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A complete Holocene lake sediment ancient DNA record reveals long-standing high Arctic plant diversity hotspot in northern Svalbard

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1. Introduction

Biodiversity hotspots are localities of high species diversity. Such localities have been suggested as crucial sites for the long-term persistence of species and are thus key sites for conservation, especially in a changing climate (Myers et al., 2000; Elvebakk, 2005; CAFF, 2013). However, long-term observations of persistence are lacking. To fill this knowledge-gap, paleoecological records can provide insight into past vegetation and species persistence valuable for conservation (Willis et al., 2007) alongside the reconstruction of past climates (e.g. Birks et al., 1994; Berglund et al., 1996; Alvos et al., 2016; Clarke et al., 2019b). Traditional paleobotanical proxies can be challenging in the Arctic due to low production of local pollen and plant macrofossils (Lamb and Edwards, 1988; Vasil’chuk 2005; Birks, 1991). In contrast, the cold conditions favor DNA preservation of what little may be produced (Hofreiter et al., 2001). Next generation sequencing methods have drastically increased the potential for DNA-based investigations in paleoecological studies over the past decade (Taberlet et al., 2007; Parducci et al., 2017), and the metabarcoding of sedaDNA has proven an efficient tool for

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reconstructing past vegetation and species diversity (Sønstebø et al., 2010; Willerslev et al., 2014; Zimmermann et al., 2017a).

Lake basins trap detrital and organic material from both the catchment and in-lake production. Lake sediment records are therefore excellent archives for inferring regional climate (Gjerde et al., 2017). Sedimentological, magnetic, geochemical and physical sediment properties can be used as proxies to reconstruct major patterns in abiotic environmental changes in the catchment. High-resolution elemental composition can be obtained from X-ray fluorescence (XRF) scans. Combined with the profiles of magnetic susceptibility (MS) and lithogenic, geochemically-stable, and conservative elements, these indicators can infer climate variability. Silica (Si), titanium (Ti), potassium (K), iron (Fe) and calcium (Ca) can, for instance, reflect changes in glacially-derived minerogenic input (Sandgren and Snowball, 2001; Schomacker et al., 2019), grain size (Cuven et al., 2010), weathering regimes (Rothwell and Croudace, 2015), the amount of inorganic detrital input to a lake (Rethe et al., 2015) and biological lake-production (Balascio et al., 2011; Kylander et al., 2011; Melles et al., 2012; Alsos et al., 2016; Gjerde et al., 2017; de Wet et al., 2018).

The majority of paleoecological and geological records indicates that the Early-Mid Holocene period in Svalbard was warmer than today (c. 9000-5000 years ago; Hyvärinen, 1970; Birks, 1991; Birks et al., 1994; Salvigsen and Høgvard, 2006; Miller et al., 2010; Alsos et al., 2016; Farnsworth, 2018). According to Mangerud and Svendsen (2018), the August sea-surface temperature was possibly as much as 6 °C higher than today. Based on present-day distributions, clone sizes, and genetic patterns, it is assumed that the most thermophilous plants in Svalbard had a broader distribution during the Early Holocene (Alsos et al., 2002; Engelskjøn et al., 2002; Birkeland et al., 2017). For some species, this has been confirmed by sedaDNA and/or plant macrofossil records from several lakes in the Svalbard archipelago (Birks, 1991; Wohlfarth et al., 1995; Alsos et al., 2016).

Ringhorndalen and the neighbouring valley Flatøyrdalen (Fig. 1) are the most botanically diverse locations in Svalbard, and assumed to have remnants from the Early Holocene vegetation (Elvebakk and Nilsen, 2002, 2016; Eidesen et al., 2013; Birkeland et al., 2017). Flatøyrdalen has been described as an ‘Arctic hotspot’ due to its “extrazonally warm climate, resulting in thermophilous

Fig. 1. (a) The North Atlantic Ocean with the location of (b) the Svalbard archipelago and (c-d) the study site in Ringhorndalen. Hatched lines around Jodavannet mark the catchment and the red star marks sampling location of the sediment core used for all analyses. Coloured circles in (c) indicate current local distributions of thermophilous vascular plant species (https://artskart.artsdatabanken.no, 2019): Arenaria humifusa (grey), Arnica angustifolia (yellow), Calamagrostis purpurascens (dark red), Carex bigelowii ssp. arctisibirica (brown), Carex krausei (dark green), Carex saxatilis ssp. laxa (pink), Campanula uniflora (dark blue), Comastoma tenellum (light blue), Empetrum nigrum ssp. hermaphroditum (purple), Luzula spicata (orange), Pinguicula alpina (white), Tofieldia pusilla (light green) and Vaccinium uliginosum ssp. microphyllum (black). Maps modified from The Norwegian Mapping Authority and The Norwegian Polar Institute.
biodiversity elements not found in its surroundings” (Elvebakk, 2005). Many of the unusually thermophilous plant species occurring in the area are spatially distant from their normal distribution range. Of the 124 vascular plant species known from the area, some populations represent the northernmost known sites for the species worldwide and the only known location in Svalbard (Pinguicula alpina, Luzula spicata, Eriogonum uniflorus, Draba aff. oblongata and Festuca ovina) or Europe (Calanagrostis purpurascens) (Elvebakk and Nilsen, 2002, 2016; Eidesen et al., 2013, 2018).

In this study, we investigate sediments from a lake in Ringhorndal using metabarcoding of sedaDNA, sedimentology, and geochemical proxy data to reconstruct changes in vegetation composition and postglacial environmental conditions. We aim to answer the following questions: (i) How has the composition of plants in the catchment area developed since the last deglaciation? (ii) Can sedaDNA together with elemental data increase our understanding of Holocene climate variability?

2. Setting

Ringhorndal is situated at the east coast of Wijdefjorden on northern Spitsbergen (Fig. 1). The sediment core was obtained from lake Jodavannet (informal name: 79.3383 °N, 16.0167 °E; Fig. 1d), which is situated 140 m a.s.l. on the northern side of the valley mouth.

2.1. Local climate and modern growth conditions

No meteorological data record exist for the area, but the current climate can partly be inferred from local vegetation and the glacier equilibrium line altitude (ELA). Elvebakk and Nilsen (2002) concluded that the vegetation in the outer areas of Ringhorndal reflects relatively high temperatures combined with aridity due to low precipitation and wind desiccation. ELAs of 800 m a.s.l. on the glacers in the inner part of Wijdefjorden indicate low precipitation in the region (Hagen et al., 1993). Based on observations of snowlines and mass balance of selected glaciers, Hagen et al. (1993) inferred a mean annual precipitation of 200 mm in the inner Wijdefjorden area, including Ringhorndal.

Eidesen et al. (2018) measured climatic (temperature, moisture, radiation) and soil abiotic variables (pH, organic content via loss-on-ignition (LOI) and carbon-to-nitrogen (C:N) ratio) during one growing season from May to July 2017. The results revealed warmer growth conditions in Ringhorndal compared to reference sites in Adventdalen (e.g. mean July soil temperature in Dryas heath in Ringhorndal was 9.4 ± 1.4 °C, whereas it was 6.8 ± 0.5 °C in Adventdalen, based on four loggers at each location). Light and moisture measurements showed few cloudy days and the main moisture to be input from snowmelt early in the season rather than summer precipitation. The recorded soil pH was close to neutral (mean = 6.7; range = 5.1–7.9), with the highest values in the valley mouth. The organic C content in soil from zonal vegetation was within the expected range for bioclimatic zone C (10–30%) according to Jónsdóttir (2005). The N content was variable between samples (0.39 ± 0.27% (±SD)), but in line with measurements in other productive areas of Svalbard such as Colesbukta and Engelsbukta on the west coast (P.B. Eidesen, unpublished data; Fig. 1b). The C:N ratio of 13.01 ± 2.71 (mean ± SD) indicates good decomposition conditions according to Eidesen et al. (2018).

2.2. Geology, topography and vegetation

The bedrock in the catchment of Jodavannet consists of Mesoproterozoic micaschist, metapsammite and amphibolite. Further upvalley the bedrock consists of Palaeoproterozoic granitic gneiss, migmatite and amphibolite. The easternmost part of Ringhorndal consists of Mesoproterozoic quartzite, micaschist, amphibolite and marble (Dallmann, 2015). The valley floor is mostly occupied by outwash, with braided meltwater rivers that originate from two confluent outlet glaciers from the Åsgardfonna ice cap in the inner part of the valley (Fig. 1c). Situated at 140 m a.s.l., Jodavannet is above the regional postglacial marine limit of c. 65 m a.s.l. (Forman et al., 2004). It has an area of 0.02 km². At present, the catchment of Jodavannet (1.31 km²; Fig. 1c–d) has no glacial meltwater input as it is separated from the rivers draining the glaciers by topographic boundaries. Runoff is brought to the lake from the northern, western, and southern slopes. A small stream enters from northwest, and the lake drains into a larger lake in southeast (Fig. 1d). Traces of erosion from water flow towards the lake can be seen on the southern and southwestern sides (Fig. 1d).

The valley mouth has distinct high-Arctic steppe vegetation with prevalent Potentilla pulchella communities (Elvebakk and Nilsen, 2002). This is where the wind tunnel effect from Wijdefjorden is strongest. Small ridges separate the outer valley where Jodavannet is located from the more wind-sheltered and thus warmer inner valley, where most of the thermophilous plant species are growing today. The catchment therefore includes only some of the most thermophilous flora elements. Upland on the steep valley sides, particularly lush gullies, open screes, and sheltered boulder fields stretch down from vertical headwalls higher up. Cassiope tetragona heath dominates further downslope.

At present, the vegetation around Jodavannet is homogenous, with a moss-dominated belt stretching up to 5 m from the lake shore. Saxifraga oppositifolia, Carex subspathacea, Dupontia fisheri, Bistorta vivipara and Salix polaris are the most frequent vascular plant species in the closest 30 m around the lake (Table C, Appendix A). In general, there are mainly temperature-indifferent species (sensu Elvebakk, 1989), but some weakly thermophilous species are also common within close proximity to the lake, e.g. Carex subspathacea, Equisetum arvense ssp. alpcrest, and Cardamine pratensis, while Dryas octopetala (weakly thermophilous) and Cassiope tetragona (distinctly thermophilous) have occurrences further away.

3. Methods

3.1. Sediment coring and subsampling

A 186 cm long sediment core (JVP1) was taken from the deepest part of the lake (79.33831 °N; 16.01902 °E) at a water depth of 6.4 m, in August 2016. Coring was performed with a hand-held lightweight piston corer equipped with 60 mm diameter and 200 cm long coring tubes. The core was kept sealed and refrigerated during transfer to the Centre for GeoGenetics, University of Copenhagen, Denmark, where c. 2–5 g subsamples were taken along the entire core length at 2 cm intervals, following the clean sampling procedures described by Pedersen et al. (2016). The subsamples were kept cold and transported to Tromsø Museum, at UiT The Arctic University of Norway, where they were stored frozen (−18 °C) until further processing.

3.2. Age-depth modelling

To establish a chronology for JVP1, nine terrestrial and aquatic macrofossils were retrieved from the core by wet sieving of c. 10 cm³ of sediment. All macrofossils were radiocarbon (14C) dated with accelerator mass spectrometry (AMS) at the Tandem Laboratory at Uppsala University, Sweden, and the Radiocarbon Dating Laboratory at Lund University, Sweden (Table 1).

Using the nine 14C ages, an age-depth model was constructed in the Online Supplement
3.3. Lithological analyses

High-resolution optical (90 μm) and X-ray imagery (200 μm), XRF (1000 μm), and MS (4000 μm) were obtained from Itrax scanning, performed at the Centre for GeoGenetics, University of Copenhagen (Itrax CS37 Cph; Croudace et al., 2006). Scans were carried out using a rhodium (Rh) tube with voltage and current set to 30 kV and 50 mA, respectively, and the XRF count time was 35 s. Variations in MS (Thompson et al., 1975) and elemental profiles in kilo counts per second (kcps) from XRF scanning (Kylander et al., 2011; Lowemark et al., 2011) were used as proxies for variations in environmental conditions in the catchment area of the lake (Rothwell and Rack, 2006; Rothwell et al., 2006). Additionally, percentage organic content of the sediments was measured by LOI (Heiri et al., 2001). Samples of 2 cm³ were sampled at 1 cm intervals throughout the core, dried at 105 °C for 24 h and ignited at 550 °C for 4 h. The profiles of LOI, MS and 6 different elements/elemental ratios are used to describe long-term environmental variation reflected in the geochemistry of the sediment record. To reduce noise, the MS and elemental values were modified with a weighted moving average (pracma package; 5-points backward window length), resulting in measurement intervals of 1.6 cm (MS) and 0.3 cm (elemental values; Gjerde et al., 2017), and the conservative element Ti was used to normalize the element profiles. Ti was normalized against the sum of the coherent (coh) and incoherent (inc) scatter from rhodium (Rh).

High MS signal in lake sediments can reflect inorganic allochthonous material (Thompson et al., 1975) and was used as proxy for minerogenic input (Snowball and Sandgren, 1996; Schomacker et al., 2019). Rh coh versus Rh inc was used as a density proxy, typically reflecting dense sediments of minerogenic origin.

Typical alloigenic elements such as Ti, Ca and K have previously been used to reflect erosion intensity. High Ti values can indicate increased detrital sediment input from glacial or aeolian deposition as well as erosion and runoff from the catchment (Rothwell and Croudace, 2015; Davies et al., 2015). High K/Ti values were evaluated as potential increased weathering, because K is relatively water-soluble compared to the more stable Ti (Rothwell and Croudace, 2015). To assess changes in biological production, Ca/Ti and Si/Ti were used to indicate biogenic silica production in the lake (Melles et al., 2012; Liu et al., 2013).

3.4. SedaDNA extraction, metabarcoding, and high throughput sequencing

SedaDNA was extracted from entire subsamples (2–5 g; n = 41) and negative extraction controls (n = 6) in the ancient DNA dedicated laboratory at Tromsø Museum. We extracted a greater proportion of subsamples from the deeper layers of JVP1 to increase resolution in the earliest time-period (8–101 cm, n = 10; 110–135 cm, n = 7; 138–185 cm, n = 24). A modified DNeasy PowerMax Soil DNA Isolation kit protocol (Qiagen Norge, Oslo, Norway) was used for extraction. In addition to the cell disruptive solution (“C1”), 100 μL of 5 mg/mL proteinase K and 400 μL of 1M dithiothreitol (DTT) were added to each sample. The subsequent vortexing of samples was conducted in a FastPrep-24 TM 5G (M. P. Biomedicals LLC, Santa Ana, CA, USA) for 2 × 20 s at 4.5 m/s and then incubated at 56 °C for c. 15 h (following Alsos et al., 2016; Zimmermann et al., 2017b). All centrifuge steps were conducted at 4200 rpm instead of 2500 rpm. In the final step, all samples were recovered in 5 mL instead of 5 mL elution buffer (Alsos et al., 2016).

For metabarcoding, all polymerase chain reactions (PCRs) were setup in the dedicated ancient DNA laboratory at Tromsø Museum, which is physically isolated from other PCR work to prevent contamination from PCR products. Two negative PCR controls (one from the aDNA lab and one from the PCR lab) and one positive control with synthetically reconstructed sequence were amplified in addition to the 41 DNA extracts from samples and six negative extraction controls, thus in total 50 samples/controls. The target region was the short P6-loop of the chloroplast tml (UAA) intron, amplified with the universal plant primers g and h (Taberlet et al., 2007). These primers amplify vascular plant taxa, but may also sporadically detect other non-vascular plants (Taberlet et al., 2018). Unique flanking sequences (tags) 8 or 9 base pairs (bp) long were added at the 5’ end to allow for PCR product pooling. Seven PCR replicates were conducted per sample and control to increase the chance of detecting rare taxa and taxa with low template DNA representation in the sediment record, and to facilitate distinguishing probable true from false positives (Ficetola et al., 2015;
Alsos et al., 2018a). DNA amplification was conducted in 40 μL final volumes, containing 4 μL of undiluted DNA extract, 1X Gold buffer, 1.6 U of AmpliTaq Gold® DNA Polymerase (Life Technologies, Carlsbad, CA, USA), 2.5 mM MgCl₂, 0.2 mM dNTPs, 0.2 μM forward primer, 0.2 μM reverse primer, and 160 ng/μL Bovine Serum Albumin. The PCR mixtures underwent enzyme activation for 10 min at 95 ºC, followed by 45 cycles of denaturation for 30 s at 95 ºC, annealing for 30 s at 50 ºC, and elongation for 1 min at 72 ºC, plus a final elongation step for 7 min at 72 ºC.

10 μL from each PCR product was pooled and the resulting mix purified using the QiaGen MinElute PCR purification kit (Qiagen GmbH, Hilden, Germany), following the manufacturer’s instructions (Alsos et al., 2016). The purified amplion pool was sent to Fasteris (Fasteris SA, Switzerland), where it was converted into an Illumina-compatible DNA library using the single-indexed, PCR-free MetaFast protocol. The library was sequenced on an Illumina NextSeq 500 sequencing platform (Illumina, Inc., CA, USA) using 10% of a mid-output, 2 × 150 cycle flow cell.

3.5. Bioinformatic analyses and taxonomical assignment

The OBITools software package (Boyer et al., 2016) was used to analyze sequence data, and the analysis was run through the Abel computing cluster at University of Oslo. Amplicons were reconstituted by aligning paired-end reads with Illuminapairedend, and sequences having an alignment score lower than 40 (Alsos et al., 2016) were removed with obigrep. The remaining sequences were assigned to samples according to their unique sample tags with ngsfilter (demultiplexing), requiring 100% match with tags and maximum 2 bp mismatch in the primer region (default options in OBITools). As ngsfilter does not allow different tag lengths, this step was run twice and resulted in two output files that were subsequently merged with the UNIX cat command. Amplicons with unexpected tag combinations were considered chimerical sequences and thus removed with obigrep. To exclude sequences shorter than those in the reference library, obigrep was also used to remove sequences shorter than 10 bp.

Identical sequences were clustered (deduplication) using obiu-niq, keeping the information about their distribution among samples. Sequences with only one copy in the dataset were removed (Alsos et al., 2016) with obigrep, before using obiclean to identify sequences potentially resulting from PCR and sequencing errors. Using information about sequence record counts and sequence similarities across all samples, sequences were classified as head (ideally true sequences), singleton (potentially rare true sequences) or internal (assumed erroneous sequences; Boyer et al., 2016). A maximum of a single base-pair difference was allowed between two variant sequences, and the abundance threshold ratio for the uncommon internal versus common head sequence was 5%. This threshold retained relatively rare sequences, as small sequence differences may make a taxonomically important difference to PG-loop sequence identification (Sanstebø et al., 2010; Alsos et al., 2016). Sequences with no close resemblance to others (neither head nor internal), were classified as singleton. To avoid potential PCR or sequencing errors, only head and singleton sequences were kept.

Finally, sequences were assigned to taxa based on sequence similarity with two taxonomic reference libraries, using ecotag (part of the OBITools package). The primary reference library (arctoborbyo) contains local taxa of 815 Arctic (Sanstebø et al., 2010) and 835 boreal (Willerslev et al., 2014) vascular taxa in addition to 455 bryophytes (Soininen et al., 2015), which covers the majority of plant species growing in Svalbard at present (Alsos et al., 2018b). The secondary reference library contains sequences from running ecopcr on the global EMBL Nucleotide Sequence Database (R134, 2018). The EMBL reference library was used to detect possible non-local contaminants. This resulted in two files containing the unique sequences and their taxonomic assignment from each reference library.

After initial data processing with OBITools, further filtering of the sequences was conducted in R. To avoid misidentifications, only sequences with a 100% match to reference library sequences were kept. Next, the two result files were merged, keeping only the arctoborbyo-assigned sequence in the case of conflicts. To minimize the chance of including false positives, we registered sequences as present in a sample if the sequence: 1) had a minimum of 10 reads per PCR replicate and occurred in a minimum of two PCR replicates, 2) was not detected in the negative controls, and 3) occurred in a minimum of 100 reads across the entire dataset. Sequences matching non-native or marine taxa were compared to the NCBI nucleotide database using BLAST (http://www.ncbi.nlm.nih.gov/blast/; Accessed April 2018) to confirm if they were correctly assigned to unlikely taxa in the study area. Some sequences were assigned with low taxonomic resolution from their taxon ID in the NCBI taxonomy database. These sequences were also compared to the NCBI nucleotide database to improve taxonomic assignment. Consequently, some taxonomic names were modified according to the BLAST-results and accepted taxonomy in the local botanical literature (Elven et al., 2011) (Carex sp. changed to Carex saxatilis, Cassiope lycopodioides to Cassiope tetragona, Pedicularis dasyantha/hirsuta, Festuca pratensis to Festuca sp. (5 species), Ranunculus sceleratus to Ranunculus hyperboreus, Polydopiales to Cystopteris fragilis and Nannochloropsis granulata to Nanochloropsis sp.; Table 2). The resulting taxonomy after modifications was used in all subsequent analyses. Two exotic taxa (Cedrus and Juniperus) were suspected contaminants and therefore removed from the dataset.

3.6. Constrained hierarchical cluster analysis

All statistical analyses were conducted in R. To categorize the Holocene development of species communities as found by metabarcoding, the vegdist function in R-package vegan (Oksanen et al., 2017) was used to create a matrix of Bray-Curtis dissimilarity indices of all identified unique taxa based on number of PCR replicates per sample. Sequences that were undifferentiated between several different species or identified to taxonomic levels excluding ecological interpretation, were merged before subsequent analysis (Festuca sp. and Festuca baffinensis/brachyphylla/edulindiae/hyperborea/ovina/rubra; five sequences of Saxifraga sp.; four sequences of ‘Hypnales’; Ranunculus hyperboreus/gmelinii and two sequences of Ranunculus sceleratus; and two sequences of Pedicularis hirsuta/dasyantha). The dissimilarity matrix was then clustered with the chclust function from vegan, using constrained hierarchical clustering with clusters constrained by sample depth and the coniss algorithm (Grimm, 1987). To identify the number of statistically significant groups from the constrained cluster analysis, a broken stick distribution (Bennett, 1996) was created with the bstick function from the rioja package (Juggins, 2017).

4. Results

4.1. Chronology

The age-depth model returned a median age range of 11,870–306 cal. yr BP (Table 1; Fig. 2). Age-depth model convergence was indicated by the relatively stationary and unstructured adjacent MCMC iterations (Fig. A, Appendix A).
Table 2
All taxa from metabarcoding of sedaDNA from Jodavannet, Svalbard, after sequence filtering. Names in brackets represent known local species resulting from sequence match in reference libraries or BLAST result when no species were identified in reference libraries.

| Family/order | Taxa sedaDNA | Sum samples (out of 41) | Max. repeats (out of 7) | Sum repeats (out of 287) | Sum reads | Unique sequences assigned | Therm. (Elvebakk, 1985) | Bioclimatic subzone |
|--------------|--------------|-------------------------|------------------------|-------------------------|-----------|--------------------------|------------------------|----------------------|
| Vascular plants | | | | | | | | |
| Asteraceae | | | | | | | | |
| Arnica (angustifolia) | | 3 | 2 | 4 | 2212 | 1 (a) | II | C(s) |
| Brassicaceae | | | | | | | | |
| Brassicaeae | | 1 | 2 | 2 | 2267 | 1 (b) | | |
| Caryophyllaceae | | 6 | 4 | 11 | 1420 | 1 (a) | IV | A(r) |
| Cyperaceae | | 5 | 3 | 8 | 1389 | 1 (a) | V, II | C(s), A(r) |
| Lycopodiaceae | | | | | | | | |
| Bryaceae | | | | | | | | |
| Caryophyllaceae | | 7 | 3 | 11 | 4380 | 1 (a) | III, NA | A(f) |
| Cyperaceae | | 9 | 6 | 15 | 9846 | 1 (a) | | |
| Papaveraceae | | | | | | | | |
| Papaver (dahliana, cornwalliensi) | | 14 | 7 | 26 | 18198 | 1 (a) | IV | A(r) |
| Poaceae | | 19 | 18 | 93 | 7487 | 3 (a, b) | II | C(f) |
| Ranunculaceae | | 15 | 6 | 31 | 23812 | 1 (a) | II | B(r) |
| Juncaceae | | 6 | 6 | 19 | 3463 | 1 (a) | V | A(f) |
| Lyszula (arcuata, confusa, nivalis, wahlenbergii) | | 7 | 7 | 31 | 2517 | 1 (a) | II, V, I | C(r), A(f), A(f), C(s) |
| Lycopodiceae | | 2 | 2 | 3 | 2483 | 1 (a) | III | B(c) |
| Papaveraceae | | 9 | 6 | 27 | 34204 | 1 (a) | V, NA | A(f) |
| Poaceae | | 8 | 12 | 44 | 9479 | 2 (a, b) | III, IV, NA, IV | B(r), A(r), C(s), B(c) |
| Ranunculaceae | | 5 | 3 | 7 | 1692 | 1 (a) | V | A(f) |
| Saxifragaceae | | | | | | | | |
| Micranthes | | 10 | 5 | 18 | 1321 | 1 (a) | IV, V | B(r), C(r), A(r), C(s) |
| Micranthes | | | | | | | | |
| Saxifraga (cernua, hyperborea, rivularis) | | 10 | 5 | 30 | 10162 | 1 (a) | V, V, NA | A(f), A(f), A(r) |
| Saxifraga cernua | | | | | | | | |
| Saxifraga oppositifolia | | 25 | 7 | 105 | 292727 | 1 (a) | V | A(f) |
| Saxifraga sp. | | 25 | 12 | 105 | 27412 | 5 (b) | | |
| Algae | | | | | | | | |
| Closteriaceae | | 9 | 7 | 29 | 139905 | 1 (b) | | |
| Desmidieae | | 17 | 7 | 57 | 22787 | 1 (a) | | |
| Desmidieae | | 2 | 2 | 3 | 155 | 1 (b) | | |
| Monobasidieae | | 21 | 7 | 94 | 512683 | 1 (a) | | |
| Oocystaceae | | 4 | 2 | 5 | 357 | 1 (a) | | |
| Bryophytes | | | | | | | | |
| Andreaeaceae | | 1 | 2 | 2 | 104 | 1 (a) | | |
| Bartramieaeae | | 5 | 3 | 9 | 163 | 1 (b) | | |
| Bryceae | | 5 | 7 | 16 | 11746 | 1 (a) | | |
| Dicranaceae | | 3 | 3 | 6 | 1358 | 1 (a) | | |
| Distichieae | | 8 | 3 | 10 | 1012 | 1 (a) | | |
| Encalyptaceae | | 4 | 2 | 5 | 103 | 1 (a) | | |
| Hypnales | | 5 species | | | | | | |

*(Elvebakk, 1985)*
The lines show the age-depth curve with the best model from the respective order, but only once if the same classification applies to all species. Hypnales is possibly *Scorpidium cossomi/revolvens/scarpoidea, Pseudolepilagion turgescens and/or Tomentypnum nitens* according to present day flora. *Polytrichastrum* is possibly *Polytrichastrum sexangulare, Polytrichum hyperboreum/commune/juniperinum/strictum, Diphilum caviolium/laevigatum or Pogonatum urinigerum.*

**Fig. 2.** Bayesian age-depth model for the nine calibrated $^{14}C$ ages (blue) from JVPI in Jodavannet, Svalbard. The lines show the age-depth curve with the best model from weighted average of the mean (red), more likely calibrated ages (darker grey) and the 95% confidence interval (outer stippled grey lines).

### 4.2. Lithostratigraphy

Changes in the depositional environment were revealed based on lithology, geochemical composition and organic content (Fig. 3). Four lithological units (LU 1–4) were identified.

**LU 1:** Core depth 186–176 cm, c. 11,900–10,800 cal. yr BP

LU 1 consists of light grey to very light green, clayey silt with interbeds of organic material interrupted by occasional light tan silt beds. The XRF profiles are relatively unstable and variable, and LOI decreases gradually. We interpret LU 1 as deposited by accumulation of organic material interrupted by increasing episodic minerogenic input.

**LU 2:** Core depth 176–81 cm, c. 10,800–4300 cal. yr BP

There is a large reduction in indicators of glacially derived minerogenic input (MS, X-ray derived density and Ti), increasing LOI, and a peak in Ca at the transition from LU 1 to LU 2. LU 2 consists of well-stratified and yellow-brown to grey gyttja with sporadic interlaminated organic material. The bedding transitions from well-stratified to crudely stratified from the base of the unit towards the top. We interpret LU 2 as deposited by the accumulation of organic material with minimal minerogenic input.

**LU 3:** Core depth 81–19 cm, c. 4300–400 cal. yr BP

LU 3 consists of dark tan to very dark brown silty gyttja with organic-rich lamina interrupted by occasional light tan silt beds. The XRF profiles are relatively unstable and variable, and LOI decreases gradually. We interpret LU 3 as deposited by accumulation of organic material interrupted by increasing episodic minerogenic input.

**LU 4:** Core depth 19–0 cm, after c. 400 cal. yr BP

LU 4 consists of light grey to light tan weakly laminated silty fine sand with occasional laminae of brown organic material. We interpret LU 4 as deposited by minerogenic-rich sedimentation with minimal biogenic accumulation. The sediment source is interpreted as nival or aeolian, or a combination of both.

### 4.3. SedaDNA analysis

During bioinformatic processing, the number of reads and sequences were reduced from an initial raw count of c.167 million paired-end reads to c.11 million reads of which there were 7158 unique sequences (after OBITools, but prior to R filtering). After R filtering, the resulting counts were 2,396,240 reads represented by 77 unique sequences. Full details of the bioinformatic approach and the number of reads and sequences remaining after each step are given in Table A (Appendix A). The resulting sequences were assigned to vascular plants (73.8%), algae (7.7%) and bryophytes (18.5%) from 65 different taxa (Table 2).

No taxa were present in all samples. The most abundant vascular plants in the sedaDNA record were *Bistorta vivipara* (61%), *Saxifraga sp.* (61%), *Saxifraga oppositifolia* (61%), and *Salix* (59%) (based on overall presence in samples; Table 2). *Cassiope tetragona* (46%), *Oxyria digyna* (44%) and *Dryas octopetala* (44%) were also frequently detected, followed by *Empetrum nigrum* (37%), *Equisetum arvense* (34%), and *Equisetum variegatum* (27%).

Three significant sedaDNA zones were found based on the constrained hierarchical cluster analysis of Bray-Curtis community distance on PCR replicates per sample (Figs. 4 and 5 and Fig. B, Appendix A) and the comparison with a broken stick model.
distribution (Fig. C, Appendix A). The greatest assembly distance among adjacent samples was between sample depth 78 and 90 cm (c. 4400 cal. yr BP), followed by 154 and 156 cm (c. 9900 cal. yr BP), making the youngest zone the most distinct. The identified sedaDNA zones correspond to visually marked transitions in the composition of sedaDNA taxa (Figs. 4 and 5 and Table B, Appendix A).

4.3.1. SedaDNA zone 1: sample depth 185-156 cm, c. 11,700-9900 cal. yr BP (n = 15)

Most of the taxa dominating in the oldest part of the sediment core were herbaceous, non-graminoid vascular plants and bryophytes typical of wet habitats, e.g. Ranunculus hyperboreus, Saxifraga cernua/hyperborea/ribularis, Hypnales and Pottiaceae (see Table 2 for species). Overall, we found taxa with very different ecological characteristics, for example Saxifraga oppositifolia (widely distributed generalist), Saxifraga cespitosa (relatively drought-tolerant), Papaver cornwallisense/dahlianum (hardy pioneer), and Cystopteris fragilis (strong thermophile, sensu Elvebakk, 1989).

There was a marked reduction in vascular plant and bryophyte taxa around 10,600 cal. yr BP, while algae became dominant (especially Nannochloropsis sp.). The first occurrence of the distinct thermophiles Arnica angustifolia (c. 10,200 cal. yr BP) and Empetrum nigrum (c. 10,400-10,200 cal. yr BP) appeared. The overall taxonomic richness in sedaDNA zone 1 was 65% of all taxa present (26 vascular, 8 bryophyte and 4 algal taxa) with an average of 2.7 plant taxa per sample. The taxonomic diversity was lower from 10,600 cal. yr BP onwards. Some taxa were exclusively found in sedaDNA zone 1, namely Carex marina/ursina/glaeosa, Ranunculus hyperboreus, Pottiaceae and Neglectella solitaria.

4.3.2. SedaDNA zone 2: sample depth 155-90 cm, c. 9900-4600 cal. yr BP (n = 18)

The sedaDNA record in zone 2 was still dominated by Nannochloropsis sp. until c. 8200 cal. yr BP, but overall algal read abundance was reduced compared to the youngest part of sedaDNA zone 1. Nannochloropsis sp. also occurred sporadically c. 5600–4600 cal. yr BP, and two other algal taxa (Cosmarium botrytis and Closterium baillyanum) were present c. 9100–8800, 5400–5200 and 4900 cal. yr BP. There were relatively few bryophytes, with Hypnales as the most abundant bryophyte taxa.

The percentage of vascular plant taxa was similar between sedaDNA zone 1 and 2 (63% and 60%, respectively). The dwarf shrubs Empetrum nigrum and Salix were relatively dominant throughout sedaDNA zone 2, while Dryas octopetala and Cassiope tetragona followed c. 6400 and 4900 cal. yr BP, respectively. Among the herbs, Bistorta vivipara, Saxifraga oppositifolia, Saxifraga sp., and Oxyria digyna dominate. Graminoids were generally infrequent, but a few thermophilous sedges were present. The distinctly thermophilous Carex lachenalia was recorded exclusively in sedaDNA zone 2, c. 9770 cal. yr BP, and the distinctly thermophilous Carex parallelala occurred for the first time in the record c. 5300 cal. yr BP. Additionally, the moderately thermophilous club moss Huperzia arctica occurred exclusively in this zone, c. 5500 cal. yr BP. The thermophilous species (sensu Elvebakk, 1989) Cystopteris fragilis (strongly thermophilous) and Arnica angustifolia (distinctly thermophilous) reappeared in the record c. 5300 and c. 4900 cal. yr BP, respectively. The widespread Arctic-alpine species Silene acaulis occurred for the first time in the record c. 8200 cal. yr BP. The overall taxonomic richness in sedaDNA zone 2 (~57% of all detected taxa; 29 vascular, 5 bryophyte and 3 algae; average 2.5 plant taxa per sample) was similar to the previous zone.
4.3.3. SedaDNA zone 3: sample depth 79-8 cm, c. 4200-150 cal. yr BP (n = 8)

Most vascular plant taxa were present in sedaDNA zone 3 (~85%), and several occurred for the first time in the record (Braya glabella, Cardamine bellidifolia, Stellaria longipes, Carex saxatilis, Luzula, Calamagrostis, Deschampsia and Poinae). Graminoids had a marked increase with 10 out of 12 taxa present. Dwarf shrubs were consistently represented throughout sedaDNA zone 3 with
relatively high read abundance. Several thermophilous taxa were present (Arnica angustifolia, Calamagrostis, Carex pallescens, C. saxatilis, Cassiope tetragona and Empetrum nigrum), especially C.

4200–560 cal. yr BP. After this period, the thermophilous indicators decreased. There was also a pronounced change in dwarf shrubs, as Cassiope tetragona was consistently present in almost all samples,
while *Empetrum nigrum* disappeared from the record after c. 3400 cal. yr BP.

Algal and bryophyte taxa were also abundant with almost all taxa present, and sedaDNA zone 3 was the zone with the overall highest total and average taxonomic richness: ~86% of all detected taxa were present (41 vascular, 11 bryophyte and 4 algae), with an average of 7 taxa per sample.

### 4.3.4. Comparison to current vegetation

More than 50% of taxa found in the sediment record were not registered during surveys of contemporary vegetation close to the lake (Table C, Appendix A). However, the majority of the inferred taxa are found in the Ringhorndalen valley (taxa in bold, Table 2). Only *Arctophila fulva* and *Calamagrostis neglecta* have not been found in Ringhorndalen before, but these taxa are found elsewhere in Svalbard. *Draba* sp., Asteraceae and Brassicaceae had too low taxonomic resolution for classification of local presence, because the taxa included both local and non-local representatives.

### 4.4. Comparison between lithology and vegetation records

The stratigraphic zones identified for each data record were largely congruent (Fig. 6): SedaDNA zone 1 was comparable to LU 1. LU 2 comprised a small part of sedaDNA zone 1 and the complete sedaDNA zone 2. The transitions to sedaDNA zone 3 and LU 3 appeared largely synchronous. LU 4 was not identified as significantly distinct in the sedaDNA record (n = 2 within that lithological unit). Together, the sedaDNA zones and lithostratigraphic units defined three characteristic time periods based on environmental differences (Fig. 6).

### 5. Discussion

#### 5.1. Holocene development of vegetation and climate

##### 5.1.1. Rapid colonization during the late glacial and Early Holocene (c. 11,900–9900 cal. yr BP)

The high proportion of species recorded in this early period (>50%) and relatively high assemblage turnover around 10,600 cal. yr BP occurring over a relatively short time span (~2000 years) could be due to either an early deglaciation or rapid colonization (Figs. 4 and 5). We do not know the exact timing of deglaciation at the site, but cosmogenic exposure ages from Delingstupa (135 m a.s.l. and 145 m a.s.l.; Fig. 1c) suggest ice-free conditions as early as 14,600–13,800 ± 1000 yr ago (Hormes et al., 2013). The lithology of LU 1 (Fig. 3) reflects minerogenic-rich sedimentation, suggesting inflow of glacial meltwater across the eastern threshold of the Jodavannet catchment (Fig. 1). This indicates the presence of glaciers in the catchment. Thus, the site was most likely rapidly colonized at the time that glaciers left the watershed. Recent studies of lacustrine and raised marine sediments from northern and western Spitsbergen also suggest glacier retreat and species colonization during the late glacial (Gjerde et al., 2017; Farnsworth et al., 2018; Larsen et al., 2018). Diverse pollen records are known from further north in Wijdefjorden at Lake Stroen and Nordaustlandet (both Hyvärinen, 1970), Edgeøya (Rennike and Hedenås, 1995), and the more southern Bjørnøya (Hyvärinen, 1970; Wohlfarth et al., 1995). This includes several of the taxa we recorded, such as *Ranunculus*, *Papaver*, and *Saxifraga*.

High frequency of immigration to Svalbard has also been interpreted based on studies of modern DNA (Alsos et al., 2007). Mangerud and Svendsen (2018) suggested that the August sea-

![Fig. 6. Details of the sedaDNA samples (modelled median age, lithological unit (LU) statistically identified sedaDNA zone, and the number of taxa within vascular plants, bryophytes, and algae obtained from sedaDNA analysis). Reconstructed sea-surface temperature for the west coast of Spitsbergen from Mangerud and Svendsen (2018) is shown to the right.](image)
surface temperatures were up to about 6 °C warmer than present between 11,000 and 10,500 cal. yr BP. Our identification of the strongly thermophilous species *Cystopteris fragilis* in the sedaDNA record c. 11,200-10,900 cal. yr BP further suggests that this was a period considerably warmer than today, which allowed rapid establishment of a diverse flora.

The marked transition from LU 1 to LU 2, c. 10,800 cal. yr BP (Fig. 3), indicates an abrupt termination of glacial meltwater inflow. This likely changed the nutrient conditions of the lake causing the recorded algal bloom (Fig. 5). The relative decrease of bryophytes and vascular plants at this time is likely attributed to the dominance of algal DNA, as this may cause underestimation of other taxa (Alsos et al., 2018a). Indeed, the appearance of more thermophilous species like *Empetrum* and *Arnica* suggests that the climate was warmer than today. This is also in accordance with a rise in sea-surface temperature (Hald et al., 2004; Mangerud and Svendsen, 2018) and lake-water temperatures (van der Blit, 2016).

### 5.1.2. Dry and warm middle Holocene (9900– 4300 cal. yr BP)

Our records suggest climatically stable, dry and warm conditions throughout most of the middle Holocene (from the beginning of sedaDNA zone 2 to the onset of LU 3 and sedaDNA zone 3). The sedaDNA record shows a relatively species-rich vegetation with the establishment of several dwarf-shrubs and herbs common in Svalbard today, and a consistent presence of thermophilous indicator taxa (Fig. 6). The low catchment erosion intensity inferred from the lithostratigraphical record strengthens this interpretation. The August sea-surface temperatures are suggested to have been about 4 °C higher than present from c. 8000 to 6500 cal. yr BP, followed by a gradual decrease in temperature until present day levels around 3500 cal. yr BP (Mangerud and Svendsen, 2018). The vegetation and lithological reconstructions from Jodavannet support these findings. This is also a period with increased pollen production (Hyvärinen, 1968; 1969, 1970), peat formation (Gottlich and Hornburg, 1982; van der Knaap, 1989), and rich macrofossil records (Birks, 1991; Alsos et al., 2016). On the contrary, Mangerud and Svendsen (2018) describe cooler conditions in Svalbard between c. 8800 and 8200 cal. yr BP. This is not apparent in our data, potentially because the two samples we have are from just before and just after, rather than from this cooler period.

Dryas appeared around 6400 cal. yr BP, suggesting a shift in vegetation from moist snowbed communities dominated by *Salix polaris* and *Bistorta vivipara* to the inclusion of semi-dry heath vegetation with *Saxifraga oppositifolia* and *Dryas octopetala* (Elvebakk, 1994). Similarly, Alsos et al. (2016) revealed a shift towards more dry tolerant taxa with an increase of Dryas after c. 6400 cal. yr BP in western Spitsbergen. This shift could be explained by a mid-Holocene increase in Fram Strait sea-ice extent, resulting in reduced moisture supply to Svalbard (Müller et al. 2012). Mollicus data indicate a cooling from 6500 cal. yr BP (Mangerud and Svendsen, 2018), suggesting a combined effect of cooling and drying.

Reestablishment and/or confident detection of thermophilous indicator species, such as *Empetrum*, *Cystopteris fragilis* and *Arnica angustifolia*, from c. 5500 to 5000 cal. yr BP, reflects high temperatures, consistent with suggestions by Luoto et al. (2017). This is also a period with high taxonomic richness recorded in sedaDNA data from a western Spitsbergen lake sediment core (Alsos et al., 2016).

### 5.1.3. Neoglacial environmental changes in the Late Holocene (c. 4300– 150 cal. yr BP)

The lithology and sedaDNA data show a distinct shift c. 4300 cal. yr BP, reflecting the onset of the Neoglacial period (4200 cal. yr BP; Fig. 3; Farnsworth, 2018; Bradley and Bakke, 2019). This period is characterized by an increasing amount of minerogenic material interrupting the accumulation of organic material and a shift in vegetation, combined with the first appearance of many new taxa (Figs. 3– 5). The overall increase in the number of taxa identified from sedaDNA can be explained by: 1) better DNA quality in recent samples due to younger age and/or cooler conditions during deposition, favouring preservation, 2) the cumulative effect of colonization over time, 3) sediment properties and/or 4) more frequent stochastic dispersal caused by extreme weather events. Some studies report higher diversity in more recent than older sediments (Pansu et al., 2015; Clarke et al., 2019b). However, other studies show higher diversity in older samples and from warmer periods (Alsos et al., 2016; Zimmermann et al., 2017a; Clarke et al., 2019a). Given the lower temperatures on Svalbard relative to the majority of these other studied regions, we do not believe that age or temperature during deposition within the ranges studied here had a major impact on number of detected species. Dispersal lags may have affected arrival of some species (see below), but neither pollen, macro, nor previous DNA records from Svalbard show a clear increase in diversity over time (Hyvärinen, 1970; van der Knaap, 1989; Birks, 1991; Bennike and Hedenås, 1995; Wohlfarth et al. 1995; Alsos et al., 2016). This suggests that colonization lags are not enough to explain the high increase in diversity in recent samples. We consider a combination of explanations 3 and 4 to be more likely. Notably, the marked increase in number of taxa from sedaDNA zone 2 to 3 is in concordance with a shift from algal gyttja to increased minerogenic input (LU2 to LU3). More fluctuations and lamination suggest increasingly stormy conditions, potentially with changes in main wind directions, bringing neighbouring terrestrial material into the catchment. The corresponding peaks in terrigenous input, coarse sediment signal, and white sediment colour are likely to reflect nival and/or aeolian deposition of sandy sediments (Rathe et al., 2018). More minerogenic input is often related to better DNA preservation (Torti et al., 2015). The increased diversity may thus be a direct effect of minerogenic input, potentially combined with input from outside the catchment. An increase in bryophytes combined with more variable runoff was also recorded in western Spitsbergen from around 5500 cal. yr BP (Alsos et al., 2016), suggesting that the vegetation shift is caused by a regional change in climate.

All dwarf shrubs are common in the Late Holocene period, but *Empetrum* disappears from the record after c. 3400 cal. yr BP. Local extinction of this shrub may be related to more competition with *Cassiope tetragona* and *Dryas octopetala*. Experimental warming studies in Svalbard have shown that *Empetrum nigrum* is favoured by warmer conditions, whereas it is outcompeted by *Cassiope tetragona* under cooler conditions (Buizer et al., 2012). This species replacement would suggest increasingly cooler Neoglacial conditions. However, the presence of several thermophilous indicator species (i.e. *Calamagrostis*, *Cassiope tetragona*, *Carex saxatilis* and *Carex paradoxa*) between c. 750-570 cal. yr BP suggests relatively warm local conditions. These contrasting patterns can be explained by regional glacial expansion in surrounding areas combined with a favourable local microclimate, as well as the ability of clonal plants to persist for long periods of climate deterioration (Alsos et al., 2002). Today, there are favourable conditions for plant growth as well as large ice caps in close proximity to Ringhorndalen, and it is likely that these conditions developed during the Neoglacial period (Gjerde et al., 2017; Miller et al., 2017).

The lithology of LU 4 (c. 4000 cal. yr BP; Fig. 3) indicates minerogenic-rich sedimentation with minimal biogenic accumulation. We only have two sedaDNA samples from LU 4, with highly
contrast species richness (10 vs. 44 taxa). The sediment source is likely nival, aeolian, or a combination of both. DNA binds better to smaller clay particles than to sand (Torti et al., 2015). Thus, the variation in species richness between these two samples may be due to taphonomic issues in LU 4.

5.2. Future prospects and implications for conservation of species

While half of the taxa recorded were already present in the oldest samples, the total species richness almost doubles over the 12,000-year period. Arrival time may relate to climate, dispersal mode, stochastic events and/or dispersal distance (Alsos et al., 2007, 2016). One of the major dwarf shrubs of the northern hemisphere, Empetrum, colonized the catchment as early as 10,400 cal. yr BP, about the same time as it appears in records from eastern Greenland (Bennike et al., 1999) and northern Norway (Clarke et al., 2019a). This species has low genetic structure, suggesting broad-fronted colonization, perhaps assisted by bird dispersal (Eidesen et al., 2013; Alsos et al., 2015). As the lake catchment of Jodavannet is outside the current distribution range of Empetrum (Fig. 1), our aDNA records support the interpretation that current distribution represents relics of a once wider distribution (Engelskjøn et al., 2003; Alsos et al., 2007). In contrast, Cassiope Tetragona is harder than Empetrum, but not detected in the record until 4900 cal. yr BP. This species was not found in northern Greenland until 7800 cal. yr BP (Wagner et al., 2010), probably due to a long migration route from the glacial survival site in Beringia across Canada and Greenland before its appearance in Svalbard, as indicated by genetic analyses (Eidesen et al., 2007).

Although some species are only recorded in the older samples, they are not regionally extinct (Eidesen et al., 2018). For example, the thermophilous species Cystopteris and Empetrum are not found in any samples from the Neoglacial period. Thus, their current restricted distribution in the valley (Fig. 1, Eidesen et al., 2018) may represent remnants of a once larger local population, as has also been inferred for other plant taxa (Alsos et al., 2002, 2007). The heterogeneous environment, together with exceptionally favourable climatic conditions for this high latitude, have ensured long-term persistence of plant species and confirm the high conservation value of this locality.

With ongoing climatic warming, we may expect thermophilous species recorded in the aDNA (Table 2) and further in the valley (Eidesen et al., 2018) to expand. There are also a range of thermophilous taxa in the neighbouring areas of Greenland, Fennoscandia, and Russia that may colonize Svalbard (Alsos et al., 2007) and would most likely establish at sites with favourable local climate, such as Ringhorndalen. Furthermore, increasing human traffic to the region increases the risk of anthropogenic species introductions (Alsos et al., 2015). This expanded expectation of thermophilous species may pose a threat to the less competitive, high-Arctic species in the region. However, Clarke et al. (2019b) found evidence of a refuge site for cold tolerant species from a 24,000-year old record from the Polar Urals. They suggest that mountainous landscapes may provide refuge sites for cold tolerant species in warmer climate. We believe that sites like Ringhorndalen, with high species richness and topographic diversity that facilitates for microclimatic variation, are important for long-term survival of both cold and warm adapted species.

6. Conclusions

Our combined geochemical and molecular evidence suggests that isolated populations of unusually thermophilous plant species found in Ringhorndalen today are relics from a more widespread distribution during warmer Holocene periods. Our results support previous evidence of an Early Holocene warming, indicating good agreement between marine and terrestrial archives. We show that Arctic hotspots like Ringhorndalen are not only important for the biodiversity today, but may represent sites of long-term survival of species, making them an important conservation priority.

Author contributions

PBE, LH, AS, and IGA developed the idea and supervised the study; WRF and AS cored the lake; AS, WRF, SEK, AR, and LH described and interpreted the lithology and identified plant macrofossils for radiocarbon dating; SEK conducted the LOI analysis; LHV did the age–depth model; LHV and PBE did the vegetation surveys; AR and WRF did the aDNA clean sub-sampling; LHV did the bioinformatics analyses; LHV drafted the manuscript with contributions from all co-authors.

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Appendix A. Supplementary data

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