, we have no information as to whether such a relationship holds with DNA in lake sediments.

Several studies based on a range of depositional environments, different floras and varying levels of taxonomic resolution have compared *sed*aDNA and pollen. The conclusions are not readily generalized; results tend to show higher taxonomic resolution in *sed*aDNA but more taxa identified overall in pollen. A generally low floristic overlap between pollen and *sed*aDNA (Jørgensen et al., 2012; Parducci et al., 2012b, 2013; Pedersen et al., 2013) may indicate that *sed*aDNA is of local origin (Haile et al., 2007, 2009; Willerslev et al., 2007), whereas in the localities studied, pollen is probably derived from the regional vegetation. Indeed, comparisons of *sed*aDNA and plant macrofossils show higher taxonomic overlap, which would be expected if both reflect local sources (Jørgensen et al., 2012; Parducci et al., 2012b; Pedersen et al., 2013; Porter et al., 2013), with one exception (Parducci et al., 2015).

The *sed*aDNA technique potentially has several advantages: a standardized and objective mode of identification, high taxonomic resolution and (when techniques are well established) more time-efficient production of results. The detailed floristic information retrieved (as with macrofossils) can contribute to, for example, biodiversity estimates and interpretations of environmental changes using indicator taxa. An effective exploration of the ability of *sed*aDNA to reveal past plant community composition should ideally be based on floras that are well known and should employ careful comparisons across levels of taxonomic resolution. The Svalbard archipelago is an ideal study system to investigate the potential of *sed*aDNA. The majority of the flora is available in a DNA taxonomic reference library (Sønstebø et al., 2010), assuring reliable assignment to taxon. The number of vascular plant species is low (176), and plant distributions, thermal requirements and geological preferences are well known (Alsos et al., 2015; Elvebakk, 1982, 1989). Potential modern vegetation analogues are well described from the archipelago (Elvebakk, 1994, 2005; Klimešová et al., 2012).

Spitsbergen, the largest island in the Svalbard archipelago, was almost completely glaciated at the Last Glacial Maximum (Hormes et al., 2013; Ingólfsson and Landvik, 2013; Landvik et al., 1998). The fjords on western Spitsbergen were deglaciated between *c.* 14,100 and 11,200 cal. BP (e.g. Baeten et al., 2010; Forwick and Vorren, 2009; Hald et al., 2004; Mangerud et al., 1992). While late-glacial vegetation records are lacking from this region (reviewed in Birks et al., 1994), a number of Holocene palaeoecological records (9000 cal. BP and onwards) are available (see Bernardova and Kosnar, 2012). Based on a plant macrofossil record from the same lake that is the subject of this study, Birks (1991) concluded that while vegetation cover has decreased over time, the flora has not changed substantially in the last 8000years, suggesting long-term stability in species composition. This contention provides an interesting target to assess using the *sed*aDNA approach.

Lake Skartjørna was chosen because of the detailed plant macrofossil record mentioned above, which covers most of the Holocene and includes several species outside their present geographical distribution (*Arabis alpina, Salix herbacea, Harrimanella hypnoides*; Birks, 1991). In addition, the depositional environment of this site is well studied (Holmgren et al., 2010; Landvik et al., 1987). The main goals for this study were to (1) compare taxonomic resolution, taxonomic overlap and detection success of the *sed*aDNA and plant macrofossil records; (2) use *sed*aDNA data, plant macrofossil and sediment analyses to infer past environmental change; (3) consider what Holocene vegetation change can tell us about the impact on vegetation of expected future warming in the High Arctic; and (4) explore whether *sed*aDNA increases our understanding of past flora and vegetation when combined with other proxy approaches.

Methods

Study site

Lake Skartjørna (previously ~~named~~ Skardtjørna) is located on the west coast of Spitsbergen (61m.a.s.l., Figure 1). It is dammed by a prominent raised beach ridge that forms the postglacial marine limit at 65m.a.s.l., and it has been cut off from the sea since formation about 13,000 cal. BP (Landvik et al., 1987). An almost circular 7.5m deep basin east of the centre constitutes the deepest part of the lake. The lake area is 0.10km^2 and the total catchment is 1.24km^2 (Holmgren et al., 2010). Air photos from recent decades show the lake level fluctuating by 1–2m (Figure 1). The site was visited for coring from lake ice in March 2013 and then again ~~on 9~~ September 2015 to record the flora and geology of the catchment area. The catchment of the lake is located on the South facing thrustal scarp of the junction between the Southwestern Basement Province dominated by metamorphic rocks (phyllites and quartzite) and the post Caledonian orogeny lithologies that make up the bedrock slopes draining into the lake (Hjelle et al., 1986; Ohta et al., 1991; Dallmann, 2015).**[AQ: 2]** The northern bedrock slopes ~~are on~~ the Neoprotozoic Løvliebreen formation of psammo-pelitic phyllite, while the southern slope ~~is on~~ limestone with magnetite and haematite layers of the Malmberget unit. The north eastern bedrock free-face also revealed a zone of mineralization and enhanced weathering. However, parts of the northern and all of the southern bedrock slopes are obscured by ice-cored and vegetation-free ~~moraini~~c ridges that contain a wider range of lithologies including marbles, shales, siltstones, dolomitic limestone and sandstones predominantly of ~~carboniferous~~ age. These lateral moraines also contain a sand and silt-rich matrix which contributes sediment directly into the lake. It is also pertinent that most of these slopes are steep with free faces above steep debris-cones. There is therefore in this small catchment a direct coupling between slope conditions and sediment delivery into the lake, making it highly sensitive to changes in snowmelt runoff, active layer instability and vegetation cover. Although not observable, it is likely that the upper raised beach ridge that bounds the lake to the west ~~overviews~~ a terminal moraine. The axial drainage of the catchment to the

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lake is from the ~~drainage from~~ Tjørnskaret pass (Figure 1), which has formed a fan delta along the northeast shore of the lake. A small component of clastic sediment is also expected to derive from lakeshore erosion and wind transport.
The site is within the bioclimatic zone B (northern arctic tundra zone, Elvebakk, 2005; Walker et al., 2005). The catchment area is characterized by polygon features and vegetation with

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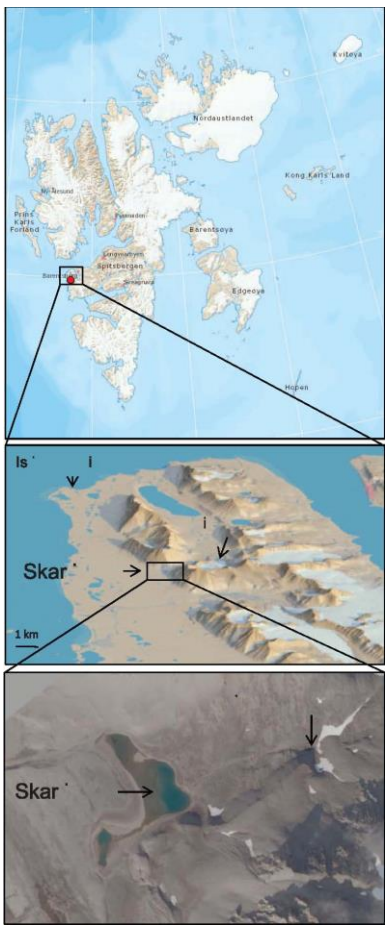

Figure 1. The arctic archipelago Svalbard (with the exception of the island Bjørnøya to the south) and the Lake Skartjørna.

overall only 10% cover, except locally in more stable sites where there is up to 100% vegetation. The vegetation mosaic comprises wet sites dominated by bryophytes, unstable mesic sites dominated by *Saxifraga aizoides* and/or the trailing form of *Saxifraga oppositifolia*, stable mesic sites dominated by *Salix polaris*, *Silene acaulis* and *Bistorta vivipara*, and, locally, dry *Dryas octopetala* heath. Other common species are *Saxifraga cespitosa*, *Oxyria digyna*, *Luzula nivalis*, *Luzula confusa* and *Papaver dahlianum*. There are scattered occurrences of *Puccinellia vahliana*, *Saxifraga svalbardensis*, *Micranthes tenuis*, *Cochlearia groenlandica*, *Sagina nivalis*, *Poa alpina*, *P. pratensis*, *Cerastium arcticum* and *C. regelii*. In the upper part of the catchment area, at Tjørnskaret, patches of 100% bryophyte cover with 10% cover of *Huperzia arctica* occur, indicating slightly warmer growth conditions (Alsos and Brown, 2015, personal observation). The annual precipitation measured at Isfjord Radio, 12 km north of the lake (Figure 1), is 320–470 mm (Førland et al., 2011). Mean July and February temperatures in the period 1961–1990 were 4.8°C and −12.4°C, respectively, with an annual mean of −5.1°C (http://eklima.met.no).

Sediment coring and sub-sampling

Ground-penetrating radar was used to locate the deepest sediment package (77.96167° N, 13.81958°E). A 5.3 m sediment core (7.40–12.70m below ice surface) was retrieved using a Nesje corer (Nesje, 1992) loaded with a 6m long, 10cm diameter PVC tube. The upper 65cm of the core consisted of soft/liquid sediments that had to be discarded in the field. The core was split into three sections to facilitate transport and handling. Core tops were plugged and taped immediately to prevent contamination. When splitting the core, some sediment was pushed out under pressure, and the lowermost 5 cm from the uppermost section was lost (section C, 166cm below sediment surface). In order to account for this, a 5cm hiatus was added (166–171cm) and the applied depths of the core segment were adjusted upwards. All depths are given as centimetres below sediment surface.

The core was kept at 1–10°C during transport and subsequently stored at 4°C at the University of Tromsø (UiT). It was later transported to the Centre for GeoGenetics, Copenhagen University, where it was split in half. From one half, sub-samples of 8g were taken using sterile disposable syringes. The core was returned to Tromsø, where the same core half was sub-sampled for radiocarbon dating, loss-on-ignition (LOI), grain-size analysis and plant macrofossil analysis. The second half was kept intact as a reference core and exclusively used for line-scan imaging and non-destructive x-ray fluorescence core scanning.

Radiocarbon dating and chronology

Twelve samples of plant macrofossils were AMS radiocarbon dated at the Poznan Radiocarbon Laboratory of the Adam Mickiewicz University, Poland (Table 1). Calibration was done using IntCal13 (Reimer et al., 2013), and the age–depth relationship was modelled by Bayesian statistics using the program MacBacon 2.2 with default settings (Blaauw and Christen, 2011). The sedimentation rate was based on the age–depth relationship and rounded off to closest 0.1 mm/yr.

Lithological analyses

Colour line-scan images with a resolution of approximately 70 µm were acquired using a Jai L-107CC 3 CCD RGB Line Scan Camera installed on an Avaatech XRF core scanner. The sediment surface was subsequently covered with 4-µm-thick Ultralene foil, and qualitative element-geochemical analyses were carried out with the Avaatech XRF core scanner. The measurements were carried out at 10 mm steps (each step covered 10 mm down-core and 12 mm cross-core). Instrument settings were 10kV, 1000µA, 10s count time and no filter. Data processing was performed using WinAxil version 4.5.6. The results are presented as ratios of selected elements divided by the most conservative element, Ti, to minimize the influence of water and matrix effects (Tjallingii et al., 2007; Weltje and Tjallingii, 2008). Scanning failed for a part of the core (319–341cm), and re-scanning revealed problems with the calibration; we thus had to exclude this part to avoid risks of bias.

LOI and water content were measured using 10g sub-samples every 4cm, with intervals occasionally adjusted in relation to lithological boundaries. Samples were weighed, dried overnight at

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105°C, weighed again, combusted for 3h at 550°C, allowed to cool in a desiccator and re-weighed. Water content is based on the dry weight and expressed as a percentage of the wet (original) weight. LOI is based on the post-ignition weight and expressed as a percentage of the dry weight (see Heiri et al., 2001).

Table 1. Radiocarbon dates for ~~the~~ Lake Skartjørna sequences shown with their 1σ error, calibrated weighted (W) mean, calibrated median and calibrated 95% confidence age ranges. Radiocarbon ages were calibrated (cal. BP) following IntCal13 (Reimer et al., 2013) and an age–depth model was obtained with the software Bacon (Blaauw and Christen, 2011).

Depth (cm)	Laboratory ID	^{14}C age	Cal. W. mean	Cal. median	Cal. 2σ range	Sample contents
75.0	Poz-58874	1305±30	1238	1235.5	1120–1302	*Salix polaris* leaves, leaf frag.
135.0	Poz-58875	1930±30	1884	1874.0	1781–1988	*Salix polaris* leaves
204.0	Poz-58870	2600±30	2746	2746.0	2613–2863	*Salix polaris* leaves, leaf frag.
259.0	Poz-58871	3580 ±50	3810	3902.0	3629–3970	*Salix polaris* leaves, leaf frag.
309.0	Poz-58872	4025 ±30	4497	4483.0	4400–4624	*Salix polaris* leaves
341.0	Poz-58873	4420 ±50	4946	5045.0	4825–5080	*Salix polaris* leaves, leaf frag.
375.5	Poz-65652	4560 ±40	5337	5194.5	5186–5472	*Salix polaris* leaves
383.0	Poz-58865	4700 ± 160	5424	5342.0	5294–5551	*Salix polaris* leaves
387.0	Poz-65653	4700± 40	5479	5449.5	5350–5595	*Salix polaris* leaves, leaf frag.
434.0	Poz-58866	5650± 40	6421	6443.5	6271–6594	*Salix polaris* leaves
496.0	Poz-58868	7170± 40	7945	7985.5	7736–8098	Moss (*Drepancladus, Cinclidium*)
526.0	Poz-58869	7740± 40	8477	8506.5	8330–8604	*Salix polaris* leaves

Family	Taxa sedaDNA	Taxa macrofossils	Max. repeats	Sum repeats	Sum reads	Birks *n*	Therm.	Zone
Betulaceae	*Betula* (*nana* ssp. *tundrarum*)		2	5	786	5	I	C(r)
Brassicaceae		Brassicaceae undiff.2						
Brassicaceae		*Draba glacialis* ssp. *purpurascens*2				1	IV	A(r)
Brassicaceae	***Arabis alpina***	*Arabis alpina*1,2	2	5	1023	1	I	B(r)
Brassicaceae	*Cardamine* (*bellidifolia*)		2	4	218		V	A(f)
Brassicaceae	*Cochlearia* (*groenlandica*)		2	8	524		V	A(f)
Brassicaceae	***Draba* (13 species)**	*Draba*2	3	47	5,211	2		
Caryophyllaceae		Caryophyllaceae1,2				1		
Caryophyllaceae	***Cerastium* (*arcticum*, *alpinum*, *regelii*)**	*Cerastium arcticum*/ *Cerastium alpinum*2	4	44	2,507	1		
Caryophyllaceae	***Minuartia* (*rubella*)**	*Minuartia rubella*2	1	3	86	1	V	A(f)
Caryophyllaceae	***Sagina* (*nivalis*, *cespitosa*)**	*Sagina nivalis*2	1	3	109	2	V	A(r)
Caryophyllaceae	*Silene* (*uralensis*, *involucrata*)		1	3	112			
Caryophyllaceae	***Silene acaulis* (ssp. *acaulis*)**	*Silene acaulis*1,2	4	47	15,824	6	IV	A(s)
Ericaceae		*Harrimanella hypnoides*2				1	II	C(r)
Juncaceae	***Juncus biglumis***	*Juncus*2	1	3	50	5	V	A(f)
Juncaceae	***Luzula* (*arcuata*, *confusa*, *nivalis*, *wahlenbergii*)**	*Luzula*2	4	77	10,584	11		
Lycopodiaceae	Lycopodiaceae (*Huperzia arctica*)		2	4	84		III	B(s)
Orobanchaceae	***Pedicularis* (*hirsuta*, *dasyantha*)**		4	43	1,759			
Papaveraceae	***Papaver* (*dahlianum*, *cornwallisense*)**	*Papaver*2	4	120	29,857	7		
Poaceae	Agrostidinae (*Calamagrostis neglecta*)		2	4	164		II	C(s)
Poaceae	***Festuca* (*rubra*, *baffinensis*, *hyperborea*, *edlundiae*, *brachyphylla*)**		4	23	2,148			
Poaceae	(*Phippsia algida*, *Phippsia concinna*, *Hierochloe alpina*)		2	5	460			
Poaceae	***Deschampsia* (*alpina*, *sukatschewii*)**		3	9	298			
Poaceae	**Poeae (*Poa alpina* var. *alpina*, *Poa alpina* var. *vivipara*)**		4	38	2107			
Poaceae	***Puccinellia* (7 species)**		3	12	612			
Polygonaceae	***Bistorta vivipara***	*Bistorta vivipara*1,2	4	156	406,904	25	V	A(f)

Approximately 0.5 cm^3 of sediment was sub-sampled for grain-size analysis from the same intervals as for LOI. The analysis was carried with a Beckman Coulter Laser Particle Size Analyser (LS 13320) at the geological laboratory at UiT. The results were analysed using Gradistat v8 (Blott and Pye, 2001), and presented as fractions of clay (<2μm), fine silt (2–8μm), medium silt (8–16μm), coarse silt (16–63μm) and sand (63–500μm). Although small stones (gravel sized particles) were observed occasionally in the sediments, no such particles were recorded, probably because of the small sample size.

DNA extraction and amplification

DNA was extracted from 40 samples and 15 negative controls at the ancient DNA (aDNA) dedicated laboratories at the Centre for GeoGenetics. For whole genome extraction, we used PowerMax Soil DNA Isolation kit (MO BIO Laboratories, Carlsbad, CA, USA), following the manufacturer's instructions, with the exception that the centrifuge steps were done at 4600 r/min, and samples were placed in a Fast-Prep for 2 × 20 s at 4.0 m/s at step 2. At step 4, samples were incubated at 65°C for 30 min while continuously rotated. All samples were finally recovered in 3 mL elution buffer.

All PCRs were performed in an aDNA dedicated room at the Laboratoire d'ECologie Alpine, University Grenoble Alpes, using the *g* and *h* universal plant primers for the short and variable P6 loop region of the chloroplast *trn*L (UAA) intron (Taberlet et al., 2007), and including a unique 8bp long flanking sequence (tag) at the 5′ end to allow parallel sequencing of multiple samples (Binladen et al., 2007; Valentini et al., 2009).

DNA amplifications were carried out in 50μL final volumes containing 3μL of DNA sample; 2U of AmpliTaq Gold® DNA Polymerase (Life Technologies, Carlsbad, CA, USA), 15mM Tris-HCl, 50mM KCl, 2.5mM $MgCl_2$, 0.2mM each dNTP, 0.2μM each primer and 8μg Bovine Serum Albumin. One PCR negative control was carried out. All PCR samples (DNA and controls) were randomly placed on PCR plates. Following the enzyme activation step (10min at 95°C), PCR mixtures underwent 45 cycles of 30s at 95°C, 30s at 50°C and 1min at 72°C, plus a final elongation step (7 min at 72°C). Four individually tagged PCR repeats were made for each sample to increase the chance of detecting taxa represented by low quantities of DNA, as well as to increase confidence in the taxa identified (Ficetola et al., 2015).**[AQ: 3]** Equal volumes of PCR products were mixed (15 μL of each), and 10 aliquots of 100 μL of the resulting mix were then purified using MinElute Purification kit (Qiagen GmbH, Hilden, Germany). Purified products were then pooled together before sequencing; 2×100+7 paired-end sequencing was performed on an Illumina HiSeq 2500 platform using TruSeq SBS Kit v3 (FASTERIS SA, Switzerland).

DNA sequences analysis and filtering

Sequence data were analysed using the OBITools software package (http://metabarcoding.org/obitools/doc/index.html). First, direct and corresponding reverse reads were assembled using *illuminapairedend*, and sequences having a low alignment quality score (threshold set at 40) were filtered out (Supplementary Table S1, available online). The retained reads were assigned to relevant samples using *ngsfilter*, keeping sequences matching 100% with tags and allowing a maximum of three mismatches with primers (Bienert et al., 2012). Strictly identical sequences were then merged together (dereplication) using *obiuniq*, keeping information on their distribution among samples. All sequences with only a single copy and/or shorter than 12 bp were filtered out using *obigrep*. *Obiclean* was then used to identify amplification and sequencing errors, using a threshold ratio of 5% for reclassifying 'internal' sequences to their relative 'head' sequence (Bellemain et al., 2013; De Barba et al., 2014). Finally, using the *ecotag* program (Yoccoz et al., 2012), sequences were compared with a local taxonomic reference library containing 2445 sequences of 815 arctic (Sønstebø et al., 2010) and 835 boreal (Willerslev et al., 2014) vascular taxa as well as 455 bryophytes (Soininen et al., 2015), and assigned to the relevant taxon. Using the local reference library confers the advantage of a more accurate match with species that are found in the local environment. As almost all vas-

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cular plant species in Svalbard and the majority of those found in neighbouring territories, as well as the circum-arctic region, are in the local reference library, we prioritized matches against this database. On the other hand, if taxa were lacking in the local library, there may have been no assignment, or an erroneous one. Therefore, we also made comparisons with a second reference library generated after running *ecopcr* on the global EMBL database (release r117 from October 2013). Sequences assigned to non-native taxa were blasted to check for potential wrong assignments (http://www.ncbi.nlm.nih.gov/blast/).

Extreme caution must be taken before accepting a taxonomic assignment in an environmental sample (Pedersen et al., 2015). Accordingly, to avoid any misidentifications, only sequences matching 100% to reference library entries and occurring as at least 10 reads per PCR repeat were kept. The following were also removed: (1) sequences having higher frequencies in negative controls than in samples, (2) sequences occurring in <3 repeats in total (i.e. across all samples), (3) sequences belonging to food plants and thus suspected to be contaminants, and (4) sequences suspected to be droplet contaminants or overflow from samples from another study run at the same time. One complete sample, which appeared as an outlier in terms of low number of reads and repeats, was excluded (Supplementary Table S1, available online). By applying these thresholds, rare taxa were possibly missed but potential errors were removed. In four cases, two sequences were assigned to the same taxon and combined (*Saxifraga oppositifolia*, *Micranthes*, *Pedicularis* and Dicranaceae). For sequences that matched several taxa or were assigned to genus level only, the likely taxa based on the current native flora of Svalbard were listed as potential species. Taxa identified in more than two of the four PCR repeats per sample were assumed to be certain, whereas the validity of taxa found in fewer repeats was assessed carefully.

Plant macrofossil analysis

Macrofossils were analysed for direct comparison with the *sed*aDNA record as well as for biostratigraphic correlation with the record of Birks (1991). In total, 36 samples were collected contiguously as 4–6cm slices. Sediment volume was determined by water displacement. Sample volumes were approximately 50–60cm^3 (cf. Birks (1991), *c.* 160cm^3). Prior to sieving each sample,

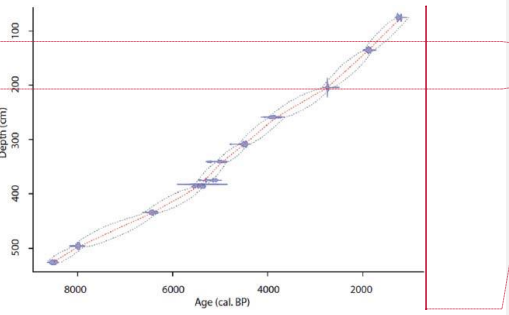
c. 10g sodium pyrophosphate ($Na_4P_2O_7 \times 10H_2O$) was added, mixed and left for at least 1h to disaggregate clay materials. For some samples, this step was repeated. Samples were sieved using 250µm meshes and plant macrofossils were identified using a binocular microscope and based on the reference collection at the herbarium TROM. The analyses were not intended to be exhaustive and no attempt was made to identify bryophytes.

Results

Chronology and lithostratigraphy

All 12 AMS radiocarbon dates returned plausible ages spanning 7740±40 to 1305±30 ^{14}C years, corresponding to a calibrated weighted-mean range of 8477–1238 cal. BP (Table 1). The age– depth model revealed a fairly even sedimentation overall, although periods of lower sedimentation rate occurred around 530–390cm depth (8500–5500 cal. BP) and 260–210cm depth (3800–2800 cal. BP, Figure 2). Five lithostratigraphic units (L1–L5) were identified, based on sediment structure (Supplementary Figure S1, available online), geochemical and lithological compositions and organic content (Figure 3). The elements Si, K and Ca are assumed to characterize relative changes in input of terrigenous sediments derived from the local metamorphic bedrock. Variations in sulphur appear to track changes in organic content, and changes in Fe likely relate to redox variation. The presence of the delta close to the northern part of the basin strongly suggests that the main terrigenous sediment source has been direct input from the northern slopes rather than the drainage from Tjørnskaret pass. Aeolian input is regarded as a minor sediment source, as the catchment area wind speeds are relatively low. Consequently, the changes in minerogenic content can be regarded as a proxy for changes in slope stability and erosion from the catchment through time.

L1 (531–455cm depth, 8600–6900 cal. BP). The unit is dominated by alternating clayey laminae (5–10mm) and silty beds (up to 40mm thick), which are reflected in frequent (reciprocal) fluctuations in percent LOI and the elements Si, K, Fe and S (Figure 3). The thin laminae are characterized by higher LOI (up to 10%), high S and lower Si and K ratios. In the thicker, silty strata, distinct peaks in Si and K, together with lower S and percent LOI, suggest sedimentation dominated by terrigenous minerogenic input. The upper unit boundary is defined by the top



of the uppermost silty

Figure 2. Age–depth model for Lake Skartjørna, Svalbard.The calibrated ^{14}C dates are shown in blue, and the lines show the age–depth curve (darker grey indicate more likely calendar ages, grey stippled line show 95% confidence interval and red line show best model based on weighted average of the mean).

bed and a marked drop in Ca. Unit L1 likely reflects alternation between low-energy hydrologic conditions with minerogenic deposition from suspension (clay), interrupted by phases of enhanced slope input from the catchment.

L2 (455–387cm depth, 6900–5500 cal. ~~BP~~). A transition to more regularly alternating 2–5 mm thick clayey laminae occurs at 455cm. Si and K are lower and their fluctuations are smaller. This suggests more stable sedimentation, mainly from suspension, and less variable influx of minerogenic sediments, probably because of reduced runoff from the catchment. The organic contribution to the sediments as shown by the percent LOI is the highest of any unit (average LOI 8.3% as compared with average 7.3% of all samples below and average 5.6% of all samples above). Also, the S and Fe are high, likely indicating higher organic input and/or a change in redox conditions.

L3 (387–315cm depth, 5500–4600 cal. ~~BP~~). A distinct drop in LOI to ca 5% and abrupt increases in Si, K and sedimentation rate define the base of unit L3. The unit is dominated by 1- to 3-mmthick laminae interrupted by successions of coarser beds up to 50–100 mm thick. There are large fluctuations in Si and K, with peaks also associated with the reappearance of Ca. These variations suggest a reversion to sedimentation driven by relatively high-energy terrigenous inputs.

L4 (315–165cm depth, 4600–2300 cal. ~~BP~~). The unit is dominated by clayey beds. The thicker silty beds characterizing units L1 and L3 are absent. At the base of the unit is a small but distinct increase in LOI to about 6%; Si and K fluctuate less than in L3 and increase gradually from 227cm depth. The more fine-grained character of the sediments, the absence of the thick silty laminae and the increase in the percent LOI indicate sedimentation mainly from suspension, probably with less slope-derived influx to the basin.

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L5 (165–60cm depth, 2300–1100 cal. ~~BP~~). At 165 cm, a transition to more weakly laminated sediment occurs. The lower boundary is also characterized by a distinct temporary drop in Si and K, and the start of synchronous, larger amplitude fluctuations of these elements. The percent LOI is generally low. Periodic highs in Si and K may indicate episodes of higher influx of terrigenous sediments to the lake from the catchment, but they are not evident as major textural changes.

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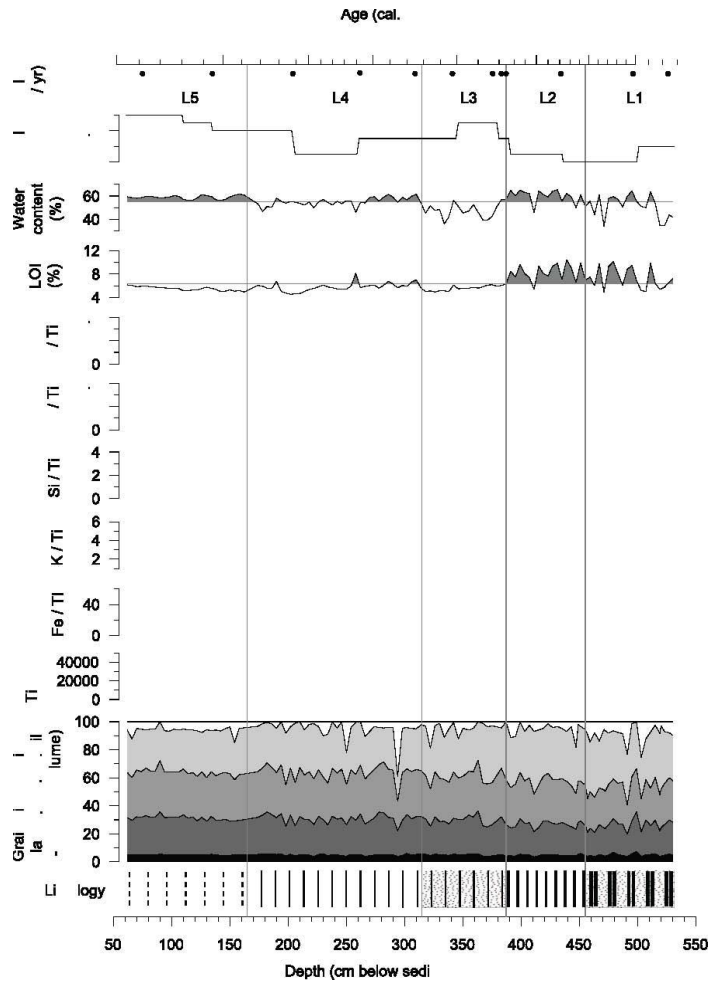

Figure 3. Sediment properties of the core from Lake Skartjørna, Svalbard. Black dots (•) indicate the depth of ^{14}C-samples. Lithostratigraphic units (L1–L5) are marked.Water content is given as percentage wet weight. Loss-on-ignition (LOI) is given as percentage dry weight. Selected elements analysed by XRF are given as ratio to Ti, see methods). Filled areas mark values above the mean. Grain size fractions are given for clay (<2µm), fine silt (2–8µm) medium silt (8–16µ), coarse silt (16–63µm) and sand (63–2000µm) as percentages of total volume. ~~The width of the core has been increased (×3) to enhance visibility~~, for high resolution photo see electronic (Supplementary Figure S1, available online).

sedaDNA

We obtained 13,030,207 reads of 62,568 unique sequences assigned to 40 samples (Supplementary Table S1, available online). After subsequent filtering, 53 taxa (9,197,220 reads) remained, of which 48 (9,191,407 reads) were assumed to be of local origin, whereas five taxa (5813 reads) were exotics (Table 2, S3). **[AQ: 4]** Of the 48 local taxa, 27 taxa were recorded in three or more repeats, and a further five were confirmed by macrofossils (Table 2). The identities of these 32 taxa were assumed certain. A further 16 were found in only one or two out of four PCR repeats in a sample, many likely the same as identified macrofossils, but they are interpreted with caution (Table 2, Figure 4). In total, 34 taxa of 13 families of vascular plant were identified, 32 (94%) determined to genus level and 19 (56%) to species level (when assuming a correct match to local taxa). In addition to the vascular plants for which the primers are designed, we also detected 12 bryophytes and two algal taxa (Table 2). Salicaceae, *Oxyria digyna*, *Bistorta vivipara*, *Micranthes*, *Papaver* and *Saxifraga oppositifolia* were present nearly all PCR

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repeats of all samples (Figure 4). Further taxa present in most samples, but with lower repeats, were the herbs *Pedicularis*, *Silene acaulis*, *Cerastium*, *Draba* and the rush *Luzula*. The majority of taxa identified are temperature-indifferent or weakly thermophilous in Svalbard and common in the northern arctic tundra and polar desert zones (Table 2). Three taxa that are only present in climatically more favourable sites in Svalbard today, *Arabis alpina*, *Betula* and Agrostinae, have scattered occurrences in one to two repeats between 8600 and ~~2900,~~ 1800 ~~and 5100~~ cal. B~~P,~~

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Table 2. All taxa recorded from Lake Skartjørna from *sed*aDNA (this study) and macrofossils (1 is this study, 2 is Birks, 1991).

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Depth (cm)	Laboratory ID	^{14}C age	Cal. W. mean	Cal. median	Cal. 2o range	Sample contents
75.0	Poz-58874	1305±30	1238	1235.5	1120–1302	*Salix polaris* leaves, leaf frag.
135.0	Poz-58875	1930±30	1884	1874.0	1781–1988	*Salix polaris* leaves
204.0	Poz-58870	2600±30	2746	2746.0	2613–2863	*Salix polaris* leaves, leaf frag.
259.0	Poz-58871	3580 ±50	3810	3902.0	3629–3970	*Salix polaris* leaves, leaf frag.
309.0	Poz-58872	4025 ±30	4497	4483.0	4400–4624	*Salix polaris* leaves
341.0	Poz-58873	4420 ±50	4946	5045.0	4825–5080	*Salix polaris* leaves, leaf frag.
375.5	Poz-65652	4560 ±40	5337	5194.5	5186–5472	*Salix polaris* leaves
383.0	Poz-58865	4700 ± 160	5424	5342.0	5294–5551	*Salix polaris* leaves
387.0	Poz-65653	4700± 40	5479	5449.5	5350–5595	*Salix polaris* leaves, leaf frag.
434.0	Poz-58866	5650± 40	6421	6443.5	6271–6594	*Salix polaris* leaves
496.0	Poz-58868	7170± 40	7945	7985.5	7736–8098	Moss (*Drepancladus, Cinclidium*)
526.0	Poz-58869	7740± 40	8477	8506.5	8330–8604	*Salix polaris* leaves

Family	Taxa *sed*aDNA	Taxa macrofossils	Max. repeats	Sum repeats	Sum reads	Birks *n*	Therm.	Zone
Betulaceae	*Betula* (*nana* ssp. *tundrarum*)		2	5	786		**I**	C(r)
Brassicaceae		Brassicaceae undiff.[2]				5		
Brassicaceae		cf. *Braya glabella* ssp. *purpurascens*[2]				1	**IV**	A(r)
Brassicaceae	***Arabis alpina***	*Arabis alpina*[1,2]	2	5	223	1	II	B(r)
Brassicaceae	*Cardamine* (*bellidifolia*)		2	4	218		V	A(f)
Brassicaceae	*Cochlearia* (*groenlandica*)		2	8	524		V	A(f)
Brassicaceae	***Draba* (13 species)**	*Draba*[2]	3	47	5,211	2		
Caryophyllaceae		Caryophyllaceae[1,2]				1		
Caryophyllaceae	***Cerastium* (*arcticum, alpinum, regelii*)**	*Cerastium arcticum*/ *Cerastium alpinum*[2]	4	44	2,507	1		
Caryophyllaceae	***Minuartia* (*rubella*)**	*Minuartia rubella*[2]	1	3	86	1	V	A(f)
Caryophyllaceae	***Sagina* (*nivalis, cespitosa*)**	*Sagina nivalis*[2]	1	3	109	2	V	A(r)
Caryophyllaceae	*Silene* (*uralensis, involucrata*)		1	3	112			
Caryophyllaceae	***Silene acaulis* (ssp. *acaulis*)**	*Silene acaulis*[1,2]	4	47	15,824	6	IV	A(s)
Ericaceae		*Harrimanella hypnoides*[2]				1	**II**	**C(r)**
Juncaceae	***Juncus biglumis***	*Juncus*[2]	1	3	50	5	V	A(f)
Juncaceae	***Luzula* (*arcuata, confusa, nivalis, wahlenbergii*)**	*Luzula*[2]	4	77	10,584	11		
Lycopodiaceae	Lycopodiaceae (*Huperzia arctica*)		2	4	84		III	B(s)
Orobanchaceae	***Pedicularis* (*hirsuta, dasyantha*)**		4	43	1,759			
Papaveraceae	***Papaver* (*dahlianum, cornwallisense*)**	*Papaver*[2]	4	120	29,857	7		
Poaceae	Agrostidinae (*Calamagrostis neglecta*)		2	4	164		**II**	**C(s)**
Poaceae	***Festuca* (*rubra, baffinensis, hyperborea, edlundiae, brachyphylla*)**		4	23	2,148			
Poaceae	(*Phippsia algida, Phippsia concinna, Hierochloë alpina*)		2	5	460			
Poaceae	***Deschampsia* (*alpina, sukatschewii*)**		3	9	298			
Poaceae	**Poeae (*Poa alpina* var. *alpina, Poa alpina* var. *vivipara*)**		4	38	2107			
Poaceae	***Puccinellia* (7 species)**		3	12	612			
Polygonaceae	***Bistorta vivipara***	*Bistorta vivipara*[1,2]	4	156	406,904	25	V	A(f)
Polygonaceae	***Koenigia islandica***		3	6	154		III	B(r)

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Table 2. (Continued)

Family Taxa *sed*aDNA Taxa macrofossils Max. repeats Sum repeats Sum reads Birks *n* Therm. Zone

Bryaceae ***Bryum*** 3 13 430 N/A Bryaceae Bryaceae 2 6 146 N/A Dicranaceae Dicranaceae 2 9 283 N/A Ditrichaceae *Distichium* (*capillaceum, hagenii,* 1 4 90 N/A
inclinatum) Encalyptaceae ***Encalypta alpina*** 3 33 934 N/A Grimmiaceae **Grimmiaceae** 3 13 416 N/A *Arctoa* (*fulvella*, cf. *anderssonii*) 2 9 339N/A

Rhabdowei-
siaceae Seligeriaceae *Blindia acuta* 1 4 132 N/A Sphagnaceae *Sphagnum* 2 9 337 N/A Timmiaceae *Timmia* 2 9 322 N/A

Taxa only identified by one of the proxies are shown in blue (*sed*aDNA) and purple (macrofossils). Green indicates that the same taxa might have been detected with both methods; darker green indicates that the taxa were identified with a higher taxonomic resolution. Taxa identified by *sed*aDNA in minimum three of the four PCR repeats and/or ~~confirmation~~ by macrofossils ~~record~~ are regarded certain identifications and shown in bold. For DNA sequences matching to several species, the Svalbard representatives are given in brackets. 'Max repeats' are the maximum number of PCR repeats within one sample, whereas 'sum repeats' are sum of PCR repeats where the taxa occur across all samples. 'Birks n' is number of samples where the taxa were found out of 36 samples analysed (Birks, 1991). Division of species into thermal groups (I = Strongly thermophilous, II = Distinctly thermophilous, III = Moderately thermophilous, IV = Weakly thermophilous, and V = Temperature indifferent) follows Elvebakk (1989). Nomenclature and northernmost bioclimatic zone (A = Polar desert zone, B = Northern arctic tundra zone, C = Middle arctic tundra zone) where the species occurs as rare (r), scattered
(s) or frequent (f) follows the PanArctic Flora checklist (Elven et al., 2011). Thermal groups and bioclimatic zones are given for the taxa identified to the lowest taxonomic level; bold indicates thermal conditions warmer than present.

Figure 4. Results of *sed*aDNA analyses from Lake Skartjørna, Svalbard. The x-axis refers to number of PCR repeats with 10 or more reads. Only taxa with 100% match to reference database are included. Taxa with either >50% successful PCR repeats in minimum one sample and/or confirmation by macrofossil are regarded as certain taxa (black), whereas taxa found in lower numbers of repeats (white) should be inferred with some caution (see Table 2). Tentative zonation based on the DNA results are indicated (D1–D3). Saxifraga taxa abbreviated as cern. (cernua), riv. (rivularis) and hyp. (hyperborea).

~~respectively~~. Three zones were identified visually based on the flora. Twenty-six of the 34 vascular plant taxa (~~85~~%) and six of DNA data (D1–D3), the two lowermost roughly corresponding to the 12 bryophyte taxa (50%) appear in this lowermost zone. the lithostratigraphic units L1 and L2 (Figure 4).

D2 (441–387 cm depth, 6600–5500 cal. BP). In this zone, *KoeD1 (525–441 cm depth, 8500–6600 cal. BP)*. The zone is char-*nigia islandica* disappears, whereas *Dryas* and *Saxifraga cespi-* acterized by relatively high values of *Festuca* and *Poa alpina*, *tosa* become more frequent. Ranunculaceae and *Festuca* are still along with Ranunculaceae. *Koenigia islandica* and Lycopodia-present. Also, there is a turnover of bryophytes, with four taxa ceae are limited to this zone, which also features the richest grass disappearing and three new ones appearing; *Encalypta alpina* appears regularly from this zone

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onwards. Twenty-three taxa of vascular plants and five taxa of bryophytes are found in this zone. This is a lower taxon count than the other two zones, but also the time span and number of samples is lower.

D3 (387–74cm depth, 5500–1200 cal. BP). At the transition to this zone, *Festuca* and Ranunculaceae disappear (but the latter reappears at 340cm), whereas nearly all species of bryophytes begin to appear regularly, including *Blindia acuta* and *Arctoa*, which are rare in Svalbard today. Overall, this zone has the highest diversity of vascular plants (32 of 34 taxa) and includes all 12 bryophyte taxa. ~~Except for Ranunculaceae, which is restricted to the first part of this zone, t~~here is no clear biostratigraphic pattern.

Plant macrofossils

Three zones (M1–M3) are apparent, but the zone boundaries are not ~~that~~ well demarcated and do not coincide with the lithologic or *sed*aDNA zones ~~(Figure 4)~~. M1 corresponds to L1 and D1, whereas the M2/M3 boundary falls within D3 and L4 (Figure 6).

M1 (529–434cm depth, 8500–6400 cal. BP). The zone has the highest abundance and diversity of plant macrofossils. There is high but variable abundance of *Salix polaris* leaves. Other frequent macrofossils in this zone include *Salix reticulata*, *Saxifraga oppositifolia*, *Saxifraga* undiff. and *Silene acaulis* seeds, as well as *Nostoc pruniforme* gelatinous spheres. Single seeds of *Arabis alpina* and Brassicaceae undiff. were identified from the lowermost two samples (529–521cm depth, 8500–8400 cal. BP). Samples with fewer macrofossils appear to correlate with the coarser sedimentary beds of L1.

M2 (434–291cm depth, 6400–4200 cal. BP). There is a strong drop in the overall concentration of macrofossils, ~~and~~ whereas the ~~diversity~~ is variable. This zone is characterized by a relatively high abundance of *Dryas octopetala* leaves. The abundance of *Salix polaris* leaves decreases; *Salix reticulata* leaves are present in the lowermost part but disappear above 397cm (5700 cal. BP). No *Saxifraga* undiff. seeds occur above this zone. The zone displays a gradual decline in species richness from 12 taxa to four.

M3 (291–63cm depth, 4200–1100 cal. BP). This zone is defined by low abundance and ~~diversity~~ of macrofossils. Only *Salix polaris* leaves and *Saxifraga oppositifolia* seeds occur frequently.

Correlation with other records from Skartjørna

The core lengths and time intervals covered in this study (530 cm; 8600 years) and those of Holmgren et al. (2010; 550 cm; 8200 years) are similar. The most significant sedimentological shift reported by Holmgren et al. (2010), a major decline in organic carbon at 415cm depth (5400 cal. BP), can be correlated with the decline in percent LOI at the L2/L3 boundary at 387 cm (5500 cal. BP) in the present core (Figure 3).

The chronology of the 335cm core analysed by Birks (1991) rests on a single basal radiocarbon date (330–335cm): 8110±115 ^{14}C BP (9000±400 cal. BP 2σ error interval), plus palaeomagnetic correlation. However, an additional unpublished date at 155– 160cm of 2470±80 ^{14}C BP (2550±130 cal. BP) was done by G. Miller 2005 (H. H. Birks and J. Mangerud, personal communica tion) and supports the palaeomagnetic correlation.[AQ: 5] ~~A~~ major decline in organic carbon occurs, but earlier than in the core of Holmgren et al. (2010). Birks' (1991) macrofossil record is also divided into three zones. The lowermost and uppermost zones have a similar composition to our M1 and M3. However, in Birks' record, the high concentration of *Salix polaris* leaves is maintained throughout the middle zone, and the boundary between the middle and upper zones is based on a subsequent decline at *c*. 150cm (2600 cal. BP). The *S. polaris* leaves aside, it is possible that our M2/M3 boundary at 291cm correlates with the change in Birks' (1991) record at 190cm depth, as *Dryas octopetala* leaves occur more frequently below both these boundaries, which date to approximately 4000 cal. BP. Thus, we conclude that the zones of Birks (1991) can be roughly correlated with our zones M1–M3.

Comparison between sedaDNA and macrofossils

All families recorded as macrofossils in either this study or the more extensive study by Birks (1991) were also found in the *sed*aDNA (Table 2). In addition, *sed*aDNA identified Betulaceae (the only local species is *Betula nana* ssp. *tundrarum*), Lycopodiaceae (only local species *Huperzia arctica*), Orobanchaceae (identified to *Pedicularis*), Poaceae (*Poa alpina* identified to species, *Deschampsia*, *Festuca* and *Puccinellia* identified to genera, one sequence identified to *Phippsia* or *Hierochloë*, and Agrostidinae, assigned to its only local representative, *Calamagrostis neglecta*).

At lower taxonomic levels, direct comparison is not always possible. For example, Brassicaceae identified in macrofossils could correspond to any or none of the four Brassicaceae taxa identified with *sed*aDNA (Table 2). However, all taxa found in more than one sample of macrofossils were identified with high certainty in the *sed*aDNA analyses (except *Juncus*). All but two genera identified as macrofossils (cf. *Braya glabella* ssp. *purpurascens* and *Harrimanella hypnoides*) were identified with *sed*aDNA, although some occurred in rather few PCR repeats (Table 2). Other taxa are only detected by *sed*aDNA, for example, *Koenigia islandica*, *Pedicularis* and different species of Poaceae (Figure 4–6, Table 2). Overall, 34 and 28 taxa of vascular plants were identified with *sed*aDNA and macrofossils, respectively. In many cases, the taxa were identified to species level with both methods. Macrofossils were superior in distinguishing species of Salicaceae. The numbers of taxa detected per sample were much higher for *sed*aDNA(mean±SE =15.9±0.42) than for macrofossils (2.37±0.19 and 5.83±0.57 for this study and that of Birks (1991), respectively; Figure 6 and Supplementary Figure S2, available online).

In our record, the pattern of declining macrofossil taxon richness with time follows that of the *sed*aDNA, but with a much lower number of taxa overall (Figure 6). A similar decline is also observed in study of Birks (1991, Supplementary Figure S2, available online). Dominant taxa such as Salicaceae, *Bistorta vivipara* and *Saxifraga oppositifolia* are represented in all samples of both proxies. For most taxa identified with both proxies (*Oxyria digyna*, *Saxifraga aizoides*, *Papaver*, *Arabis alpina*, *Minuartia*, *Sagina*, *Ranunculus pygmaeus*, *Dryas*

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octopetala, *Cerastium* and *Draba*), *sed*aDNA detects them in more samples than do the macrofossils (compare Figure 4 with Figure 5 and Birks, 1991). For example, while macrofossils of *D. octopetala* only were found in nine samples, it was detected in 34 samples of *sed*aDNA (Figure 6).

Discussion

This new, well-dated record and the previous detailed plant macrofossil study of Birks (1991) together provide an excellent opportunity to assess how *sed*aDNA augments and/or modifies interpretations of past flora and climate in high-arctic settings. The plant material contributing to the *sed*aDNA is likely derived from overland flow from melting snow beds or from rain events, erosion of material by small streams or at the lake shore and via wind deposition (possibly from more distant sources; Birks, 1991, ~~2004~~; Glaser, 1981).[AQ: 6] These are essentially the same sources as macrofossils, but *sed*aDNA is possibly not represented in the same proportions. In addition, DNA from soil may be

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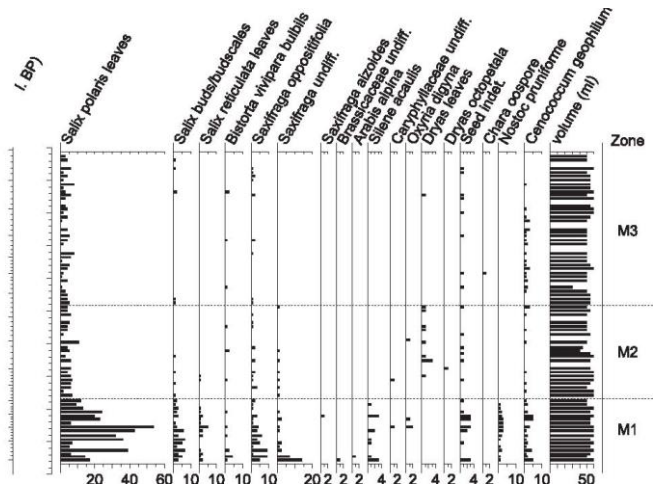

Figure 5. Macrofossils found in Lake Skartjørna, Svalbard.The x-axis refers to number of occurrences in a total volume of 50–60mL except for *Nostoc pruniforme* gelatinous spheres which are presented on a relative scale: (1) few (1–5), (2) moderate (6–20) and (3) abundant (>20). All plant macrofossil are seeds unless otherwise stated.Analysed by P. Sjögren, 2015.Tentative zonation based on the macrofossil results are indicated (M1–M3).

transported as complexes with other particles (England et al., 2004; Taberlet et al., 2012).

The capacity of sedaDNA to provide a record of past flora

The *seda*DNA provides a coherent record that is, in some instances, complementary to that of the plant macrofossils. For example, while *seda*DNA has lower taxonomic resolution than macrofossils for the important family Salicaceae, it reveals taxa that were not present as macrofossils, such as *Huperzia* and several species within the Poaceae, taxa less commonly recorded in macrofossil studies. Previous *seda*DNA studies from the Arctic are characterized by less overlap between *seda*DNA and macro-fossils (10–35%: Jørgensen et al., 2012; Parducci et al., 2012a, 2012b, 2013, 2015; Pedersen et al., 2013; 56%: Porter et al., 2013). Our higher taxonomic recovery from *seda*DNA likely reflects the almost complete reference library available, and possibly also the quantity of sediment used for extraction and the PCR and sequencing conditions. Only 27 of 48 taxa occurred in all four PCR repeats, suggesting that some taxa would be missed in studies that used fewer repeats. This argues for using multiple repeats to detect species with low concentrations of DNA, even though it may increase the occurrence of false positives (Ficetola et al., 2015). However, in our case, single repeats seemed to be reliable as using this threshold increased the number of local taxa by 16 (Table 2) but added only one new exotic taxon (*Convallaria majalis*, Supplementary Table S2, available online). Overall, the large degree of overlap in species composition between *seda*DNA and macrofossils (Table 2) confirms that *seda*DNA may be a reliable approach to reconstructing past vegetation.

All but one of the taxa found as macrofossils in more than one sample were identified with a conservative evaluation of the *seda*DNA results (i.e. three or more PCR repeats). These taxa are widespread in Svalbard today (Alsos et al., 2015), most are typically vegetation dominants and they produce a relatively high biomass (e.g. *Silene acaulis*, *Luzula* spp., *Dryas*, *Salix polaris*, *Saxifraga oppositifolia*; Elvebakk, 1985, 2005), and they are currently common in the catchment vegetation. The high abundance of macrofossils of *Salix polaris* and *Saxifraga oppositifolia*, for example, indicate relative high abundance in the catchment vegetation. DNA of several taxa with a generally more scattered occurrence today (e.g. *Minuartia*, *Juncus biglumis*) or at their thermal limit (*Betula*, *Arabis alpina*) was also identified, although in less than three of four PCR repeats (Table 2). This suggests that the positive correlation between biomass in vegetation and recovery in DNA found in modern soil samples (Yoccoz et al., 2012) may also apply to fossil samples from lake sediments. While Yoccoz et al. (2012) used number of reads as quantification of plant abundance in their modern soil samples, we used number of PCR repeats (0–4), which is a more conservative interpretation more appropriate for aDNA.

Variation in macrofossil abundance can often be interpreted as changes in past plant abundances (Birks, 2003, 2014), but infrequent occurrences of macrofossil taxa likely reflect changes in source area (e.g. fluvial input vs slope input) and a degree of serendipity in detection, with absence particularly hard to interpret. For example, macrofossils of *Dryas octopetala* are rarely recorded in the top zone of both macrofossil records, whereas it is present in most *seda*DNA samples (Figure 6). Here, the molecular approach may be superior, as it exhibits more consistent detection of taxa.

Holocene environmental change at Skartjørna

The set of proxies retrieved from Lake ~~Skjartorna~~ gives insight into past environmental conditions, but many of the signals are subtle,

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indicating moderate environmental changes. The observed changes are discussed here primarily in conjunction with two other records from Lake ~~Skjartorna~~ (Birks, 1991; Holmgren et al. 2010). Sediment properties can be heterogeneous across lake basins for a variety of reasons, and not all the proxy records agree. We assume that the ~~longer cores of~~ the current study ~~as well as~~ the one ~~collected~~ by Holmgren et al. (2010) ~~have~~ higher influx from the erosion of the northern slope, whereas the shorter one collected by Birks (1991) might be closer to the axial inflow (Figure 1).

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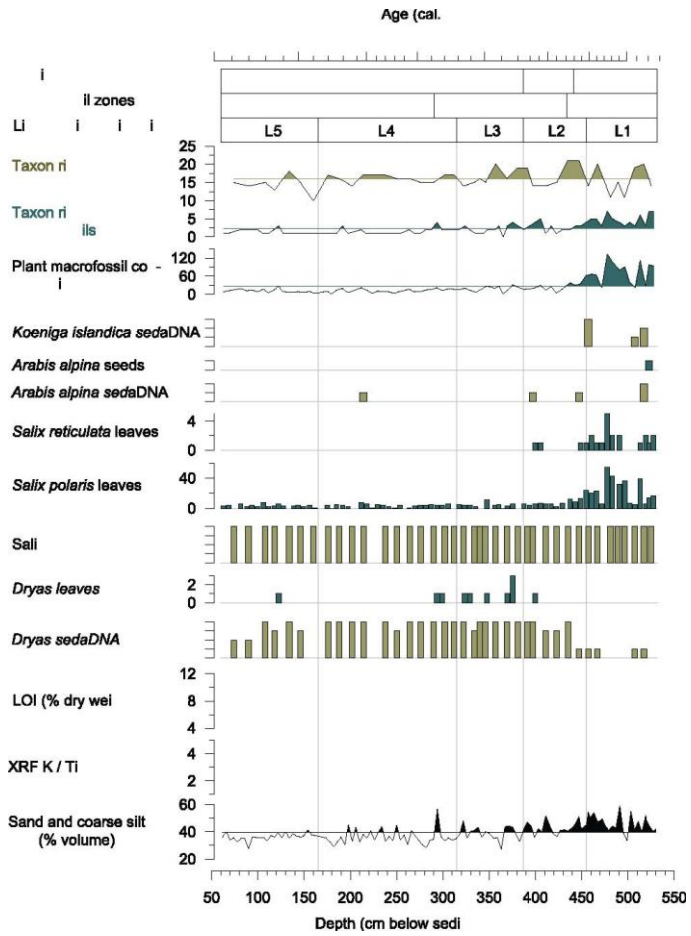

Figure 6. Comparison of zones and selected proxies.Taxon richness *sed*aDNA – number of vascular plant taxa per samples with one or more PCR repeats with 10 or more reads.Taxon richness macrofossils – number of identified taxa per sample as given in Figure 5. Scales of *sed*aDNA results are in number of PCR repeats with 10 or more reads. ~~XRF K/sum is given as the ratio of the 11 most abundant elements.~~ Sand and coarse silt (16–2000µm) is given as percentage volume of all fine fraction. Filled areas mark values above the mean.

However, the main patterns in the datasets suggest a warm early Holocene and a subsequent cooling trend until the present.

Early and mid-Holocene (c. 8600–5400 cal. BP; 560–375cm)

In the early part of our record (8600–7000 cal. BP), the high-frequency variation in %LOI reflects the alternation of bands of silty material and more organic, fine-grained sediment, suggesting an episodic and dynamic runoff regime to the lake, possibly accentuated in the sediment record by the relative proximity of the coring site to the northern slope. The maximum organic content, as expressed by percent LOI, is relatively high for a high-arctic lake (~10%). Values decline ~5500 cal. BP to 4–5%. A similar overall trend in carbon content is recorded by Holmgren et al. (2010): ~3% in the early record dropping to ~1.5% after ~~5200~~ cal. BP. In our record, *Nostoc*, which fixes atmospheric nitrogen, is continually present from 8600–6500 cal. BP. Holmgren et al. (2010) report high diatom concentrations between 8100 and 6600 cal. BP, interpreted as relatively high overall algal productivity. Furthermore, they record low (<10) C:N ratios for this period, which indicate dominance of autochthonous over allochthonous sources. Thus, the most likely explanation for the relatively high levels of organic ~~carbon~~ observed in the early part of the two records is higher biologic productivity driven by warmer growing-season

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temperatures and/or a longer ice-free period. The distinct drop in percentage LOI around 5500 cal. BP co-occur with the emergence of silty beds in the sediment and an increased sedimentation rate, and the %LOI values could have been suppressed by increased minerogenic inflow. High biological productivity as perceived by the %LOI record could thus have continued for some time, and/or the decline been more subtle. The overall higher concentration and diversity of macrofossils found in our and Birks (1991) records, the higher diversity of plants found in the *sed*aDNA, ~~as well as~~ the occurrence of relatively thermophilous species in all three records also indicate that a more lush terrestrial flora was present.

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High levels of plant macrofossil delivery to the lake occurred between 8500 and ~7000 cal. BP, declining by 6500 cal. BP to lower values~~, which~~ persist through the remaining record. For much of this time (until ~7000 cal. BP), strong banding of the sediments suggests multiple high runoff events, which could have entrained plant material. The runoff events may have been intense but short-lived with much of the influx of plant material occurring gradually between events, suggesting relatively high ~~*Salix*~~ cover. Birks (1991) also reports relatively high concentrations early in the record, but they persist to ~2500 cal. BP. The difference in the records may be largely because of proximity to the axial influx versus erosion of the northern slope. Given the difference between the records and the possibility of local sedimentary variations, we recommend caution in interpreting environmental change based on this change (or lack of change) in macrofossil concentrations.

The DNA record includes ~~two~~ taxa indicative of relative warmth (*Arabis alpina* and *Betula nana*), which first occur early (8500–6500 cal. BP) and reappear sporadically through the record until ~1500 cal. BP. *Arabis alpina* was also found as macrofossil both in our and Birks record. In addition, Birks (1991) recorded several other thermophilous species: *Harrimanella hypnoides* (synonym *Cassiope hypnoides*, early Holocene only, ~9000–8000 cal. BP), *Salix herbacea* and *Salix glauca* (sporadic to ~4000 and ~2500 cal. BP, respectively). All the above-mentioned taxa (except *Salix* cf. *glauca* which is extinct) are all classified as strongly or distinctly thermophilous in Svalbard today and have a northern limit in the Middle arctic tundra zone (Table 2); the records at Skjartørna lie outside their modern range limits (as much as 30km outside for *Arabis alpina* (nearest site Diabasbukta) and *Betula* (presumably *Betula nana*, nearest site Colesdalen; Alsos et al., 2015). All the above species also require consistent winter snow cover. Thus, the combined vegetation records, and our lithologic record, are consistent with enhanced summer warmth and relatively high levels of precipitation. Mean July temperatures may have corresponded to Middle arctic tundra zone values (minimum 6°C, Elvebakk, 2005; Walker et al., 2005), that is, 1–2°C warmer than today. Other records show increased local pollen production approximately 8000–5200 cal. BP (Hyvärinen, 1968, 1969, 1970), and peat formation ~~in western~~ Spitsbergen ~~island~~ during the period 8800–4200 cal. BP also suggests a warmer climate (Göttlich and Hornburg, 1982).

The *sed*aDNA record after ~6400 cal. BP contains taxa tolerant of dry conditions (e.g. *Dryas*, *Andreaea*, *Encalypta alpina*). The increase in *Dryas* is also clearly seen in the macrofossil record. At about the same time, the lithostratigraphic record suggests reduced and/or less variable runoff. Overall macrofossil input to the lake dropped dramatically, also ~6400 cal. BP (Figure 6). The changes in terrestrial vegetation composition, macrofossil abundance and lithology likely reflect a change in precipitation, such as an overall reduction in winter snow cover that favoured the development of *Dryas* heaths on open slope and tops.

Mid- and late Holocene (5400–c. 1000 cal. BP~~, c.~~ 375–60cm)

The sediment record (particularly lithology, K, Si and Ca) suggest that the magnitude and variability of runoff increased between 5600 and 4600 cal. BP. More stable sedimentation characterized the period 4600–2300 cal. BP and then was followed by stronger pulses of minerogenic input between 2300 and 1100 cal. BP. After 5400 cal. BP, macrofossil plant species richness per sample is generally low (Figure 6, Supplementary Figure S2, available online). However, the *sed*aDNA data show that all taxa except three vascular plants and one bryophyte persisted after 4200 cal.
BP. Thus, most taxa recorded from the early Holocene survived locally. Drier and more open conditions are suggested by consistent presence of *Dryas* and *Draba* and the bryophytes ~~Andreaea~~ and *Timmia*. The catchment probably supported a geomorphologically and aspect-controlled mosaic of communities, including *Dryas octopetala* heath, open herb communities and moist snow-bed and drainage communities, as it does today.

~~The find of a *Chara* oospore at about 3500 cal. BP is remarkable. Today, *Chara canescens* is found only in warm springs on Spitsbergen. The single find (not identified to species) in Skartjørna might originate from long-distance transport by birds, particularly geese (see Langangen, 2000).~~

The changes at Skartjørna that may signal sparser vegetation and lower biomass 5500–4000 cal. BP reflect other inferred changes in the marine and terrestrial environments. Sea-surface temperatures started to decline *c*. 7000–5000 cal. BP, and further cooling began around 4000 cal. BP (Rasmussen et al., 2012). On land, the nearby glacier Linnébreen re-formed around 4600 cal. BP and advanced *c*. 2800 and 2400 cal. BP (Reusche et al., 2014; Svendsen and Mangerud, 1997). Overall, these observations are consistent with the onset of the Neoglaciation *c*. 5000–4000 cal. BP in the North Atlantic region (Miller et al., 2010).

Resilience of tundra communities in the face of climate change

A striking feature of the molecular record is that there has been little floristic turnover in the local vegetation through the Holocene, despite a decrease in vegetation productivity inferred from the macrofossils. This provides firm evidence in support of Birks' (1991) conjecture that was based on macrofossils alone. Even some thermophiles persisted (but only a single repeat of the thermophilic species *Arabis alpina* is recorded after 5700 cal. BP). The scattered occurrence of *Betula* until 1800 cal. BP, long after cooler conditions were established, may reflect its ability to survive by clonal growth under conditions too cold for sexual recruitment (Alsos et al., 2002, 2003). The combined ~~vegetation~~ records suggest the gradual attrition of suitable habitats for thermophiles in response to cooling and drying of the climate, but nevertheless, survival of most taxa in situ. This may be related to fine-scale heterogeneity of the landscape that supports a range of microclimates (Armbruster et al., 2007) that ~~buffer~~ against the overall lowering of temperature by 1–2°C.
The Skartjørna plant records (this study, Birks, 1991) show that thermophilic species had broader distributions on Spitsbergen in the early Holocene. This is consistent with early Holocene range extensions of thermophilic species in the southernmost island of Svalbard (Bjørnøya, Wohlfarth et al., 1995), East Greenland and northern Eurasia (Bennike et al., 1999; Binney et al., 2009), and also range expansion of *Betula nana* in Svalbard in the early Holocene (Andersson, 1910). Based on this history, we might expect future warming of 1–2°C mean July temperature to drive an increase in cover and productivity and the expansion of local thermophilic species (given sufficient precipitation and conditions conducive to establishment), but not major floristic change. However, future warming is likely to reach at least 2–4°C mean July temperature above present; this temperature increase is unprecedented in the Holocene and may see the

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appearance of new elements in the flora, assuming effective dispersal and establishment.

Added understanding of vegetation ~~by~~ sedaDNA compared with only macrofossils

Because of the large degree of concurrence of taxa detected as *sed*aDNA and macrofossils (Table 2), one may argue that the proxies show overlap rather than being complimentary as suggested by others (Jørgensen et al., 2012; Parducci et al., 2013, 2015; Pedersen et al., 2013). However, the timing of ~~zonation~~ based on *sed*aDNA differs from that of macrofossils (Figure 6 and Supplementary Figure S2, available online), indicating that the proxies pick up different signals of change; indeed, the majority of taxa changing around 6600 and 5500 were only recorded in *sed*aDNA. A more important contribution of the *sed*aDNA from this site is the observation that most taxa persisted throughout the period studied even though they are only found in scattered parts of the period as macrofossils. For example, only one and two macrofossils of *Cerastium* and *Draba* were found, respectively (Birks, 1991), whereas they were recorded in most *sed*aDNA samples (Figure 4). While these taxa tend to be ubiquitous and are components of most vegetation association in Svalbard (Elvebakk, 1994, 2005), thus not adding much information about ecological conditions or vegetation type, the more or less continuous record of these and other taxa in the *sed*aDNA increases our understanding of persistence of species over time.

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Conclusion

Our results show *sed*aDNA to be an effective tool for reconstructing past vegetation change in the Arctic. The taxonomic resolution was similar to macrofossil, but the latter was superior in distinguishing, for example, *Salix* ssp., whereas *sed*aDNA was superior in detecting Poaceae. Using the number of repeats as a basic estimate of abundance, *sed*aDNA reflects the higher biomass of common arctic taxa, and these are identified with high certainty. As with macrofossils, the likelihood of detection is probably related to abundance in the vegetation. However, more taxa were detected with *sed*aDNA than with macrofossil analysis, suggesting that it is more sensitive in detecting less abundant taxa. The Skartjørna record corroborates other studies in that its record of thermophilic species indicates temperatures 1–2°C higher than present on Spitsbergen during the early part of the Holocene. In our record, the main environmental changes occurred *c.* 7000–5500 cal. BP, as either gradual or stepwise shifts in temperature, precipitation regime, terrestrial ecosystems and lake sedimentation. The molecular data indicate that even species with highly intermittent occurrence as macrofossil persisted throughout most of the study period. We might expect that thermophilous species that are currently highly restricted on Spitsbergen will expand again (assuming sufficient precipitation) and that both terrestrial and lacustrine productivity will increase. However, as future warming is likely to reach 2–4°C, we may also see responses that cannot be anticipated by reference to the available Holocene records.

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Declaration of conflicting interests

Ludovic Gielly is one of the co-inventors of patents related to *g-h* primers and the subsequent use of the P6 loop of the chloroplast *trn*L (UAA) intron for plant identification using degraded template DNA. These patents only restrict commercial applications and have no impact on the use of this locus by academic researchers.

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