Holocene plant diversity revealed by ancient DNA from 10 lakes in northern Fennoscandia

Dilli P. Rijal[1,2*], Peter D. Heintzman[1*], Youri Lammers[1], Nigel G. Yoccoz[2], Kelsey E. Lorberau[2], Iva Pitelkova[1], Tomasz Goslar[3], Francisco Javier Ancin Murguzur[2], J. Sakari Salonen[4], Karin F. Helmens[5,6], Jostein Bakke[7], Mary E. Edwards[1,8], Torbjørn Alm[1], Kari Anne Bråthen[2], Antony G. Brown[1,8], Inger G. Alsos[1]

[1] The Arctic University Museum of Norway, UiT - The Arctic University of Norway, Tromsø, Norway; [2] Department of Arctic and Marine Biology, UiT - The Arctic University of Norway, Tromsø, Norway; [3] Faculty of Physics, Adam Mickiewicz University, Poznań, Poland; [4] Department of Geosciences and Geography, University of Helsinki, Finland; [5]Swedish Museum of Natural History, P.O. Box 50007, 10405 Stockholm, Sweden; [6] Värriö Research Station, Institute for Atmospheric and Earth System Research INAR/Physics, P.O. Box 64, 00014 University of Helsinki, Finland; [7] Department of Earth Science, University of Bergen, Norway; [8] School of Geography and Environmental Science, University of Southampton, UK Southampton

Authors for correspondence: Dilli P. Rijal (dilliprijal@gmail.com, Tel: +47 77620786), Peter D. Heintzman (peter.d.heintzman@uit.no, Tel: +47 77620741) and Inger G. Alsos (inger.g.alsos@uit.no, Tel: +47 77620796).

*These authors contributed equally to this work.

Summary

- It is crucial to understand how climate warming and other environmental factors affect biodiversity, especially in the rapidly changing northern latitudes.
- We use sedimentary ancient DNA (*sed*aDNA) metabarcoding to estimate taxonomic richness, and local and regional species pools of terrestrial plants for 10 lakes in northern Fennoscandia over the Holocene.
- In total, 288 taxa were found in the 316 samples analysed, with local species pools of 89-200 and mean taxonomic richness of 21-65 per catchment. Quality control showed that *sed*aDNA is a reliable estimate of richness. Local and regional species pools showed a steep increase in the Early Holocene, when the highest rate of warming took place, and continued to increase through the Middle and into the Late Holocene, although temperature decreased over these periods. Only the regional species pool levels off during the last two millennia. Richness and local species pools were always higher in catchments with higher bedrock nutrient availability.
- We find *sed*aDNA to be a good proxy for diversity, opening avenues to detect patterns hereto unknown, and we provide a robust methodological approach to its application. Our findings suggest we can expect time lags and environmental factors to affect species richness also of the following global warming.

Keywords (5-8): Ancient DNA, metabarcoding, taxonomic richness, terrestrial plants, time lags, species pools

Introduction

Our ability to counter the current loss of biodiversity is dependent on how well we understand the causes of its global, regional and local patterns. However, the trajectory of biodiversity, especially in response to ongoing climate change, is debated (Gonzalez *et al.*, 2016; Suggitt *et al.*, 2019; Harrison, 2020; Le Roux *et al.*, 2020). Changes in species richness due to climate change, nutrient levels and species introductions are often context-dependent (Vellend *et al.*, 2017), and hence hard to predict. There is also a discrepancy among temporal biodiversity patterns at global, regional and local scales, and local processes may compensate or even counteract global trends (Pilotto *et al.*, 2020). Most evident is the discrepancy whereby the temporal decline in biodiversity at the global and regional scales does not match that of local scale where, on average, there is less or even no decline (Vellend *et al.*, 2013; Dornelas *et al.*, 2014; Blowes *et al.*, 2019). In addition, short-term studies may not detect the underlying long-term trends, and there is a need for longer time series at the regional and local scales (Gonzalez *et al.*, 2016; Nogués-Bravo *et al.*, 2018; Fordham *et al.*, 2020). Palaeobotanical proxies such as pollen (Giesecke *et al.*, 2012) and plant macrofossils (Birks & Birks, 2000) provide direct long-term evidence of plant biodiversity change. However, plant macrofossils are variably preserved (Allen & Huntley, 1999), and the problem of taxonomic resolution and known biases in pollen records, especially above the treeline, may also bias species richness estimation (Birks *et al.*, 2016a; Reitalu *et al.*, 2019). Recent studies indicate that sedimentary ancient DNA (*sed*aDNA) can provide higher taxonomic resolution and be better at detecting the local presence of plant species than macrofossils and pollen (Willerslev *et al.*, 2014; Alsos *et al.*, 2016; Parducci *et al.*, 2017; Clarke *et al.*, 2020), and it may therefore advance our estimates of long-term changes in species pools and richness. This paper uses the taxonomic and provenance advantage to produce the first multi-site estimate of changing vascular plant richness for northern Fennoscandia using *sed*aDNA.

The largest impact of ongoing climate change is expected at high latitudes (CAFF, 2013; Bjorkman *et al.*, 2018). Field and modelling studies have shown an increase in plant species richness (Niskanen *et al.*, 2019) and phylogenetic diversity (Thuiller *et al.*, 2011) at high latitudes in Europe as summer temperature increases. Further, comparative vegetation surveys of mountain summits across Europe show an increase in richness over the last 145 years (Steinbauer *et al.*, 2018). Similarly, an increase in richness has been found across the forest-tundra ecotone (Løkken *et al.*, 2020). Short-term observational studies, however, suggest that colonization by terrestrial species is lagging behind shifts in temperature isotherms (Lenoir *et*

al., 2020), which can be compensated on the short term by local extinction lags (Dullinger et al. 2012, 2013). Furthermore, a circumpolar study suggested that regional plant species richness is still affected by past glaciations, whereas local richness is determined by local habitat factors (Stewart *et al.*, 2016). Empirical and conceptual advances on how species pools affect biodiversity patterns are limited (Zobel, 2016), in part because constructing complete species pools is difficult (Lessard *et al.*, 2012). Therefore, studies addressing species pools and local richness at high latitudes and at different scales are warranted to further our understanding of biodiversity patterns.

Paleoecological studies, especially pollen analyses, have been widely used to estimate effects of climate changes on plant species richness (Willis *et al.*, 2010; Felde *et al.*, 2018; Giesecke *et al.*, 2019). A study in the boreal ecoregion of North America showed a homogenous decrease in richness over the Holocene in eastern North America, whereas a more heterogeneous pattern was found in western North America (Blarquez *et al.*, 2014). In Europe, pollen studies show an overall increase in richness over the Holocene for the Alps, temperate oceanic region, and continental region. However, the northern boreal region (Scotland, Fennoscandia, Iceland, Baltic States, NW Russia) show a deviating pattern with a peak around 12 000 calibrated years before present (cal BP), an overall decrease during Early Holocene (11 700-7000 cal BP) followed by an increase to nearly peak levels recent times (Giesecke *et al.*, 2019). The decrease in richness during the Early Holocene at mid to high latitude sites is ascribed to closure of the forest. To what extent dispersal lags are affecting the richness trend is debated. Giesecke et al. (2012) argue that if plant dispersal had been generally slow, then diversity in previously glaciated areas would be expected to increase over time (Giesecke *et al.*, 2012). As little change was found in palynological richness over the Holocene at three sites in Central Sweden, they argue that there is no evidence for delayed immigration of species affecting richness. In contrast, Felde et al. (2018) find increasing richness over the last 8000 years from 30 sites in Norway and two in northern Sweden and argue that the results are consistent with the hypothesis of post-glacial dispersal limitations. In the far north of Fennoscandia, a comparison among four lakes spanning a gradient from the northernmost spruce forest across birch forest to the shrub-tundra, shows an inconsistent pattern in palynological richness (Seppa, 1998). These studies highlight the challenges of comparing pollen richness across different vegetation zones.

As treelines have shifted over the Holocene (Seppä, 1996; Sjögren & Damm, 2019), sites in northernmost Fennoscandia would have been within or outside the treeline at different times,

complicating inferences based on pollen. *Sed*aDNA on the other hand, detects species growing within the lake catchment, has lower problems with swamping, no long-distance component, generally higher taxonomic resolution, and shows a strong correlation with the species richness of modern vegetation (Sønstebø *et al.*, 2010; Alsos *et al.*, 2018). When the two approaches are compared, *seda*DNA better reflects the local plant community and detects more forbs and overall more taxa than pollen analyses (Sjögren *et al.*, 2017; Zimmermann *et al.*, 2017a,b; Clarke *et al.*, 2020). Especially in a small catchment, *sed*aDNA may, therefore, also register the effect of drivers on a local rather than regional scale (Liu *et al.*, 2020).

Edaphic variation is hypothesized to strongly influence establishment, ecological drift, and niche selection, which all affect the local species pool, and this in turn affects species richness (Hulshof & Spasojevic, 2020). An overall greater species richness has been reported from calcareous as compared with siliceous bedrock areas in the eastern Swiss Alps (Holzinger *et al.*, 2008). Leaching produces neutral to acidic microenvironments and provides a mosaic of habitats which may promote species establishment and increase local richness (Holzinger *et al.*, 2008). A pertinent example of this is provided by Arnesen *et al*. (2007) who investigated the floristic diversity in a 120 km^2 mountain area of northern Norway containing both felsic and calcareous bedrock types. They found significant floristic differences between bedrock types with an overall higher Shannon's diversity index for the whole area than any of the individual bedrock types. Human land use may also increase soil fertility and thereby richness (Birks *et al*., 2016b), but the overall human impact in the north is low although not negligible (Sjögren & Damm, 2019). There is also evidence from Norwegian mountain systems that the trajectory of succession after glacier retreat varies due to abiotic factors, and particularly the trajectory of soil formation (Matthews & Vater, 2015).

Here, we analyse *sed*aDNA from 10 lakes in northern Fennoscandia to estimate taxonomic richness (Hill-N0), local species pools (accumulated number of taxa per catchment), and the regional species pool (accumulated number of taxa for all 10 lakes) over the last 11,700 years. Sites were selected at the northernmost outpost of boreal deciduous (birch) and coniferous (pine and spruce) forest as well as shrub-tundra sites in Northern Fennoscandia. After establishing chronologies for each site and quality checking the *sed*aDNA data, we trace temporal species diversity patterns throughout the Holocene on a subregional edaphic (nutrient) and climatic gradient. In doing so, we address the size of a range of local species pools through the Holocene and assess to what extent ecological limits may have regulated these pools. In particular we ask:

i) Does *sed*aDNA data reliably reflect richness through time?

ii) What are the temporal patterns in local species pools and do these patterns indicate that the species pools have reached a post-glacial equilibrium?

iii) What are the temporal patterns of species richness within localities and how do they relate to the local and regional species pools?

iv) To what extent has climate change, in terms of changing temperature, represented an ecological driver that modified richness through time?

v) To what extent have edaphic factors affected local species richness through time?

Materials and Methods

Study area, site selection, and fieldwork

The study area covers northernmost Fennoscandia above the Arctic Circle (at 67.75-70.43 N and 19.62-30.02 E) with nine lakes in Norway and one in Finland (Fig. 1, Table 1). We selected these 10 sites based on environmental and climatic variables, geographic spread, and vegetation types. Full geological and vegetation descriptions are in Methods S1 and Table S1. We retrieved sediment cores at seven sites and used previously collected cores for the remaining three. Coring was conducted using a modified Nesje piston-corer (Nesje, 1992), with a DNA tracer applied to monitor for contamination (Notes S1), or a rod-operated Multisampler (Methods S2). All cores were kept cold during transport to, and storage at, The Arctic University Museum of Norway in Tromsø (TMU).

Sampling, photography, and LOI analysis

We split 110 mm piston core sections longitudinally and sampled one half for *sed*aDNA and loss-on-ignition (LOI analysis) in a dedicated clean room facility at TMU (Methods S3). We took sampling negative controls to monitor for contamination. The remaining core half was retained for high-resolution imagery. We extruded and sampled Multisampler cores in the same dedicated clean room. For three previously collected cores (Otterå, 2012; Wittmeier *et al.*, 2015), we took *sed*aDNA samples in the clean labs at the Department of Earth Science, University of Bergen, Norway (Langfjordvannet, Jøkelvatnet) or in the Physical Geography department at Stockholm University, Sweden (Kuutsjärvi), taking sampling negative controls as above. We performed high-resolution imagery at the Department of Geosciences, The Arctic University of Norway in Tromsø. We calculated dry mass LOI by igniting the sample at 550 °C (Heiri *et al.*, 2001) (Methods S4).

Composite core construction and age-depth models

We either opportunistically collected macrofossils during sampling or systematically sieved for them from our seven newly collected cores; these were used for radiocarbon (^{14}C) dating (Methods S5). All macrofossils were photographed and identified. We used Accelerator Mass Spectrometry (AMS) at the Poznań Radiocarbon Laboratory of the Adam Mickiewicz University, Poland for ^{14}C dating. For multiple-core records from the same site, we created composite core records based on alignment of LOI values, visible stratigraphy, and/or radiocarbon dates (Methods S5; Fig. S1**a-b, d-f**; Tables S2, S3). We constructed Bayesian

age-depth models for all sites using *bacon* v.2.3.4 (Blaauw & Andrés Christen, 2011) in *R* v3.4.4 (R Core Team, 2019). For previously collected cores, we constructed age-depth models using published information (Jensen & Vorren, 2008; Otterå, 2012; Wittmeier *et al.*, 2015) (Methods S5; Notes S2).

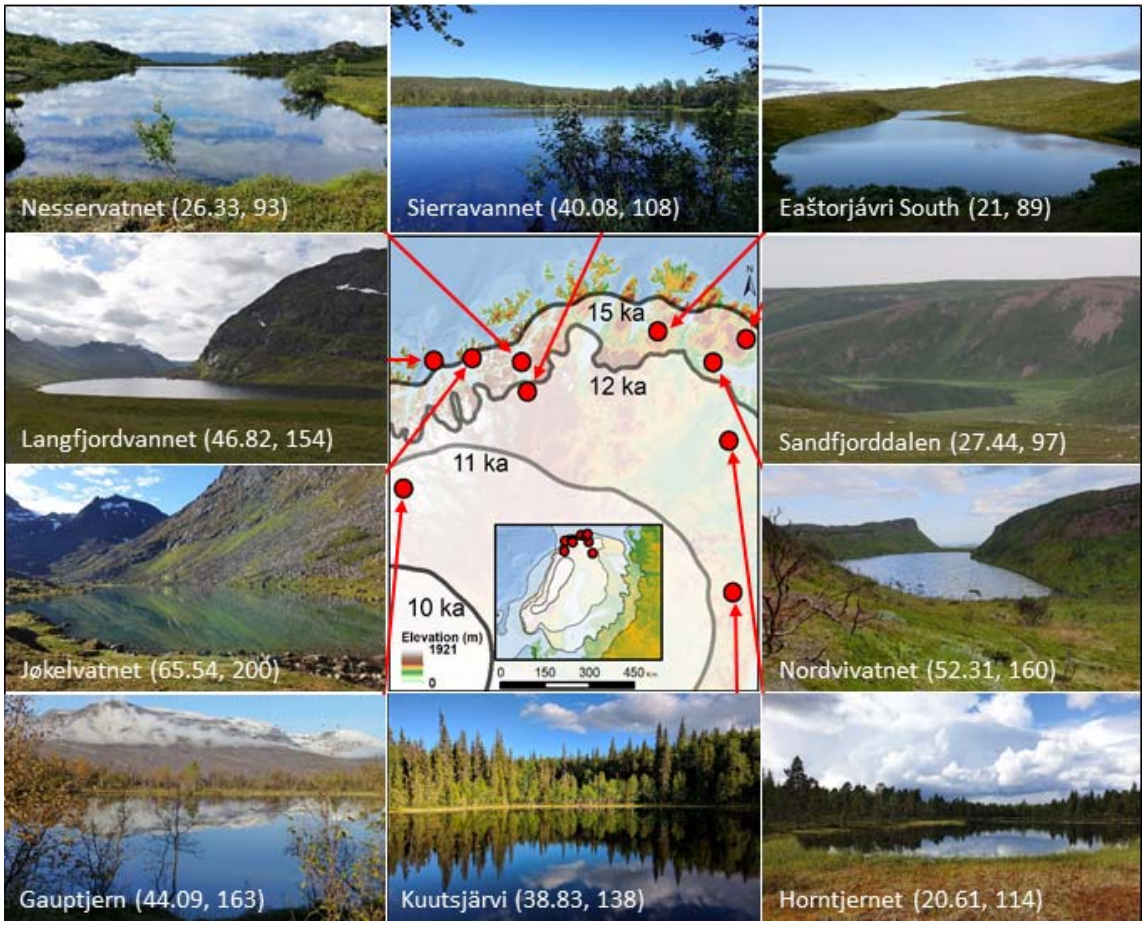

Fig. 1 Digital elevation map of the study area (data source: European Environment Agency). The extent of the Scandinavian ice sheet (the most credible extent of Hughes *et al.*, 2016)) at 15 000, 12 000, 11 000, and 10 000 cal BP are indicated by transparent layers. Inset shows the extent of the Scandinavian ice sheet at 21 000, 15 000, 12 000 and 10 000 cal BP. Lake names are followed by mean taxonomic richness and total taxa recorded in each lake (local species pool). See Table 1 for further site information. Photo credit: Jøkelvatnet, Lasse Topstad; Sandfjorddalen, Leif Einar Støvern; Langfjordvannet & Eaštorjávri South, Dilli P. Rijal; Kuutsjärvi, Karin Helmens; all others, Inger G. Alsos.

<u>Sedimentary ancient DNA data generation</u>

All pre-PCR steps were performed in the dedicated clean room facilities at TMU. We homogenized DNA samples and extracted DNA from 0.25-0.35 g of sediment (Table S4),

following the Zimmermann *et al*. (2017b) protocol, as modified by Alsos *et al*. (2020). We included one negative extraction control for every 10 sediment extractions. We also extracted DNA from 16 samples using alternative protocols (Methods S6). We amplified DNA and control extracts using unique dual-tagged primers (Taberlet *et al.*, 2007) that target the vascular plant chloroplast (*trn*L p6-loop locus) (Methods S6; Notes S3; Table S5). We pooled and then cleaned up to 384 PCR products following Clarke et al. (2019a). Each amplicon pool was then converted into a DNA library at either FASTERIS, SA (Switzerland) or in-house at TMU. We sequenced each library on a 2x 150 cycle flow cell, either on the Illumina NextSeq or MiSeq platform, at either FASTERIS or the Genomics Support Centre Tromsø (GSCT) at The Arctic University of Norway in Tromsø.

Bioinformatics and data quality control

We followed a bioinformatics pipeline that uses a combination of the ObiTools software package (Boyer *et al.*, 2016) and custom R scripts (available at https://github.com/Y-Lammers/MergeAndFilter). We merged and retained overlapping paired-end reads, demultiplexed the data based on PCR primer tag, collapsed identical sequences, and removed putative artifactual and low abundance sequences (Methods S7). We identified the remaining sequences using two reference databases; *ArctBorBryo* (Sønstebø *et al.*, 2010; Willerslev *et al.*, 2014; Soininen *et al.*, 2015) and *EMBL* (rl133). We checked for the presence of, but did not detect, the DNA tracer (Notes S1). We removed identified sequences that matched *blacklists* of potentially erroneous sequences and known contaminants (https://github.com/Y-Lammers/Metabarcoding_Blacklists; Table S6), and other low-abundance data (Methods S7). Final taxonomic assignments were determined using regional botanical taxonomic expertise and followed the taxonomy of the Panarctic Flora (Elven *et al.*, 2011) and Lid's Norsk Flora (Elven *et al.*, 2005), with only terrestrial vascular plants and bryophytes retained for all downstream statistical analyses (Table S6). We only included Holocene-aged (11 700 cal BP to present) samples for downstream analysis. We use the term *taxonomic richness* to include taxa identified to various ranks from the species to family levels.

We developed two statistics to account for data quality differences between samples both within and between lake records: the *metabarcoding technical quality (MTQ)* score to assess metabarcoding success on a per-sample basis, and a *metabarcoding analytical quality (MAQ)* score to assess the success of recovering sequences of interest (Notes S4). We required an *MTQ* of ≥0.75 and *MAQ* of ≥0.2 to pass quality control (QC), which excluded all negative

controls (Fig. S2). If DNA was extracted more than once from a sample, then we selected data from the DNA extract that yielded the greatest *MAQ* score (Table S7). We examined the relationships between observed taxonomic richness and time against four potential impactors of data quality (1) total count of raw reads (summed across PCR replicates), (2) mean barcode length (in base pairs, bp) across all retained barcodes, (3) mean proportion of weighted PCR replicates (*wtRep*; see Notes S5 for definition) across all final barcodes, and (4) proportion of raw reads assigned to terrestrial plant taxa (Methods S8; Notes S6).

Numerical and statistical analyses

We measured taxonomic richness (diversity) based on Hill numbers (N0 and N1) (Hill, 1973; Birks *et al.*, 2016b) using *wtRep* (Notes S5; Methods S9). We calculated rarefied taxonomic richness based on the lowest number of reads assigned to a sample within a lake, and calculated its correlation with Hill N0 (Table S8). We used generalized additive models (GAMs) (Wood, 2017) to evaluate temporal biodiversity changes during the Holocene (Methods S9). We treated Hill N0 and N1 as the response, and median calibrated age of the samples as predictor variables, and used the "poisson" family with log link. To account for residual temporal autocorrelation between samples, we also included a continuous time first-order autoregressive process (CAR(1)) in generalized additive mixed models (GAMM; (Simpson, 2018)). We found near identical results for taxonomic richness trends between GAM and GAMM models (Fig. S3; Tables 2, S9). In the case of two shorter cores from Nesservatnet and Sierrvannet, GAMM(CAR(1)) provided a reasonable fit to the data, and hence was included in the main results.

We evaluated how local and regional species pools affected richness estimates at their respective scales. In our case, the regional species pool is the total number of taxa found across all samples. In addition, we also generated a regional species pool for each 500-year time bin. We define a local species pool as the number of taxa recorded within a lake. First, we calculated and compared the actual and estimated cumulative number of taxa and taxonomic richness of samples through time by combining all the samples. We also used GAM to highlight the regional trend in taxonomic richness through time. We then performed linear regression by considering the mean number of taxa within a lake and within a 500-year time bin as the response variables, and the respective species pools as the predictor variables to test whether observed richness is correlated with the species pools of respective scales.

To examine the relationship of climate and diversity estimates, we have used oxygen isotope ($\delta^{18}O$) values from the North Greenland Ice Core Project (NGRIP) (Andersen *et al.*, 2004) as a proxy for temperature. We evaluated how temperature affected the richness pattern of the different Holocene periods (Early: 11 700-8300, Middle: 8300-4250, and Late: 4250-0 cal BP following (Walker *et al.*, 2019)) by comparing regression slopes of the Middle and Late Holocene to the Early Holocene using a linear mixed model with taxonomic richness as the response and an interaction between $\delta^{18}O$ and the Holocene period as predictor along with lakes as the random variable.

We used a new semi-quantitative nutrient index derived from the sum of the phosphorus, potassium, and calcium content of the bedrock modified by a measure of weatherability (Methods S9). We performed linear regression treating mean taxonomic richness of different periods of the Holocene as the response and nutrient index as the predictor. Unless otherwise stated, all analyses were performed using the *vegan* (Oksanen *et al.*, 2019), *mgcv* (Wood, 2017), and *ggplot2* (Wickham, 2016) packages in *R*.

Results

The dating of the individual DNA samples was dependent on the age-depth models for each lake. Since the cores were all central or near central lake locations and the lakes were medium-small with in most cases only one depositional basin, the age-depth curves were approximately linear or curvilinear with three exceptions (Fig. S1). Sandfjorddalen had a step in the sedimentation rate with possible hiatus in the Early Holocene (11 000-8000 cal BP) and again in the Late Holocene between 6000 and 2000 cal BP (Fig. S1**j**). This probably reflects its position in the valley floor as a flow-through lake. Sierravannet had a distinct upturn in the accumulation rate around 600 cal BP to present (see Notes S2; Fig. S1**d**) and Kuutsjärvi had a distinct reduction in sedimentation rate from around 4000 cal BP (Fig. S1**i**). Some age-depth models showed minor concavities and convexities, which was common, with the concavities typical of deeper funnel-shaped lakes and the convex models typical of more trapezoidal lakes (Bennett & Buck, 2016). For the interpretation of the *sed*aDNA records the age-depth models provided similar temporal resolution with all except one (Sierravannet) being in the range of 158-616 years per sample. Six of the sedimentary records covered the entire Holocene (Fig. S1) and all except one (Sierravannet) covered the three periods of the Holocene, although the usable Nesservatnet record was reduced to the Late Holocene after removal of low quality *sed*aDNA samples (see below).

Across our 10 lake sediment records, we generated 91.6 million raw sequence reads from 387 sediment samples and 90 control samples. We retained 316 samples after removing duplicates and applying our QC thresholds (Fig. 2), with 12-55 samples retained per record (Tables S4, S10; Fig. S4). Based on our measures of *sed*aDNA data quality, we found that the *MTQ* and *MAQ* score QC thresholds removed the worst performing samples. The records with the best *sed*aDNA quality are Gauptjern, Horntjernet, Nordvivatnet, Sandfjorddalen, and Sierravannet. Samples from the Early Holocene should be treated with caution from the Eaštorjávri South, Kuutsjärvi, Langfjordvannet, and Jøkelvatnet records (Notes S6; Figs. S5-12).

We retained 402 barcodes, which were collapsed to 346 taxa with between 89-200 taxa recorded from each lake record (Table S10). Of these, 50% could be assigned to the species level (Tables S6, S11). As our focus was on the terrestrial plant diversity, we excluded 13 algae and 36 aquatic taxa. Nine taxa were only present in samples that failed QC. Thus, our final dataset retained 288 terrestrial plant taxa detected in 316 samples.

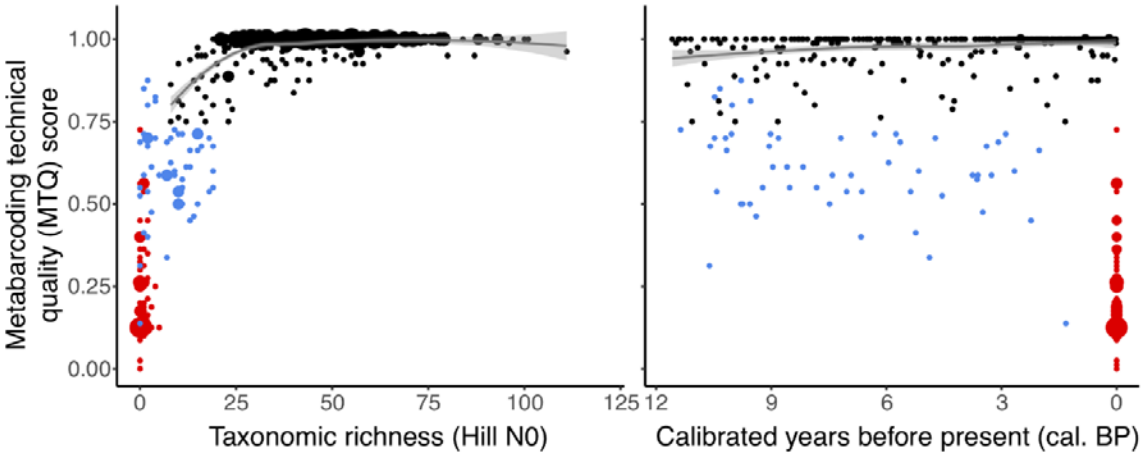

Fig. 2 Sample age minimally impacts metabarcoding technical quality across the entire data set. A fuller discussion of sample quality metrics are presented in Notes S6 and Figs S5-12. Data in black, samples that passed quality control (QC); blue, samples that failed QC; red, negative controls. Fitted loess-smoothed lines are for samples that passed QC.

Species pool and richness within the 10 catchments

The local species pools increased over time for all catchments with the highest numbers recorded at Jøkelvatnet (200 taxa), which today drains a catchment that has a Late-Holocene glacier in its upper reaches (Fig. 3). Rich species pools were also found at Gauptjern, which is at the border between pine and birch forest, and at Nordvivatnet and Langfjordvannet, which have a mixture of heathland, birch forest and scree slope in their catchments. Somewhat lower species pools were found at the two sites in pine forest, Horntjernet and Kuutsjärvi, and at Sierravannet, a site with birch forest, and pine and larch plantations. The two shrub-tundra sites, Eaštorjávri South and Sandfjorddalen, had smaller species pools, similar to Nesservatnet, which is surrounded by heathland/mires (93 taxa) and located on the small island of Årøya.

There were clear differences among lakes both in the overall levels of richness and in the change in richness over the period (Fig. 3). The mean taxonomic richness (Hill N0) ranged from 20.6 (± 6.4) at Horntjernet to 65.5 (± 24.5) at Jøkelvatnet, whereas Hill N1 ranged from 14.9 (± 7.8) at Eaštorjávri South to 52.4 (± 20.5) at Jøkelvatnet (Table S10). The rarefied richness based on the number of reads showed a strong correlation with observed taxonomic richness (R=0.82-0.99, Table S8), suggesting that the observed pattern was not affected by

sequencing depth. The Hill N1 (common taxa) showed temporal patterns that mirrored those of observed taxonomic richness for all the lakes except Sierravannet (Fig. 3).

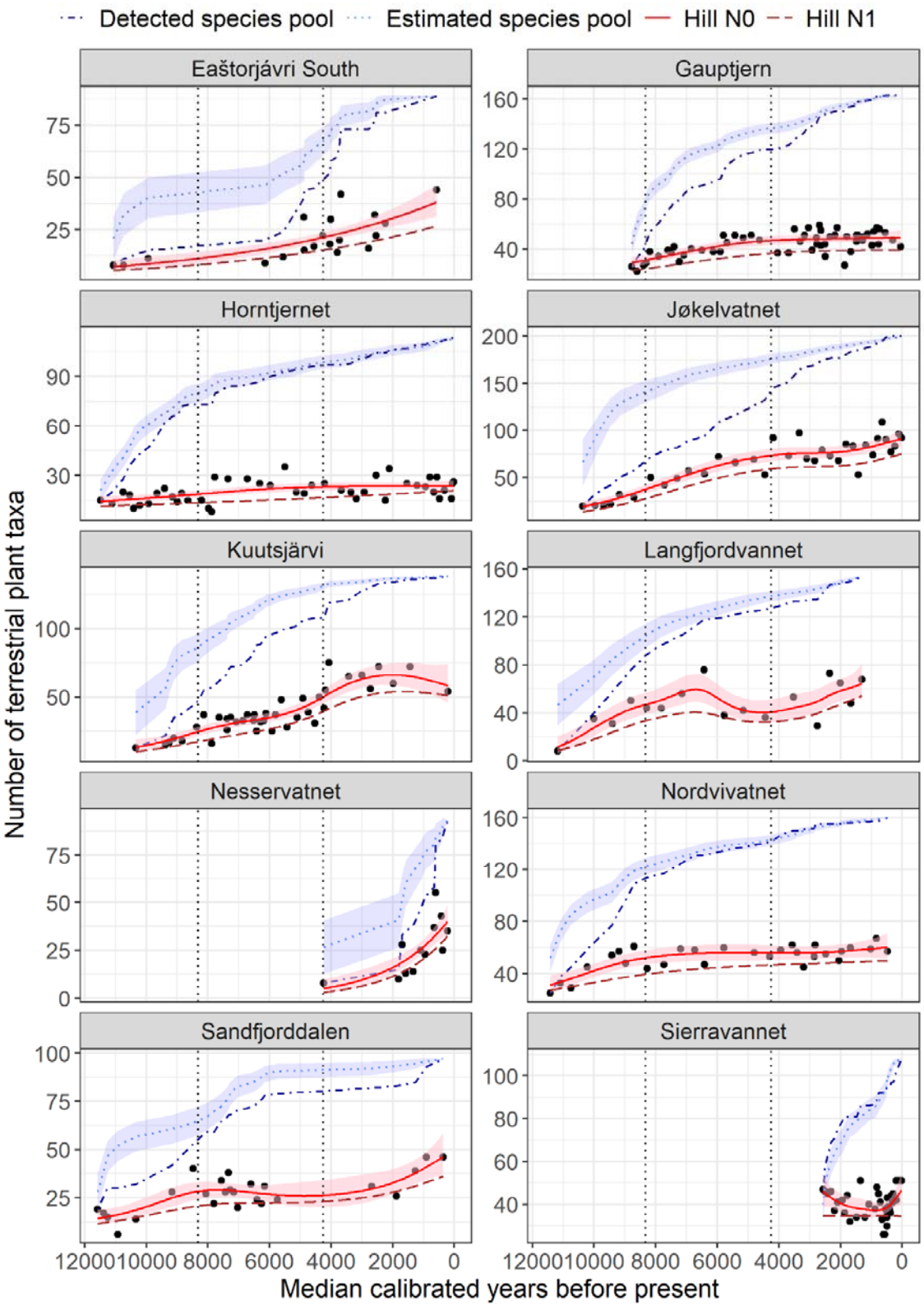

Fig. 3 Temporal pattern of local terrestrial plant richness at 10 lakes from northern Fennoscandia. Observed taxonomic richness (Hill N0) is the solid red line with 95% confidence intervals in pink shading. The fitted lines for Hill N1 are indicated by a dashed brown line. The development of local species pool is expressed in terms of detected cumulative number of taxa (blue dot-dashed line), and an estimate of cumulative number of taxa (blue dotted lines) based on 1000 permutations along with its ± 1 standard deviation (blue shading) through time. Hill N1 mirrors observed taxonomic richness (Hill N0). The Early (11 700-8300 cal BP), Middle (8300-4250), and Late Holocene (4250-0 cal BP) periods are indicated by dotted vertical lines. Note difference in scale on the y-axes.

We observed a significant effect of the age of samples on taxonomic richness as indicated by statistically significant smooth terms in GAMM models (Table 2), except for Sierravannet, which only covered 2600 years, and where diversity suddenly dropped around 670-515 cal BP, corresponding to a putative flood event (see Notes S2; Fig. S13). For two of the lakes, Eaštorjávri South and Nesservatnet, a near linear pattern of increase in taxonomic richness through time (edf=1) was recovered. On the other hand, Langfjordvannet had the most complex pattern of increase in richness (edf=5.93, Table 2). The steepest increase was seen in the Early and Middle Holocene for most lakes. Only at three sites, Nordvivatnet, Horntjernet and Gauptjern, did richness reach plateau during the Late Holocene; for most lakes no levelling off was observed suggesting that richness is still increasing (Fig. 3).

Regional species pool and richness

During the Early Holocene, there was a strong increase in both estimated and detected regional species pool size (Fig. **4a**). The detected species pool begins to stabilise in the period 7000-5000 cal BP, after which it showed a small increase again from 5000-3800 cal BP, before again stabilising from around 4000 cal BP. For the last two millennia, the estimated increase in species pool has reached a plateau, with only 4 taxa estimated (1.34% of the total). Similarly, the detected species pool also levelled off over the last two millennia with an increase of 10 taxa (3.5% of the total).

The mean (±se) predicted taxonomic richness (Hill N0) based on a GAM showed a steep increase during the Early Holocene (11 500-8500 cal BP) from 13.8±3.9 to 31.8±1.5 taxa per sample when evaluated using 500-year time windows. The richness continued to increase

during the Middle Holocene (8000-4500 cal BP, 33.4±1.5 to 42.7±1.3), and showed only a minor increase during the Late Holocene (4000-500 cal BP, 43.7±1.3 to 45.9±2.0).

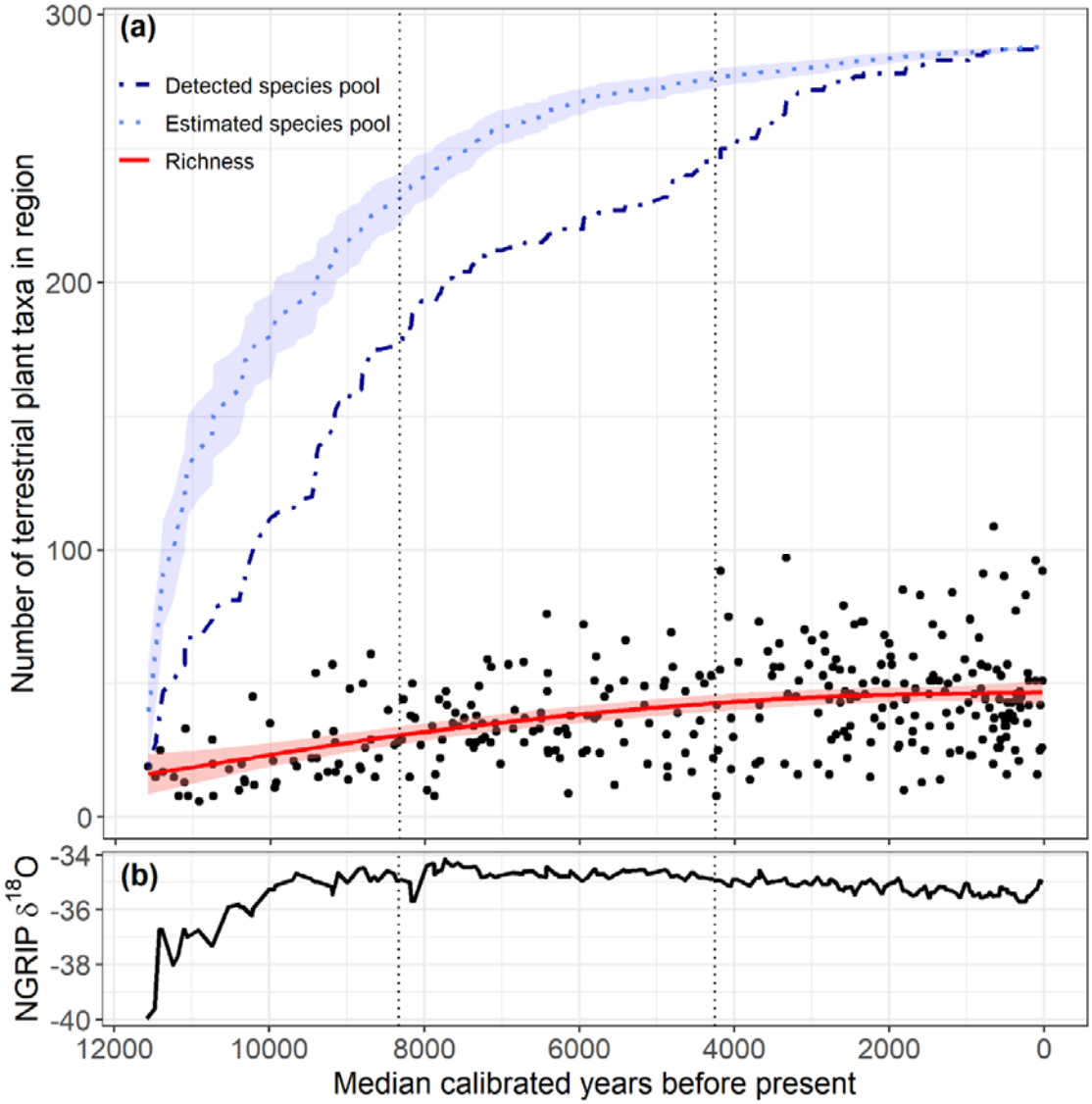

Fig. 4 Temporal pattern of regional taxonomic richness and temperature. (a) Accumulation of detected (dot-dashed line) and estimated (± 1 standard deviation, blue shading) regional species pool (defined as accumulative count of taxa) as well as number of taxa detected per sample (n=316) along with the 95% confidence interval (pink shading) of the fitted Generalized Additive Model (solid red line). The overall patterns remain the same also when excluding two shorter cores spanning only the Late Holocene (Fig. S14), and (b) variation in temperature reflected by NGRIP $\delta^{18}O$ values (Andersen *et al.*, 2004). The Early (11 700-8300 cal BP), Middle (8300-4250 cal BP), and Late Holocene (4250-0 cal BP) periods are indicated by dashed vertical lines.

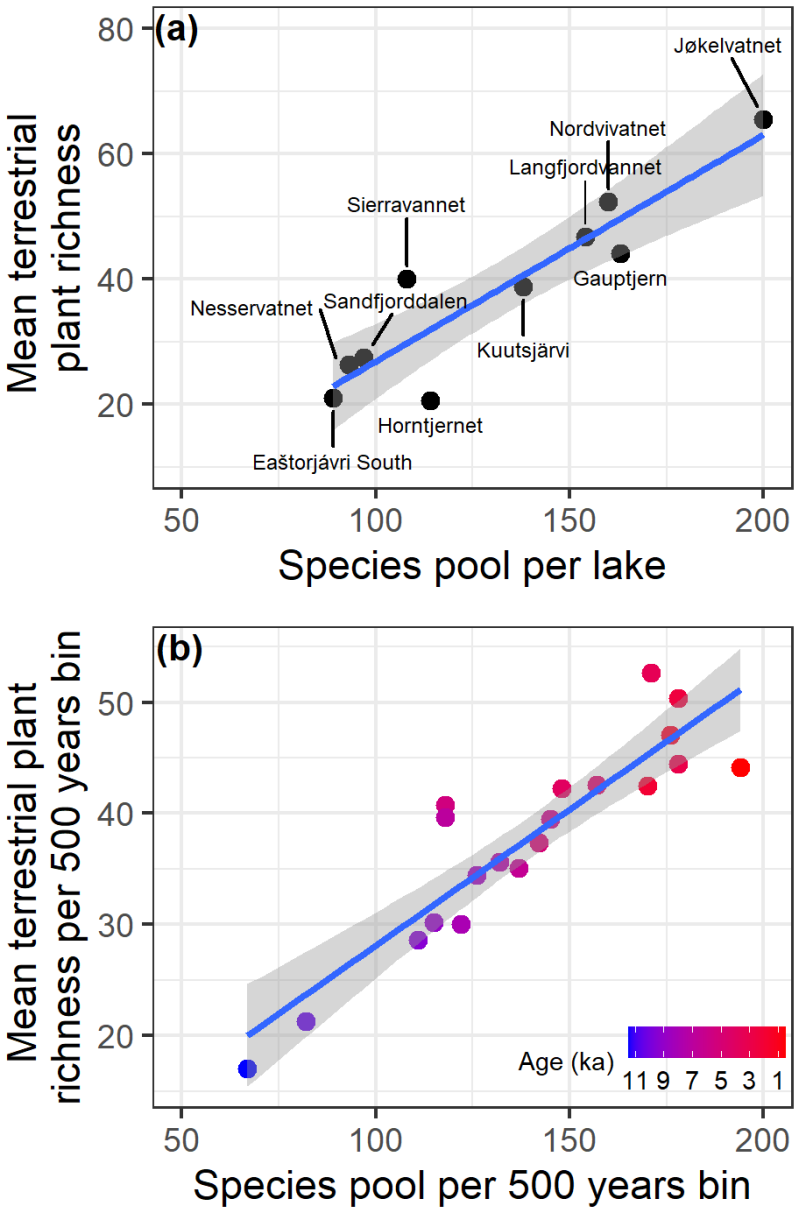

Fig. 5 Relationship between species pool and taxonomic richness. Correlation between (a) local species pool (total number of species observed over the Holocene) for individual lakes and (b) regional species pool (total number of taxa observed per 500-year time bins) and the respective mean taxonomic richness of terrestrial plants in northern Fennoscandia.

Richness in relation to local and regional species pool

There was a strong positive association between mean terrestrial plant richness and the local species pool of lakes, where 82% of the variation in local richness was explained by local species pool (R^2_{adj}=0.82, p<0.001, df=8; Fig. **5a**). The mean richness of lakes represented

about 24% to 37% taxa of the local species pool, except at Horntjernet, where richness represented only 18% taxa of the local species pool. The mean local richness increased by nearly four taxa (regression slope=0.36) when 10 taxa were added in the local species pool. Similarly, we found a strong positive correlation between mean richness per 500-year time period and total taxa available in the respective period, and 86% of the variation in richness was explained by the regional species pool (R^2_{adj}=0.86, p<0.001, df=21; Fig. **5b**) where c. 23% to 39% of the taxa from the regional species pool were represented by the mean richness. The mean regional richness increased by more than two taxa (regression slope=0.23) with the addition of 10 taxa in the regional species pool.

Impact of regional climate on richness

Climate had a significantly positive effect on richness in the Early Holocene (p<0.001), a marginal negative effect in the Middle Holocene (p=0.048), and a clear negative effect in the Late Holocene (p<0.001; Fig. **6a**; Table S12). In the Early and Late Holocene, temperature changed linearly through time with the rate of change of 0.92 (SE=0.07) and -0.13 (SE=0.01) $\delta^{18}O$/1000 year respectively, whereas there was no overall change in temperature during the Middle Holocene (Fig. **4b**).

Effect of nutrient/bedrock on richness

We observed a positive correlation between nutrient index and taxonomic richness for all three time periods although this was not significant for the Early Holocene (R^2_{adj}=0.36, p=0.07, df=6), which also had the smallest sample size. The correlation was stronger for the Middle Holocene (R^2_{adj}=0.51, p=0.02, df=7) than the Late Holocene (R^2_{adj}=0.35, p=0.04, df=8; Fig. **6b**; Table S13). The effect of nutrient index on taxonomic richness was strongest when the impact of climate was negligible during the Middle Holocene. This suggests that a significant cause of site-to-site variation and sub-regional richness patterns was soil nutrient availability which is dependent upon the bedrock and the rate of weathering.

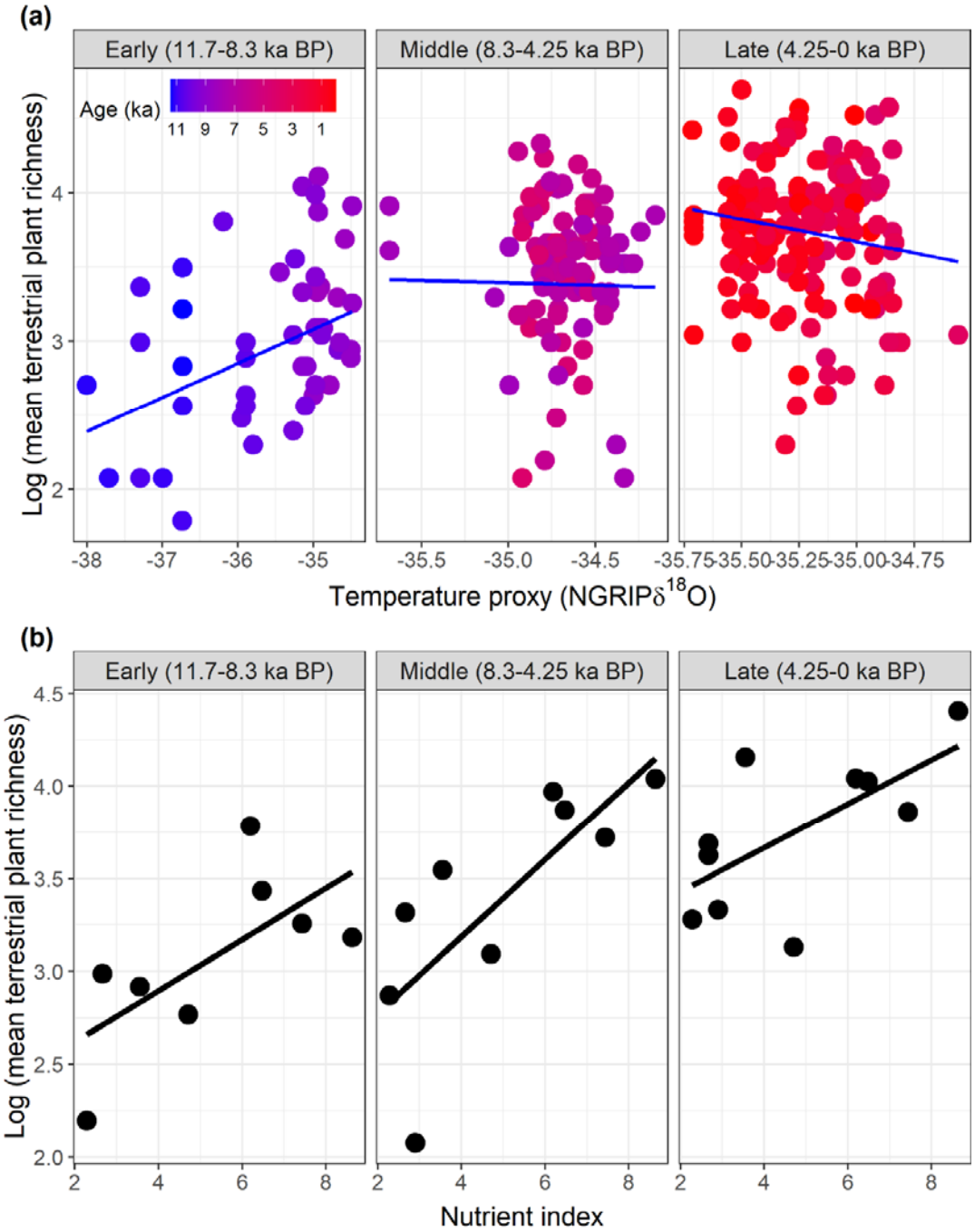

Fig. 6 Impact of climate and nutrient index on observed taxonomic richness. (a) Linear mixed effect model showing impact of regional climate on taxonomic richness of terrestrial plants for the different periods of the Holocene. Two samples with NGRIP $\delta^{18}O$ smaller than -39 were not included in the analysis. See Table S12 for the summary statistics. Note difference in scale on x-axes. (b) Linear models showing spatial pattern of mean taxonomic richness of terrestrial plants along nutrient index for different periods of the Holocene. See Table S13 for summary statistics.

Discussion

The ability of *sed*aDNA to capture plant taxonomic richness

The mean observed richness (Hill N0) of terrestrial plants found per sample and site (~21-66) is higher than that recovered for northern boreal sites based on pollen analyses (~20 taxa, Seppä, 1996; Giesecke *et al.*, 2019), but similar to pollen estimates from the Alps and Mediterranean (~30 taxa, Giesecke *et al.*, 2019). The detected richness values are within the range that has been found in other recent studies of *sed*aDNA from northern sites (20-70 taxa per sample, Zimmermann *et al.*, 2017a; Clarke *et al.*, 2019b; Liu *et al.*, 2020); although some shrub tundra (8.4 per sample, Crump *et al.*, 2019; ~13 per sample, Clarke *et al.*, 2019a) and High Arctic (15.9 per sample, Alsos *et al.*, 2016; 5-30 per sample, Voldstad *et al.*, 2020) sites notably have lower estimates. One should be aware that *sed*aDNA analyses, based on p6-loop metabarcoding, also have taxonomic biases, as some species-rich families such as Salicaceae, Poaceae and Cyperaceae are poorly resolved due to haplotype sharing (Sønstebø *et al.*, 2010; Willerslev *et al.*, 2014). Nevertheless, our results are consistent with other *sed*aDNA analyses that detect more taxa than pollen counts (Parducci *et al.*, 2017; Clarke *et al.*, 2020; Liu *et al.*, 2020). Together with improved geographic fidelity, *sed*aDNA thereby improves our understanding of the geographical patterns and scale dependency of past plant diversity.

The temporal patterns evaluated here rely on the assumption that our ability to detect plant taxa in *sed*aDNA is not impacted by differential preservation, due to sample age for example, or methodological problems such as DNA extract inhibition (e.g. Murchie *et al.*, 2020). Here we discarded samples of poor quality that had metrics comparable to negative controls and thus may have been affected by methodological problems, and broadly examined the quality of the retained samples. Half of our sites showed no evidence of declining *sed*aDNA with sample age, whereas the remainder had reduced quality in the Early Holocene interval. That our samples generally exhibited good *sed*aDNA quality throughout the study interval is likely due to a combination of excellent DNA preservation in the cold environments of high latitudes (Smith *et al.*, 2001) and the young age of the samples (<11 700 years) relative to the upper limit of ancient DNA preservation (~1 million years, Lindahl, 1993; Willerslev *et al.*, 2007; Orlando *et al.*, 2013). As multi-site *sed*aDNA studies become common, it will be crucial that data quality is scrutinized and, where possible, standardized to allow for biologically meaningful comparisons between sites.

Nutrient availability and plant richness

In considering the positive association between nutrient index and mean taxonomic richness of lakes for different periods of the Holocene, we highlight that our nutrient index is based on bedrock weathering, and the release of P, K and Ca, which acts as a surrogate for alkalinity. During the Early Holocene, it is likely that nutrient release started immediately after deglaciation when mean annual temperatures exceeded 4 °C. At this temperature, liquid water was abundant (Hall *et al.*, 2002) and light-demanding and disturbance-tolerant pioneer species could have survived on the nutrient-poor microhabitats, and thus showed weak overall association with the nutrient index. With continued warmer, and possibly wetter conditions, leaching and nutrient release would have increased thereby promoting richness in the Middle and Late Holocene. It is relevant here that the calcareous/acidic bedrock spatial pattern in northern Fennoscandia is small-scale, with small, often linear outcrops of metamorphic carbonate. This contrasts with the large limestone blocks/massifs found in younger geologies such as the European Alps, which have been shown to have effects on diversity over both short and long timescales (Gobet *et al.*, 2000; Holzinger *et al.*, 2008). Given that there is also a positive association between nutrient index and total richness (potentially representing a good subset of the regional species pool), it is reasonable to consider nutrient index as an important driver for species pool development and hence regional richness. Indeed, it is the floristic variation between sites that is the cause of a large difference between the local and regional species pools even today (Gough *et al.*, 2000; Arnesen *et al.*, 2007).

A steep Early Holocene increase in plant richness

The highest rate of increase in richness, and local and regional species pools, is observed in the Early Holocene 11 700-8300 cal BP. Due to their significant correlation, we cannot distinguish the effect of time, in the form of dispersal lags, from temperature, and both factors likely contributed to the observed increase in diversity. Climate was also the driver for deglaciation, which increased the area of landscape available for colonisation. Three of our records span a longer time period than examined here (Langfjordvannet: 16 700, Nordvivatnet: 12 700, Sandfjorddalen: 12 500 cal BP; Fig. S1**e,g,j**), and they, as well as macrofossils from Jansvatnet (14 500 cal BP, Birks *et al.*, 2012) and pollen records (Prentice, 1981; 13 900 cal BP, Huntley *et al.*, 2013), show that an Arctic pioneer vegetation established towards the end of the Younger Dryas period and into the Early Holocene. Thus, a species pool already existed at least along the coast at the start of our study period, whereas some of

the inland sites (Gauptjern, Horntjernet, Kuutsjärvi) represent records that deglaciated after the onset of the Holocene. Nevertheless, all sites exhibit a strong increase in richness independent of location relative to deglaciation.

Especially during the rapid warming at 11 700-10 000 cal BP, we find a high increase in richness. Factors other than climate and availability of land may have influenced richness in this period. For example, biotic factors such as low competition may have facilitated establishment (Pellissier *et al.*, 2010), and abiotic factors, particularly paraglacial processes, may have produced disturbance at the local scale (Ballantyne, 2002). On the other hand, dispersal lags may have limited richness and species pools, as for example a 400-year time lag between climate and arrival of birch woodland has been estimated based on macrofossils (Birks, 2015). Nevertheless, the overall rapid increase in diversity in an early phase of colonization is also recorded in pollen studies (Birks & Birks, 2016; Giesecke *et al.*, 2019), and expected given that they cover the development from pioneer to established vegetation communities.

Our richness patterns show a continued strong increase after around 11 000 cal. BP, when the major expansion of birch forest took place, and 10 000 cal. BP when pine expanded into the region (Seppa, 1998). Thus, in contrast to the decrease in richness due to forest expansion observed in pollen studies (Birks *et al.*, 2016b; Giesecke *et al.*, 2019), we found a general increase in richness through time. This may be because *sed*aDNA analyses are less sensitive to swamping by trees than pollen analyses and therefore better reflect habitat complexity (Sjögren *et al.*, 2017; Clarke *et al.*, 2020; Liu *et al.*, 2020).

Middle Holocene dispersal lags

The moderate increase in local and regional species pools during the Middle Holocene (8300 - 4200 cal BP) was not directly related to climate. The NGRIP record shows a peak (end of the Holocene Thermal Maximum) then slight cooling during this period. This is in accordance with reconstructions of local climate in northernmost Fennoscandia based on macrofossils and pollen, although local variation does exist, especially due to the proximity of the Norwegian Coastal Current, which is an extension of the Atlantic Gulf Stream (Allen *et al.*, 2007; Huntley *et al.*, 2013; Eldevik *et al.*, 2014). Richness levelled off in only two lakes (Nordvivatnet and Sandfjorddalen) and one lake (Langfjordvannet) showed a hump in richness, which we assume is due to local factors as all other lakes showed a moderate increase. For Gauptjern, palynological richness fluctuates around 8 taxa for this period

(Jensen & Vorren, 2008), whereas our *sed*aDNA data show a clear increase. Pollen studies show that two of four sites along a spruce-pine-birch tundra transect show stable levels of richness throughout the Middle Holocene (Seppa, 1998). An increase in richness has also been observed in pollen studies at most sites studied in Norway (Felde *et al.*, 2018). The closest sites studied for *sed*aDNA show stable richness at Varanger in Finnmark (Clarke *et al.*, 2019a), increasing (Voldstad *et al.*, 2020) or decreasing richness as in Svalbard (Alsos *et al.*, 2016), and fluctuating high richness in the Polar Urals (Clarke *et al.*, 2019b). Further south, pollen analyses show an increase in richness during the Middle Holocene in Southern Sweden and Germany, whereas three sites in Central Sweden level off during this period (Berglund *et al.*, 2008; Giesecke *et al.*, 2012). Seen from a European perspective, our richness curves are similar to those found in the nemoral zone of Europe, where increase is inferred to be due to human impact, but they differ from those of the boreal zone (Giesecke *et al.*, 2019), probably due to lower influence of Holocene tree expansion in the *sed*aDNA data. Thus, in contrast to many pollen studies, our *sed*aDNA data show an increase in richness and species pool for the Middle Holocene. As the climate was stable during this period, we infer the increase to mostly be due to dispersal lags and/or establishment lags.

Late Holocene richness nears a plateau

The regional species pool clearly levelled off during the past few millennia suggesting that a near saturation point was reached. The slight cooling and well-known instability in this period (Pears *et al.*, 2020) had no direct effect on our richness estimates or species pool, although it clearly caused a withdrawal of the forest in the region (Seppa, 1998; Sjögren & Damm, 2019). For Gauptjern, palynological richness also increases slightly in this period (Jensen & Vorren, 2008). Again, the four sites studied by Seppä (1998) show variable patterns of richness; only Lake Skáidejávri shows a clear increase during the Late Holocene. Palynological richness levels off at a site in Central Sweden, although a slight increase is observed in the most recent period (Giesecke *et al.*, 2012). Richness also increases in sites in southern Sweden, Germany and in general in the boreal and nemoral region, mainly due to human land use (Berglund *et al.*, 2008; Giesecke *et al.*, 2012, 2019). The reason for leveling off at the regional scale in northern Fennoscandia is likely due to the near-saturation of the regional species pool and the overall low impact of human land use within the catchments.

In contrast to the regional scale, our data suggest that the local species pools and richness are not yet saturated. This is in contrast to what has been observed in studies of modern

vegetation, where there appears to be no effect of time since glaciation for local (plot level) richness, whereas a legacy of the ice age is inferred for richness at the pan-Arctic (floristic regions) scale (Stewart *et al*., 2016). This apparent contradiction may be the result of scale and environmental spatial variation. Our catchments are larger than the plots studied by Stewart *et al*. (2016), and may allow for co-existence of different vegetation types. Soils develop slowly on hard felsic and mafic rocks and have low buffering capacity resulting in nutrient loss and the partial development of oligotrophic vegetation types such as acid heaths and ombrotrophic mires. These have their own floras and some species are restricted to these environments. Indeed, mires and heath vegetation expanded in the region during the Late Holocene (Seppa, 1998; Sjögren & Damm, 2019). Depending upon the local bedrock, a given area may thus gradually come to include additional 'poor' vegetation types, allowing additional species and the total richness to increase while retaining the more demanding species on more favourable areas. These areas can be more favourable in both edaphic and thermal terms (south and east facing slopes). In addition, infilling of the lake creates wetland zones that also may include terrestrial taxa. Thus, a continued increase in richness and local species pool may be a result of habitat diversification.

Conclusions

By using standardized field and lab methods, age-depth models, and rigorously synchronized taxonomic data from across 10 lakes covering environmental gradients in northern Fennoscandia, we have shown a unique increasing pattern of terrestrial plant richness over the Holocene. Both the QC and statistical testing reveals that the resulting plant diversity data is not biased by sample age or sequencing depth. The taxonomic precision and known source areas (hydrological catchments of the lakes) from this *sed*aDNA data set allows meaningful estimates of taxon richness, its spatial variation, and temporal patterns. The data reveal a steep increase in diversity in the Early Holocene related to the concurrent increase in temperature at that time and abundant vacant niches. However, richness, local and the regional species pool continued to increase although at a slower rate throughout most of the period, suggesting that dispersal lags and habitat diversification had a major impact on diversity also through the Middle and Late Holocene. This interpretation is strengthened by the strong correlation we observed between richness and the regional species pool. In addition, we found that local nutrient levels, calculated based on bedrock type, had a strong impact on the overall levels of richness. Individual differences were observed among our sites, but our novel combined and standardised *sed*aDNA analyses of 10 sites provides a superior representation of the overall

regional patterns in plant taxonomic richness over the Holocene. Based on these patterns from the past, we may expect time lags in species response to ongoing climate change.

Acknowledgements

We thank Roseanna Mayfield and Kristian Sirkka for assistance with fieldwork; Marie Føreid Merkel, Sandra Garces Pastor, and Yann Belov for technical assistance in the lab; Karina Monsen for assistance with high-resolution photography; Eivind Støren, Department of Earth Science, University of Bergen for providing access to cold rooms and helping to locate and transport cores; Ruth Paulssen and the Genomics Support Centre Tromsø (GSCT) at UiT-The Arctic University of Norway for amplicon sequencing; Dorothee Ehrich for fruitful discussions and coring at Sandfjorddalen; Matthias Forwick for help organizing scanning of cores; and Lasse Topstad and Leif Einar Støvern for providing photographs. DPR is thankful to Keshav Prasad Paudel for his help in map creation. The study is part of the project "ECOGEN - Ecosystem change and species persistence over time: a genome-based approach", which is financed by Research Council of Norway grant number 250963/F20.

Author Contributions

IGA, KAB, NGY, TA, and MEE designed the research and raised the funding; IGA, AGB, DPR, PDH, FJAM, YL, KAB and KEL did the fieldwork; JB, KFH, and JSS provided resources; DPR, PDH, KEL and IP did the laboratory work with input from IGA and AGB; TG performed radiocarbon dating; PDH built composite cores and performed age-depth modelling with input from AGB, TG and KFH; YL and PDH designed the bioinformatic pipeline with input from DPR and IGA; IGA and DPR verified and curated barcode taxonomic assignments; PDH and YL designed and performed the quality control checks with input from DPR and IGA; DPR did the statistical analyses with input from NGY and KAB; YL, DPR and PDH curated the data; DPR, PDH, AGB and IGA drafted the manuscript; and all authors have reviewed and approved the final manuscript.

Competing Interests

The authors do not declare any competing interests.

Data Availability Statement

Raw sequence data have been deposited in the European Nucleotide Archive (ENA) at project accession PRJEB39329, with sample accessions ERS4812035-ERS4812048. All radiocarbon, loss-on-ignition, and processed *sed*aDNA data are available in the Supplementary materials. Pre-filtered ObiTools tsv output files have been uploaded to figshare (DOI: [available upon acceptance]). Scripts are on Github with URLs cited in the Methods and Supplementary Materials.

References

Allen JRM, Huntley B. **1999**. Estimating past floristic diversity in montane regions from macrofossil assemblages. *Journal of biogeography* **26**: 55–73.

Allen JRM, Long AJ, Ottley CJ, Graham Pearson D, Huntley B. **2007**. Holocene climate variability in northernmost Europe. *Quaternary science reviews* **26**: 1432–1453.

Alsos IG, Lammers Y, Yoccoz NG, Jørgensen T, Sjögren P, Gielly L, Edwards ME. **2018**. Plant DNA metabarcoding of lake sediments: How does it represent the contemporary vegetation. *PloS one* **13**: e0195403.

Alsos IG, Sjögren P, Brown AG, Gielly L, Merkel MKF, Paus A, Lammers Y, Edwards ME, Alm T, Leng M, *et al.* 2020. Last Glacial Maximum environmental conditions at Andøya, northern Norway; evidence for a northern ice-edge ecological 'hotspot'. *Quaternary Science Reviews* **239**: 106364.

Alsos IG, Sjögren P, Edwards ME, Landvik JY, Gielly L, Forwick M, Coissac E, Brown AG, Jakobsen LV, Føreid MK, *et al.* 2016. Sedimentary ancient DNA from Lake Skartjørna, Svalbard: Assessing the resilience of arctic flora to Holocene climate change. *Holocene* **26**: 627–642.

Andersen KK, Azuma N, Barnola J-M, Bigler M, Biscaye P, Caillon N, Chappellaz J, Clausen HB, Dahl-Jensen D, Fischer H, *et al.* 2004. High-resolution record of Northern Hemisphere climate extending into the last interglacial period. *Nature* **431**: 147–151.

Arnesen G, Beck PSA, Engelskjøn T. **2007**. Soil acidity, content of carbonates, and available phosphorus are the soil factors best correlated with alpine vegetation: Evidence from

Troms, north Norway. *Arctic, antarctic, and alpine research* **39**: 189–199.

Ballantyne CK. **2002**. A general model of paraglacial landscape response. *Holocene* **12**: 371–376.

Bennett KD, Buck CE. **2016**. Interpretation of lake sediment accumulation rates. *Holocene* **26**: 1092–1102.

Berglund BE, Persson T, Björkman L. **2008**. Late Quaternary landscape and vegetation diversity in a North European perspective. *Quaternary international: the journal of the International Union for Quaternary Research* **184**: 187–194.

Birks HH. **2015**. South to north: Contrasting late-glacial and early-Holocene climate changes and vegetation responses between south and north Norway. *Holocene* **25**: 37–52.

Birks HH, Birks HJB. **2000**. Future uses of pollen analysis must include plant macrofossils. *Journal of biogeography* **27**: 31–35.

Birks HJB, Birks HH. **2016**. How have studies of ancient DNA from sediments contributed to the reconstruction of Quaternary floras? *The New phytologist* **209**: 499–506.

Birks HJB, Felde VA, Bjune AE, Grytnes J-A, Seppä H, Giesecke T. **2016a**. Does pollen-assemblage richness reflect floristic richness? A review of recent developments and future challenges. *Review of palaeobotany and palynology* **228**: 1–25.

Birks HJB, Felde VA, Seddon AWR. **2016b**. Biodiversity trends within the Holocene. *Holocene* **26**: 994–1001.

Birks HH, Jones VJ, Brooks SJ, Birks HJB, Telford RJ, Juggins S, Peglar SM. **2012**. From cold to cool in northernmost Norway: Lateglacial and early Holocene multi-proxy environmental and climate reconstructions from Jansvatnet, Hammerfest. *Quaternary science reviews* **33**: 100–120.

Bjorkman AD, Myers-Smith IH, Elmendorf SC, Normand S, Rüger N, Beck PSA, Blach-Overgaard A, Blok D, Cornelissen JHC, Forbes BC, *et al.* 2018. Plant functional trait change across a warming tundra biome. *Nature* **562**: 57–62.

Blaauw M, Andrés Christen J. **2011**. Flexible paleoclimate age-depth models using an

autoregressive gamma process. *Bayesian Analysis* **6**: 457–474.

Blarquez O, Carcaillet C, Frejaville T, Bergeron Y. **2014**. Disentangling the trajectories of alpha, beta and gamma plant diversity of North American boreal ecoregions since 15,500 years. *Frontiers in Ecology and Evolution* **2**: 6.

Blowes SA, Supp SR, Antão LH, Bates A, Bruelheide H, Chase JM, Moyes F, Magurran A, McGill B, Myers-Smith IH, *et al.* 2019. The geography of biodiversity change in marine and terrestrial assemblages. *Science* **366**: 339.

Boyer F, Mercier C, Bonin A, Le Bras Y, Taberlet P, Coissac E. **2016**. obitools: a unix-inspired software package for DNA metabarcoding. *Molecular ecology resources* **16**: 176–182.

CAFF. **2013**. *Arctic Biodiversity Assessment: The full scientific report*. Akureyri, Iceland: CAFF - Conservation of Arctic Flora and Fauna.

Clarke CL, Alsos IG, Edwards ME, Paus A, Gielly L, Haflidason H, Mangerud J, Hughes PDM, Svendsen JI, Bjune AE. **2020**. A 24,000-year ancient DNA and pollen record from the Polar Urals reveals temporal dynamics of arctic and boreal plant communities. *Quaternary Science Reviews* **247**: 106564.

Clarke CL, Edwards ME, Brown AG, Gielly L, Lammers Y, Heintzman PD, Ancin-Murguzur FJ, Bråthen K-A, Goslar T, Alsos IG. **2019a**. Holocene floristic diversity and richness in northeast Norway revealed by sedimentary ancient DNA (sedaDNA) and pollen. *Boreas* **48**: 299–316.

Clarke CL, Edwards ME, Gielly L, Ehrich D, Hughes PDM, Morozova LM, Haflidason H, Mangerud J, Svendsen JI, Alsos IG. **2019b**. Persistence of arctic-alpine flora during 24,000 years of environmental change in the Polar Urals. *Scientific reports* **9**: 19613.

Crump SE, Miller GH, Power M, Sepúlveda J, Dildar N, Coghlan M, Bunce M. **2019**. Arctic shrub colonization lagged peak postglacial warmth: Molecular evidence in lake sediment from Arctic Canada. *Global change biology* **25**: 4244–4256.

Dornelas M, Gotelli NJ, McGill B, Shimadzu H, Moyes F, Sievers C, Magurran AE. **2014**. Assemblage time series reveal biodiversity change but not systematic loss. *Science* **344**:

296–299.

Dullinger, S., F. Essl, W. Rabitsch, K.-H. Erb, S. Gingrich, H. Haberl, K. Hülber, V. Jarošík, F. Krausmann, I. Kühn, J. Pergl, P. Pyšek, and P. E. Hulme. 2013. Europe's other debt crisis caused by the long legacy of future extinctions. *Proceedings of the National Academy of Sciences* **110**:7342–7347.

Dullinger, S., A. Gattringer, W. Thuiller, D. Moser, N. E. Zimmermann, A. Guisan, W. Willner, C. Plutzar, M. Leitner, T. Mang, M. Caccianiga, T. Dirnbock, S. Ertl, A. Fischer, J. Lenoir, J. C. Svenning, A. Psomas, D. R. Schmatz, U. Silc, P. Vittoz, and K. Hulber. 2012. Extinction debt of high-mountain plants under twenty-first-century climate change. *Nature Climate Change* **2**:619–622.

Eldevik T, Risebrobakken B, Bjune AE, Andersson C, Birks HJB, Dokken TM, Drange H, Glessmer MS, Li C, Nilsen JEØ, *et al.* 2014. A brief history of climate – the northern seas from the Last Glacial Maximum to global warming. *Quaternary science reviews* **106**: 225–246.

Elven R, Alm T, Berg T, Båtvik JII, Fremstad E, Pedersen O. **2005**. Johannes Lid & Dagny Tande Lid: Norsk flora. *Det Norske Samlaget*.

Elven R, Murray DF, Razzhivin VY, Yurtsev BA. **2011**. Annotated checklist of the panarctic flora (PAF) vascular plants. *Natural History Museum, University of Oslo*.

Felde VA, Grytnes J-A, Bjune AE, Peglar SM, Birks HJB. **2018**. Are diversity trends in western Scandinavia influenced by post-glacial dispersal limitation? *Journal of vegetation science: official organ of the International Association for Vegetation Science* **29**: 360–370.

Fordham DA, Jackson ST, Brown SC, Huntley B, Brook BW, Dahl-Jensen D, Gilbert MTP, Otto-Bliesner BL, Svensson A, Theodoridis S, *et al.* 2020. Using paleo-archives to safeguard biodiversity under climate change. *Science* **369**.

Giesecke T, Wolters S, Jahns S, Brande A. **2012**. Exploring holocene changes in palynological richness in northern Europe--did postglacial immigration matter? *PloS one* **7**: e51624.

Giesecke T, Wolters S, van Leeuwen JFN, van der Knaap PWO, Leydet M, Brewer S.

2019. Postglacial change of the floristic diversity gradient in Europe. *Nature communications* **10**: 5422.

Gobet E, Tinner W, Hubschmid P, Jansen I, Wehrli M, Ammann B, Wick L. **2000**. Influence of human impact and bedrock differences on the vegetational history of the Insubrian Southern Alps. *Vegetation history and archaeobotany* **9**: 175–187.

Gonzalez A, Cardinale BJ, Allington GR, Byrnes J, Arthur Endsley K, Brown DG, Hooper DU, Isbell F, O'Connor MI, Loreau M. **2016**. Estimating local biodiversity change: a critique of papers claiming no net loss of local diversity. *Ecology* **97**: 1949–1960.

Gough L, Shaver GR, Carroll J, Royer DL, Laundre JA. **2000**. Vascular plant species richness in Alaskan arctic tundra: the importance of soil pH. *The Journal of ecology* **88**: 54–66.

Hall K, Thorn CE, Matsuoka N, Prick A. **2002**. Weathering in cold regions: some thoughts and perspectives. *Progress in Physical Geography: Earth and Environment* **26**: 577–603.

Harrison S. **2020**. Plant community diversity will decline more than increase under climatic warming. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences* **375**: 20190106.

Heiri O, Lotter AF, Lemcke G. **2001**. Loss on ignition as a method for estimating organic and carbonate content in sediments: reproducibility and comparability of results. *Journal of paleolimnology* **25**: 101–110.

Hill MO. **1973**. Diversity and evenness: a unifying notation and its consequences. *Ecology* **54**: 427–432.

Holzinger B, Hülber K, Camenisch M, Grabherr G. **2008**. Changes in plant species richness over the last century in the eastern Swiss Alps: elevational gradient, bedrock effects and migration rates. *Plant Ecology* **195**: 179–196.

Hughes ALC, Gyllencreutz R, Lohne ØS, Mangerud J, Svendsen JI. **2016**. The last Eurasian ice sheets – a chronological database and time-slice reconstruction, DATED-1. *Boreas* **45**: 1–45.

Hulshof CM, Spasojevic MJ. **2020**. The edaphic control of plant diversity (F Schrodt, Ed.).

Global ecology and biogeography: a journal of macroecology **29**: 1634–1650.

Huntley B, Long AJ, Allen JRM. **2013**. Spatio-temporal patterns in Lateglacial and Holocene vegetation and climate of Finnmark, northernmost Europe. *Quaternary science reviews* **70**: 158–175.

Jensen C, Vorren K-D. **2008**. Holocene vegetation and climate dynamics of the boreal alpine ecotone of northwestern Fennoscandia. *Journal of Quaternary Science* **23**: 719–743.

Lenoir J, Bertrand R, Comte L, Bourgeaud L, Hattab T, Murienne J, Grenouillet G. **2020**. Species better track climate warming in the oceans than on land. *Nature Ecology & Evolution* **4**: 1044–1059.

Le Roux JJ, Leishman MR, Cinantya AP, Gufu GD, Hirsch H, Keet J-H, Manea A, Saul W-C, Tabassum S, Warrington S, *et al.* 2020. Plant biodiversity in the face of global change. *Current biology: CB* **30**: R390–R391.

Lessard J-P, Belmaker J, Myers JA, Chase JM, Rahbek C. **2012**. Inferring local ecological processes amid species pool influences. *Trends in ecology & evolution* **27**: 600–607.

Lindahl T. **1993**. Instability and decay of the primary structure of DNA. *Nature* **362**: 709–715.

Liu S, Stoof-Leichsenring KR, Kruse S, Pestryakova LA, Herzschuh U. **2020**. Holocene vegetation and plant diversity changes in the north-eastern Siberian treeline region from pollen and sedimentary ancient DNA. *Frontiers in Ecology and Evolution* **8**: 304.

Løkken JO, Evju M, Söderström L, Hofgaard A. **2020**. Vegetation response to climate warming across the forest--tundra ecotone: species-dependent upward movement. *Journal of vegetation science: official organ of the International Association for Vegetation Science* **31**: 854–866.

Matthews JA, Vater AE. **2015**. Pioneer zone geo-ecological change: Observations from a chronosequence on the Storbreen glacier foreland, Jotunheimen, southern Norway. *CATENA* **135**: 219–230.

Murchie TJ, Kuch M, Duggan AT, Ledger ML, Roche K, Klunk J, Karpinski E,

Hackenberger D, Sadoway T, MacPhee R, *et al.* 2020. Optimizing extraction and targeted capture of ancient environmental DNA for reconstructing past environments using the PalaeoChip Arctic-1.0 bait-set. *Quaternary Research*: 1–24.

Nesje A. **1992**. A Piston Corer for Lacustrine and Marine Sediments. *Arctic and Alpine Research* **24**: 257.

Niskanen AKJ, Niittynen P, Aalto J, Väre H, Luoto M. **2019**. Lost at high latitudes: Arctic and endemic plants under threat as climate warms (J Diez, Ed.). *Diversity & distributions* **25**: 809–821.

Nogués-Bravo D, Rodríguez-Sánchez F, Orsini L, de Boer E, Jansson R, Morlon H, Fordham DA, Jackson ST. **2018**. Cracking the Code of Biodiversity Responses to Past Climate Change. *Trends in ecology & evolution* **33**: 765–776.

Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL, Solymos P, *et al.* 2019. *vegan: Community Ecology Package*.

Orlando L, Ginolhac A, Zhang G, Froese D, Albrechtsen A, Stiller M, Schubert M, Cappellini E, Petersen B, Moltke I, *et al.* 2013. Recalibrating Equus evolution using the genome sequence of an early Middle Pleistocene horse. *Nature* **499**: 74–78.

Otterå SM. **2012**. *Reconstruction of the landslide activity around Langfjordvatnet on Arnøy, North Troms. A study based on Quaternary geological mapping and analyses of lake cores*. Master thesis, University of Bergen, Bergen, Norway [in Norwegian].

Parducci L, Bennett KD, Ficetola GF, Alsos IG, Suyama Y, Wood JR, Pedersen MW. **2017**. Transley Reviews: Ancient plant DNA from lake sediments. *The New phytologist* **214**: 924–942.

Pears B, Brown AG, Toms PS, Wood J, Sanderson D, Jones R. **2020**. A sub-centennial-scale optically stimulated luminescence chronostratigraphy and late Holocene flood history from a temperate river confluence. *Geology* **48**: 819–825.

Pellissier L, Anne Bråthen K, Pottier J, Randin CF, Vittoz P, Dubuis A, Yoccoz NG, Alm T, Zimmermann NE, Guisan A. **2010**. Species distribution models reveal apparent competitive and facilitative effects of a dominant species on the distribution of tundra plants.

Ecography **33**: 1004–1014.

Pilotto F, Kühn I, Adrian R, Alber R, Alignier A, Andrews C, Bäck J, Barbaro L, Beaumont D, Beenaerts N, *et al.* 2020. Meta-analysis of multidecadal biodiversity trends in Europe. *Nature communications* **11**: 3486.

Prentice HR. **1981**. A Late Weichselian and early Flandrian pollen diagram from Østervatnet, Varanger Peninsula, NE Norway. *Boreas* **10**: 53–70.

R Core Team. **2019**. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria.

Reitalu T, Bjune AE, Blaus A, Giesecke T, Helm A, Matthias I, Peglar SM, Salonen JS, Seppä H, Väli V, *et al.* 2019. Patterns of modern pollen and plant richness across northern Europe. *Journal of Ecology* **107**: 1662–1677.

Seppä H. **1996**. Post-glacial dynamics of vegetation and tree-lines in the far north of Fennoscandia. *Fennia* **174**: 1–96.

Seppa H. **1998**. Postglacial trends in palynological richness in the northern Fennoscandian tree-line area and their ecological interpretation. *Holocene* **8**: 43–53.

Simpson GL. **2018**. Modelling Palaeoecological Time Series Using Generalised Additive Models. *Frontiers in Ecology and Evolution* **6**.

Sjögren P, Damm C. **2019**. Holocene vegetation change in northernmost Fennoscandia and the impact on prehistoric foragers 12 000–2000 cal. a BP – A review. *Boreas* **48**: 20–35.

Sjögren P, Edwards ME, Gielly L, Langdon CT, Croudace IW, Merkel MKF, Fonville T, Alsos IG. **2017**. Lake sedimentary DNA accurately records 20th Century introductions of exotic conifers in Scotland. *The New phytologist* **213**: 929–941.

Smith CI, Chamberlain AT, Riley MS, Cooper A, Stringer CB, Collins MJ. **2001**. Not just old but old and cold? *Nature* **410**: 771–772.

Soininen EM, Gauthier G, Bilodeau F, Berteaux D, Gielly L, Taberlet P, Gussarova G, Bellemain E, Hassel K, Stenøien HK, *et al.* 2015. Highly overlapping winter diet in two sympatric lemming species revealed by DNA metabarcoding. *PloS one* **10**: e0115335.

Sønstebø JH, Gielly L, Brysting AK, Elven R, Edwards M, Haile J, Willerslev E, Coissac E, Rioux D, Sannier J, *et al.* 2010. Using next-generation sequencing for molecular reconstruction of past Arctic vegetation and climate. *Molecular ecology resources* **10**: 1009–1018.

Steinbauer MJ, Grytnes J-A, Jurasinski G, Kulonen A, Lenoir J, Pauli H, Rixen C, Winkler M, Bardy-Durchhalter M, Barni E, *et al.* 2018. Accelerated increase in plant species richness on mountain summits is linked to warming. *Nature* **556**: 231–234.

Stewart L, Alsos IG, Bay C, Breen AL, Brochmann C, Boulanger-Lapointe N, Broennimann O, Bültmann H, Bøcher PK, Damgaard C, *et al.* 2016. The regional species richness and genetic diversity of Arctic vegetation reflect both past glaciations and current climate. *Global ecology and biogeography: a journal of macroecology* **25**: 430–442.

Suggitt AJ, Lister DG, Thomas CD. **2019**. Widespread Effects of Climate Change on Local Plant Diversity. *Current biology: CB* **29**: 2905–2911.e2.

Taberlet P, Coissac E, Pompanon F, Gielly L, Miquel C, Valentini A, Vermat T, Corthier G, Brochmann C, Willerslev E. **2007**. Power and limitations of the chloroplast trnL (UAA) intron for plant DNA barcoding. *Nucleic acids research* **35**: e14.

Thuiller W, Lavergne S, Roquet C, Boulangeat I, Lafourcade B, Araujo MB. **2011**. Consequences of climate change on the tree of life in Europe. *Nature* **470**: 531–534.

Vellend M, Baeten L, Becker-Scarpitta A, Boucher-Lalonde V, McCune JL, Messier J, Myers-Smith IH, Sax DF. **2017**. Plant Biodiversity Change Across Scales During the Anthropocene. *Annual review of plant biology* **68**: 563–586.

Vellend M, Baeten L, Myers-Smith IH, Elmendorf SC, Beauséjour R, Brown CD, De Frenne P, Verheyen K, Wipf S. **2013**. Global meta-analysis reveals no net change in local-scale plant biodiversity over time. *Proceedings of the National Academy of Sciences* **110**: 19456–19459.

Voldstad LH, Alsos IG, Farnsworth WR, Heintzman PD, Håkansson L, Kjellman SE, Rouillard A, Schomacker A, Eidesen PB. **2020**. A complete Holocene lake sediment ancient DNA record reveals long-standing high Arctic plant diversity hotspot in northern Svalbard. *Quaternary science reviews* **234**: 106207.

Walker M, Gibbard P, Head MJ, Berkelhammer M, Björck S, Cheng H, Cwynar LC, Fisher D, Gkinis V, Long A, *et al.* 2019. Formal Subdivision of the Holocene Series/Epoch: A Summary. *Journal of the Geological Society of India* **93**: 135–141.

Wickham H. **2016**. *ggplot2: Elegant Graphics for Data Analysis*. Springer.

Willerslev E, Cappellini E, Boomsma W, Nielsen R, Hebsgaard MB, Brand TB, Hofreiter M, Bunce M, Poinar HN, Dahl-Jensen D, *et al.* 2007. Ancient biomolecules from deep ice cores reveal a forested southern Greenland. *Science* **317**: 111–114.

Willerslev E, Davison J, Moora M, Zobel M, Coissac E, Edwards ME, Lorenzen ED, Vestergard M, Gussarova G, Haile J, *et al.* 2014. Fifty thousand years of Arctic vegetation and megafaunal diet. *Nature* **506**: 47–51.

Willis KJ, Bennett KD, Bhagwat SA, Birks HJB. **2010**. 4 °C and beyond: what did this mean for biodiversity in the past? *Systematics and biodiversity* **8**: 3–9.

Wittmeier HE, Bakke J, Vasskog K, Trachsel M. **2015**. Reconstructing Holocene glacier activity at Langfjordjøkelen, Arctic Norway, using multi-proxy fingerprinting of distal glacier-fed lake sediments. *Quaternary Science Reviews* **114**: 78–99.

Wood SN. **2017**. *Generalized Additive Models: An Introduction with R, Second Edition*. CRC Press.

Zimmermann HH, Raschke E, Epp LS, Stoof-Leichsenring KR, Schirrmeister L, Schwamborn G, Herzschuh U. **2017a**. The history of tree and shrub taxa on Bol'shoy Lyakhovsky island (New Siberian Archipelago) since the Last interglacial uncovered by sedimentary ancient DNA and pollen data. *Genes* **8**: 273.

Zimmermann HH, Raschke E, Epp LS, Stoof-Leichsenring KR, Schwamborn G, Schirrmeister L, Overduin PP, Herzschuh U. **2017b**. Sedimentary ancient DNA and pollen reveal the composition of plant organic matter in Late Quaternary permafrost sediments of the Buor Khaya Peninsula (north-eastern Siberia). *Biogeosciences* **14**: 575–596.

Zobel M. **2016**. The species pool concept as a framework for studying patterns of plant diversity. *Journal of vegetation science: official organ of the International Association for Vegetation Science* **27**: 8–18.

Tables

Table 1 Geographical locations and site information for the ten lakes studied (full table is available in Table S1).

Code	Lake	Latitude	Longitude	Altitude (masl)	Area (ha)	Catchment area (sq. km)	Depth (m)	Nutrient index	Present-day vegetation	Bedrock Lithology
EG02	Nesservatnet	70.13565	23.20306	86	1.21	0.09	4.10	2.90	Heath with pine/birch	Meta-sandstone/mica schist
EG03/13	Gauptjern	68.85645	19.61843	405	0.78	0.13	4.10	7.43	Birch and pine forest	Calcite marble
EG05	Horntjernet	69.34919	29.49156	88	1.19	0.15	6.97	4.70	Pine forest	Hornblend-rich gneiss
EG07	Sierravannet	69.84468	23.37671	73	3.24	10.60	14.13	2.66	Birch and pine forest	Granodiortic gneiss
EG10	Nordvivatnet	70.13305	29.01195	82	4.66	0.21	13.33	6.19	Heath and birch forest	Conglomerate
EG11	Eaštorjávri South	70.43313	27.33372	260	6.23	0.56	5.44	2.28	Sub-arctic grassland	Quartzitic sandstone
EG15	Langfjordvannet	70.15030	20.53700	66	55.31	3.83	34.80	6.47	Sub-arctic grassland with birch	Mica gneiss, slate, metasandstone
EG17	Jøkelvatnet	70.17250	21.70080	156	14.90	10.50	5.00	8.63	Arctic-alpine	Olivine gabbro,

									heath and mire	amphibolite
EG21	Kuutsjärvi	67.74723	29.61031	341	10.00	2.00	8.00	3.55	Spruce forest	Gneiss and migmatite
MS06	Sandfjorddalen	70.36016	30.01887	176	0.60	8.50	1.23	2.66	Arctic-alpine heath and mire	Feldspathic sandstone

Table 2 Summary statistics of generalized additive mixed models (GAMM) with a continuous time first-order autoregressive (CAR(1)) process. Median calibrated age of samples was treated as the predictor and taxonomic richness as the response variables

Lake	**edf**	**Ref.df**	**F**	**p-value**	**Phi (□)**	**adj.R.sq**
Eaštorjávri South	1	1	44.4	0	0.20	0.51
Gauptjern	2.58	2.58	16.83	0	0.37	0.46
Horntjernet	1.98	1.98	10.08	0	0.20	0.24
Jøkelvatnet	3.88	3.88	59.02	0	0.20	0.81
Kuutsjärvi	4.53	4.53	43.9	0	0.00	0.78
Langfjordvannet	5.93	5.93	8.91	0	0.20	0.45
Nesservatnet	1	1	33.87	0	0.20	0.49
Nordvivatnet	3.39	3.39	9.66	0	0.20	0.62
Sandfjorddalen	3.7	3.7	13.52	0	0.20	0.64
Sierravannet	2.73	2.73	3.1	0.09	0.44	0.25

edf: effective degrees of freedom; Ref.df: reference degrees of freedom; adj.R.sq.: adjusted R square

Supporting Information

Additional supporting information may be found in the online version of this article.

The following Supporting Information is available for this article:

Supplementary Methods

Methods S1. Site selection and properties

Methods S2. Fieldwork and lake sediment coring

Methods S3. Core sampling

Methods S4. Core photography and loss-on-ignition analyses

Methods S5. Composite core construction and age-depth modelling

Methods S6. Sedimentary ancient DNA data generation

Methods S7. Bioinformatics

Methods S8. Assessment of sedimentary ancient DNA data quality

Methods S9. Numerical and statistical analysis

Supplementary Notes

Notes S1. DNA tracer

Notes S2. Age-depth models

Notes S3. Positive control synthetic sequences

Notes S4. The MTQ and MAQ scores for metabarcoding data quality control

Notes S5. The proportion of weighted PCR replicates (wtRep)

Notes S6. Sedimentary ancient DNA data quality assessment

Supplementary Figures

Figure S1. Alignments of core LOI, high-res. imagery, and Bayesian age-depth models.

Figure S2. Distribution of MTQ and MAQ scores across all samples and controls.

Figure S3. Comparison between GAM and GAMM(CAR(1)) models of taxonomic richness through time.

Figure S4. Observed taxonomic richness (Hill N0) in each sample by lake and time including samples not passing quality controls.

Figure S5. Correlations between taxonomic richness and time against six measures of sedaDNA data quality.

Figure S6. Sample MTQ scores by lake and time.

Figure S7. Sample MAQ scores by lake and time.

Figure S8. Raw read counts for each sample by lake and time.

Figure S9. The mean wtRep for each sample by lake and time.

Figure S10. Barcode length for each sample by lake and time.

Figure S11. Terrestrial plant reads assigned to each sample by lake and time.

Figure S12. The assignments of reads processed by the bioinformatic pipeline.

Figure S13. A potential flood event does not impact the Sierravannet diversity trend.

Figure S14. Regional accumulated taxonomic richness during the Holocene excluding two sites.

Supplementary Tables

Table S1. Geographic and site metadata for the ten lakes.

Table S2. Composite core construction and Bayesian age-depth modelling.

Table S3. Sample metadata, including depths, LOI values, dates, and modelled ages.

Table S4. Full sample metadata including QC and bioinformatic sequence processing.

Table S5. Primer tag to sample lookup, library preparation, and accession data.

Table S6. List of all identified barcodes, including those blacklisted, and their taxonomic assignments and functional groups.

Table S7. The 16 samples that underwent DNA extraction twice.

Table S8. Correlations between observed and rarefied taxonomic richness for each lake.

Table S9. Summary of generalized additive models (GAMs).

Table S10. Summary of all data used or generated in this study.

Table S11. Read counts and PCR replicate detections for all retained taxa across all samples.

Table S12. Summary of linear mixed effect model of richness and climate.

Table S13. Summary of linear models of richness and nutrient index.

Supplementary References