







Environmental DNA of aquatic macrophytes: The potential for reconstructing past and present vegetation and environments

Aloïs Revéret¹  | Dilli P. Rijal¹  | Peter D. Heintzman^{1,2,3}  | Antony G. Brown^{1,4}  | Kathleen R. Stoof-Leichsenring⁵  | Inger G. Alsos¹ 

¹The Arctic University Museum of Norway, UiT—The Arctic University of Norway, Tromsø, Norway

²Centre for Palaeogenetics, Stockholm, Sweden

³Department of Geological Sciences, Stockholm University, Stockholm, Sweden

⁴School of Geography and Environmental Science, University of Southampton, Southampton, UK

⁵Polar Terrestrial Environmental Systems, Alfred Wegener Institute (Helmholtz Centre for Polar and Marine Research), Potsdam, Germany

Correspondence

Aloïs Revéret, The Arctic University Museum of Norway, UiT—The Arctic University of Norway, Tromsø, Norway.
Email: alois.n.reveret@uit.no

Abstract

1. Environmental DNA is increasingly being used to reconstruct past and present biodiversity including from freshwater ecosystems. Macrophytes are especially good environmental indicators, thus their environmental DNA palaeorecord might shed light on past postglacial environments.
2. Here, we first review and compare studies that use metagenomics, targeted capture, and various barcoding and metabarcoding markers, in order to explore how each of these methods can be used to capture aquatic vegetation diversity and change. We then investigate the extent to which such a record can be leveraged for reconstructing local environmental conditions, using a case study based on macrophyte ecological niches.
3. We find that, with state-of-the-art DNA barcode reference libraries, using metabarcoding to target the P6 loop region of the chloroplast *trnL* (UAA) intron is optimal to maximise taxonomic resolution and the diversity of past macrophyte communities. Shotgun sequencing also retrieves a high proportion of aquatic macrophyte diversity, but has the lowest taxonomic resolution, and targeted capture needs to be more widely applied before comparisons can be made.
4. From our case study, we infer past aquatic habitats from sedimentary ancient DNA records of macrophyte taxa. We reconstructed Holocene thermal range, continentality, water pH, trophic status, and light conditions in northern Fennoscandia. We show an overall stability since 9,000 years ago, even though individual lakes display different trends and variation in local climatic and physico-chemical conditions.
5. Combined with the availability of near-exhaustive barcode and traits databases, metabarcoding data can support wider ecological reconstructions that are not limited to aquatic plant taxonomic inventories but can also be used to infer past changes in water conditions and their environmental drivers. Sedimentary DNA

Sedimentary DNA signal from aquatic macrophytes allows reconstruction of aquatic vegetation and its drivers across millions of years, but methodological approaches have contrasting results, which depend on the study aims.

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial](https://creativecommons.org/licenses/by-nc/4.0/) License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

© 2023 The Authors. *Freshwater Biology* published by John Wiley & Sons Ltd.

is also a powerful tool to measure present diversity, as well as to reconstruct past lacustrine and fluvial communities of aquatic macrophytes.

KEYWORDS

aquatic communities, freshwater environments, palaeoecology, *sedaDNA*, vascular plants

1 | INTRODUCTION

Aquatic macrophytes are vascular plants that grow in lakes, ponds, streams, and wetlands. They are useful ecological indicators as their distributions are mainly determined by environmental factors at a local scale (Alahuhta et al., 2020; Johnson & Toprak, 2021; Poikane et al., 2018). Aquatic macrophytes play a major role in biogeochemical cycles in aquatic ecosystems and are therefore especially relevant for assessing the eutrophication of waterbodies (Penning et al., 2008) and waterways (O'Hare et al., 2018). Indeed, macrophyte communities effect the cycling of carbon (Reitsemä et al., 2018), nitrogen (Dan et al., 2021; Zhang et al., 2021), and phosphorus (Wang et al., 2022) in both water bodies and their sediments.

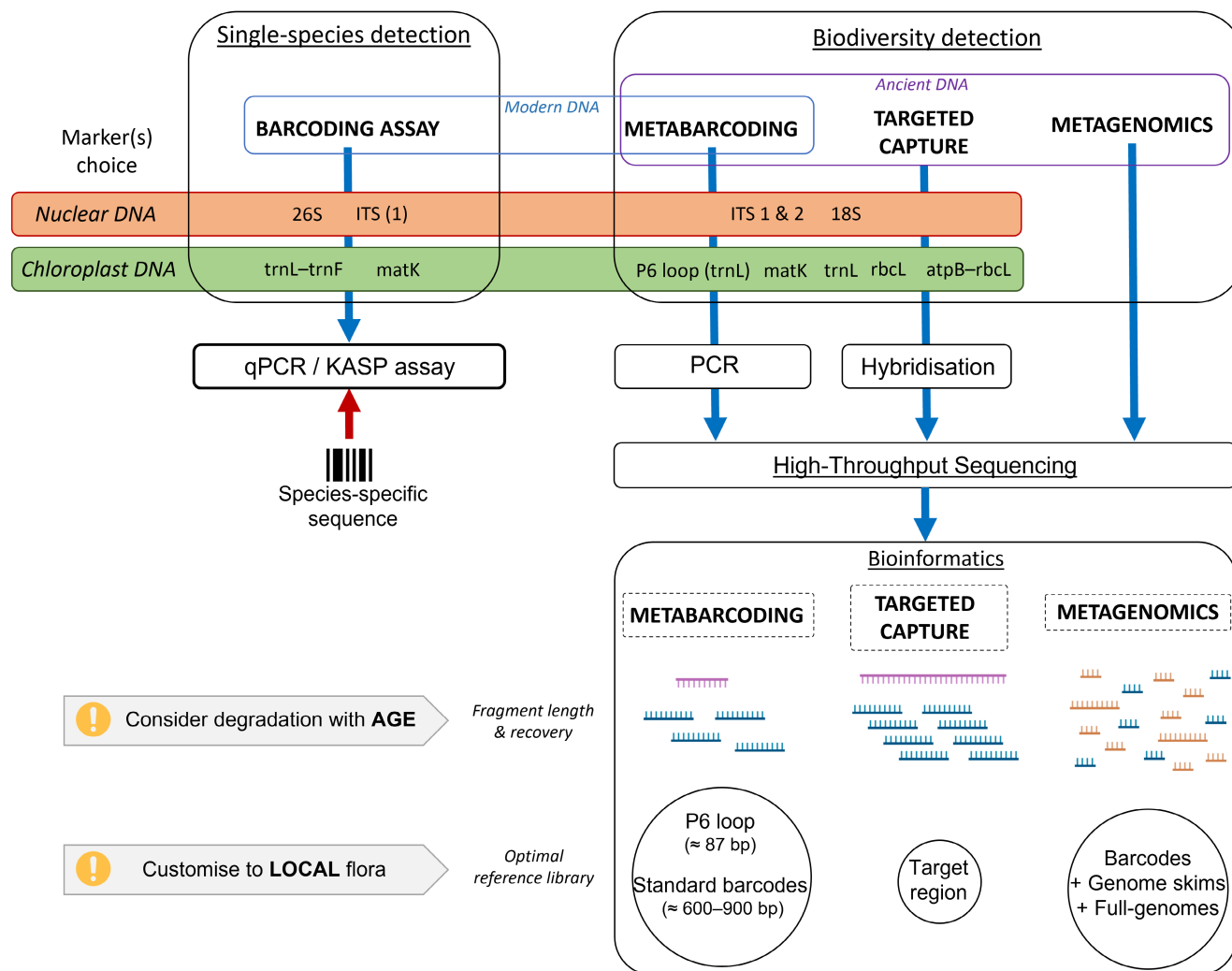
Environmental DNA (eDNA) is a rapidly growing approach for addressing ecological and genetic questions about present and past organisms, from communities to populations. There is a need for more comprehensive monitoring of freshwater biodiversity (Harper et al., 2019), and a better understanding of the ecological responses of aquatic communities to climate shifts (Maasri et al., 2022). Compared to traditional methods of aquatic community monitoring and reconstruction (e.g. field survey, macrofossils, pollen), eDNA techniques require less taxonomic expertise and sampling effort (Ji et al., 2021), and can provide higher temporal resolution (Buxton et al., 2018). For aquatic vegetation, eDNA may be comparable to, or even superior to, in-lake vegetation surveys (Alsos et al., 2018).

As a consequence of their biotope, aquatic macrophytes are readily incorporated into sediment, and are therefore especially well represented in sedimentary ancient DNA (*sedaDNA*) records (Alsos et al., 2022; Capo et al., 2021). Thus, combining a molecular palaeorecord of macrophytes with their known functional traits could allow for the reconstruction of past environmental conditions (Alsos et al., 2022; Dalla Vecchia et al., 2020; Dar et al., 2014). This has been implemented for a variety of animal and plant taxa using traditional methods, especially using macrofossil records. For instance, maximum summer temperatures in Fennoscandia throughout the Holocene have been reconstructed from chironomids (Brooks & Birks, 2000) or aquatic plant macrofossils (Väliranta et al., 2015). The latter can also help to reconstruct water level fluctuations (Väliranta et al., 2005). *SedaDNA* has been shown to enhance such reconstructions, as it can provide higher consistency in detection of species than plant macrofossils (Alsos et al., 2016) and higher taxonomic resolution than pollen (Clarke et al., 2020; Courtin et al., 2021; Garcés-Pastor et al., 2022; Niemeyer et al., 2017), and can also be

used as the sole proxy for the reconstruction of past terrestrial environments (Alsos et al., 2022). Therefore, it holds great potential for studies of past and present aquatic ecosystems (Stoof-Leichsenring et al., 2022), which would in turn expand the applicability of *sedaDNA* for abiotic reconstructions.

In addition to single-species barcoding assays, three major molecular approaches can be used to generate eDNA data, but they differ in taxonomic resolution and recovered richness estimates (Murchie et al., 2020). Shotgun sequencing is an untargeted technique (metagenomics), while (meta)barcoding and targeted capture enrich a part or parts of the genome of the taxa of interest (Box 1). Targeted capture is also called *hybridisation capture*, *capture probes*, or *target enrichment* in the literature. Markers used in plant barcoding and metabarcoding can be standard barcode regions such as *rbcl* (654 bp) and *matK* (862–910 bp); shorter ones developed to study degraded eDNA such as the P6 loop (51–135 base pairs [bp]) of the *trnL* UAA intron; or taxonomically broader loci not restricted to plants such as ITS and 18S (Hollingsworth et al., 2011). The presence of vascular plants detected in *sedaDNA* is often supported by other proxies such as present-day vegetation (Alsos et al., 2018), historical vegetation maps (Sjögren et al., 2017), macrofossils (Alsos et al., 2016), or pollen (e.g. Bjune et al., 2021; Liu et al., 2021; Parducci et al., 2019), thus confirming its reliability. However, an accurate detection of communities by eDNA heavily depends on the quality of the reference library (Wang et al., 2021; Weigand et al., 2019), which should ideally be taxonomically exhaustive within the given geographic area. Other groups are likely to yield valuable information where the aquatic macrophyte signal is unavailable, thus increasing the value of palaeo-approaches for understanding both the past and the present (Brown, 2002). For example, Tyler and Olsson (2016) have also compiled the pH niches of Swedish bryophytes, which are a bycatch of the P6 loop. Using this marker in combination with both vascular plant and bryophyte traits databases could therefore give a very accurate depiction of historical pH trends in lake palaeo-records.

Here we review studies that identified aquatic macrophytes in ancient and contemporary lake sediments, soil, and water. To assess the potential of reconstructing past vegetation changes based on aquatic macrophyte community eDNA signals, we evaluate the taxonomic resolution and richness reported between the three major molecular approaches with a focus on ancient samples. We also show the potential for such reconstructions with an aquatic macrophyte case study, where we combined a *sedaDNA* metabarcoding dataset from 10 lakes (Alsos et al., 2022) with ecological traits values from Tyler et al. (2021).



BOX 1 Methodological workflow of environmental DNA sample analysis for aquatic plants.

2 | METHODS

2.1 | Compilation of aquatic macrophyte eDNA studies

We have compiled literature, including supplementary materials, which explicitly mentioned the detection of aquatic macrophytes using any eDNA method. We performed a literature search using the following phrases: “sedimentary DNA aquatic vascular”, “sedimentary DNA macrophytes”, “freshwater environmental DNA plant diversity”, “sedimentary ancient DNA vascular”, “metabarcoding aquatic plants sediment”, “ancient sedimentary DNA vegetation”, “environmental DNA aquatic plant”, “environmental DNA macrophyte”, and “ancient DNA aquatic plant”. For each search phrase, we scanned through the first 500 results for articles with pertinent features in the title or abstract (e.g. relevant method, field experiment, possibility of aquatic species, etc.), until we could compile 10 papers that met our criteria. We replicated this search on three search engines: first Google Scholar, then the bioRxiv preprint repository, and lastly the Web of Science Core Collection; we disregarded articles already recovered

by previous search runs in our counts. In total, more than twelve thousand papers were scanned. We supplemented our search results with relevant studies from *AncientMetagenomeDir* (Fellows Yates et al., 2021), which collates metagenomics datasets, plus three studies mentioned in the bibliography of our review (Adame & Reef, 2020; Cannon et al., 2016; Schabacker et al., 2020), and relevant studies from the most recent *sedaDNA* review (Li et al., 2023).

When several studies investigated the same sites or an overlapping subset, we only considered the most recent study, unless an earlier study provided more data for aquatic macrophytes. We restricted our compilation to experiments carried out on field-derived samples that were recovered in natural conditions. We excluded marine studies of seaweeds and mangrove plants (Foster et al., 2021; Ortega et al., 2020), and in vitro and in silico studies (Du et al., 2011; Scriver et al., 2015). We also excluded non-vascular plant taxa, even when regarded as macrophytes by the authors (e.g. Characeae in Y. Wang et al., 2021). We decided to disregard three diet studies where macrophytes were detected, as our focus is on water, soil, and sediment samples (canid faeces in Wood et al., 2016; human teeth in Ottoni et al., 2021, and in Sawafuji et al., 2020). Likewise,

we disregarded Garcés-Pastor et al. (2019), where *Utricularia* sp. was detected but from modern biological samples (bryophyte tissues) and not in the sediment.

Using the resulting literature dataset, we compiled, where available: the number, type, and oldest age of eDNA samples, number of sites where aquatic macrophytes were detected through eDNA, methodological approach and loci targeted, DNA extraction method, number of polymerase chain reaction (PCR) replicates, total and filtered count of DNA reads, counts of both raw and assumed true positive operational taxonomic units (OTUs) of all vascular plants, percentages of the latter assigned to the lowest taxonomic level (family/genus/species), number of OTUs assigned to aquatic macrophyte taxa, total number of aquatic macrophyte taxa retrieved from eDNA, counts from the latter assigned to the lowest taxonomic level, number, names, and versions of the reference libraries used for taxonomic assignment, identity cut-off for such assignment, and whether the study mentioned other proxies (i.e. pollen, macrofossils, and visual survey; Table S1). As OTU terminology contains synonyms, we collated figures reported as “sequences”, “OTUs”, “Molecular OTUs”, “Amplicon Sequence Variants”, and “taxa”. Note that several sequences may match the same taxon and therefore the latter measure is the most conservative in terms of diversity. We also compiled the geographical location of all sites with detected aquatic macrophytes through eDNA (Table S2).

To consistently classify all reported vascular plant taxa as *aquatic* or not (Table S3), we followed the functional groups given by the authors whenever possible. When references were conflicting (usually between one study identifying at the genus level and another identifying at the species level), we followed the original classifications for the respective studies, but these taxa were then ignored in other studies. All taxa found in the Swedish flora were classified according to our cut-off from Tyler et al. (2021), as described in the following section, and this classification always took precedence over categories given in the literature. The remaining taxa encountered were classified using the following floras: Flora of Colorado (Ackerfield, 2015); Calflora (Powell et al., 2022); Flora of Australia (ABRS, 2015); Flora of Tropical East Africa (Clayton et al., 1974); Flora Novae Angliae (Haines et al., 2011); Info Flora (Info Flora, 2022); Field Manual of Michigan Flora (Voss & Reznicek, 2012); Online Virtual Flora of Wisconsin (Wisconsin State Herbarium, 2022); PlantZAfrica (SANBI, 2022); eFlore (Tela Botanica, 2022); SvalbardFlora (Elven et al., 2020); Flora of Victoria (Walsh & Entwisle, 1994); World Flora Online (WFO, 2022); Harrington et al. (2012); and the following online databases: Global Biodiversity Information Facility (GBIF, org, 2022); The Tree of Life Web Project (Maddison & Schulz, 2007); USDA Plants Database (USDA, 2022), all accessed in May 2022. Note that some ecologically broad genera, which include both terrestrial and aquatic species, such as *Carex*, *Equisetum*, *Juncus*, or *Ranunculus*, were included as aquatics only if originally defined as such by the study authors.

We assessed taxonomic resolution by method and marker used, in all vascular plants and in aquatic macrophytes, by computing the percentage of taxa identified to the lowest taxonomic level among

family, genus, and species. Because these are compositional data, we applied a centred log ratio transformation ahead of further analyses. Barcoding assays were disregarded because they are species-specific: their taxonomic resolution would, by definition, be 100% at the species level, with a single taxon detected (sometimes two were targeted). We also explored how total macrophyte richness correlates with macrophyte taxonomic resolution at each computed level, and how richness and species-level resolution each correlate with publication year and age of the oldest sample; using generalised linear models and excluding outliers (Figure S1). We assumed the distribution of errors to be respectively *Gaussian* and *quasi-Poisson* for continuous and discrete responses.

2.2 | Northern Fennoscandia traits case study

Our case study focuses on northern Fennoscandia, as this region has the largest standardised lake *seDNA* dataset published to date, comprising eight PCR replicates each of 355 samples (Alsos et al., 2022; Rijal et al., 2021). In addition, an exhaustive DNA reference library (PhyloNorway) covers Norwegian vascular plants (Alsos, Lavergne, et al., 2020; Alsos et al., 2022), which maximises the chances of correct species identification and enhances the accuracy of subsequent analyses. A 100% match criterion was used for PhyloNorway, along with three additional reference libraries (ArcBorBryo, PhyloAlps, and EMBL-143; Alsos et al., 2022). A traits-value database for the Swedish vascular flora (Tyler et al., 2021) lists 30 parameters that can be used as ecological indicators. These traits represent both the abiotic and biotic components of niches (Ellenberg et al., 1992). We hereafter regarded *aquatics* as species that have a moisture requirement above level 9 (wet–temporarily inundated, e.g. *Caltha palustris*); on a scale from 1 (very dry) to 12 (deep permanent water). Thus, all our selected species are temporarily inundated (10, e.g. *Hippuris* spp.); live in shallow (<0.5 m) permanent water (11, e.g. *Myriophyllum alterniflorum*), or deep permanent water (12, e.g. *Potamogeton praelongus*). As our taxonomic listing follows *seDNA* methodological constraints (i.e. some species are aggregated as they remain molecularly unresolved), taxa were attributed an averaged moisture value (e.g. 9.5 when one species is 9 and the other is 10; Alsos et al., 2022). Note that this scale emphasises water depth rather than plant growth form (free-floating, submerged, floating-leaved, emergent, or marginal), as used in some studies (e.g. a variant in Tyrrell et al., 2022) (Table S4). Depths of the lakes studied ranged from 1.2 to 34.8 m, most of them being between 4 and 15 m (see supplementary material in Rijal et al., 2021).

A *seDNA* plant metabarcoding dataset spanning across 10 lakes in northern Fennoscandia (Rijal et al., 2021) with taxonomic assignments updated by Alsos et al. (2022), was further subsetted to retain only aquatic macrophyte taxa, by comparing the recorded species list with their respective moisture requirement (Tyler et al., 2021), following the standardisation of taxonomic nomenclature in Alsos et al. (2022). The majority of the data were initially published in Rijal et al. (2021), with a focus on how climate and soil

nutrients affected overall taxonomic richness over the last 12 millennia. Alsos et al. (2022) expanded the dataset and revised the sequence identification using the new PhyloNorway DNA reference library to reconstruct post-glacial establishment across 16 millennia, and then investigated traits related to colonisation, such as dispersal mode and pollinator dependence. The scope of the present case study is to carry out a detailed examination of aquatic plant taxa, to support the proper reconstruction of major abiotic variables important for aquatic macrophytes distribution.

The final reconstructed six traits were heat and cold requirements, continentality, pH, nitrogen availability, and light optimum. Most of these factors are known to be drivers of aquatic macrophyte distribution patterns (Dar et al., 2014). Out of the 30 total traits from Tyler et al. (2021), we firstly discarded 10 traits with less than a minimum of seven distinct trait values across all aquatic taxa; thus eliminating nitrogen fixation (one level), assumed immigration time to Sweden (one), parasitism (one), carnivory (two), mycorrhiza (two), seed dispersal (three), pollinator dependence (four), seed longevity (five), nectar production (five), and tolerance to grazing (six). We additionally discarded three traits that had missing values for seven or more aquatic taxa (soil disturbance, phenology, seed dormancy); and six that were irrelevant or marginal for our environmental reconstruction (occurrence in Sweden, red-listing, photosynthetic pathway, invasiveness, longevity, vegetation type). Thus, the 11 remaining ecological traits to be tested for environmental reconstruction were: biodiversity relevance (log of trophic associations), cold- and heat requirements, temperature optimum (computed from cold and heat requirements), continentality, light optimum, moisture, pH, nitrogen and phosphorus availabilities, and salinity. We performed a Spearman's rank correlation analysis of these variables to exclude collinear explanatory variables: one of two traits was discarded whenever their paired correlation coefficient value (r) was >0.7 (Figure S2). Such high correlations were found between cold requirement and temperature optimum ($r=0.937$), pH and salinity ($r=0.770$), and nitrogen and phosphorus requirements ($r=0.746$); and therefore we retained the formers. Moisture and biodiversity relevance were dropped to simplify graphical representation, as they were less informative than other retained traits.

We investigated the possibility of reconstructing past environmental changes by looking for major shifts among traits values; both at the regional scale, combining data from all 10 lakes, and the local single-lake scale. For the regional dataset, we aggregated samples by 500-year time slices and plotted the distribution of trait values through time, using their respective proportions across all eight PCR replicates. For individual site reconstructions, we did not merge samples into time slices before plotting the distribution of traits values as their respective proportions across replicates through time.

Additionally, we checked for potential correlations between species traits and their first recorded and estimated arrival dates in the region (Alsos et al., 2022), to see if some traits could explain the arrival of aquatic macrophytes. We further evaluated how the composition of aquatic taxa was affected by our six selected traits, using multivariate analysis. First, we ran a detrended correspondence

analysis as a preliminary analysis to select an appropriate multivariate analysis. As the gradient length was short (0.76), we applied a linear ordination method, namely a redundancy analysis (RDA), with presence/absence data as the response variable. All analyses were run in R v4.1.1 (R Core Team, 2022).

3 | RESULTS

3.1 | Published data compilation

We found 62 studies that explicitly mentioned the detection of aquatic macrophytes using eDNA, encompassing 450 sampling sites (Tables S1 and S2). The majority of sampling sites originate from boreal, arctic, or high-altitude bioclimatic zones, especially for studies of ancient DNA (aDNA; Figure 1). Our review covers 4,163 samples in total, 1,124 of which are modern samples only (water, lake or river surface sediment, or undated but assumed to be recent material). The majority of studies (55%) target lacustrine or fluvial sediment, including thermokarst lakes (thus the permafrost active layer). Several studies reviewed here also used water samples (26%) from lakes and rivers, soil samples (19%) encompassing permafrost sedimentary complexes and subfossil stream deposits from river bluffs, and peat samples (8%). Four studies used samples of different types. Lastly, despite our selection criterion, two studies also included marine sediment (Figure 1): these sites are a tidal basin which later turned into a freshwater lake due to isostatic rebound (lake Nordvivatnet, see Brown et al., 2022), and a pre-transgression terrestrial area near the past coastline (Gaffney et al., 2020).

Our review is dominated by aDNA studies ($n=41$) compared to modern eDNA studies ($n=21$), although the former often include modern samples through analysis of surface layers in sediment core records. There are three main approaches to eDNA analyses for biodiversity detection (Box 1) and most of the 62 studies reviewed used only one of them, while seven combined several methods, at least on a subset of their samples. Metabarcoding is used in most studies ($n=49$), followed by shotgun sequencing ($n=10$), barcoding assays ($n=9$), and targeted capture ($n=2$). Among barcoding and metabarcoding studies, seven targeted multiple loci. Sample age is found to be strongly related to the choice of methods and markers: all shotgun sequencing studies were performed on ancient samples, whereas all barcoding assays focused on modern material (Table 1).

In total, 319 aquatic plant taxa were reported (Table S3). However, 50 of these taxa were excluded because they: (1) are not vascular plants ($n=22$); (2) were erroneously classified ($n=3$); (3) had an invalid taxonomic name ($n=1$); (4) were assumed false positives as they were outside of their continental biogeographical range ($n=6$); or (5) were only detected by methods other than eDNA ($n=18$). Thus, 269 aquatic taxa belonging to 45 families, 118 genera, and 196 species were used in this review (Table S3).

There was a clear difference in approach between ancient and modern samples (Tables 1 and 2). Shotgun sequencing has only been applied to ancient samples, whereas barcoding assays have only

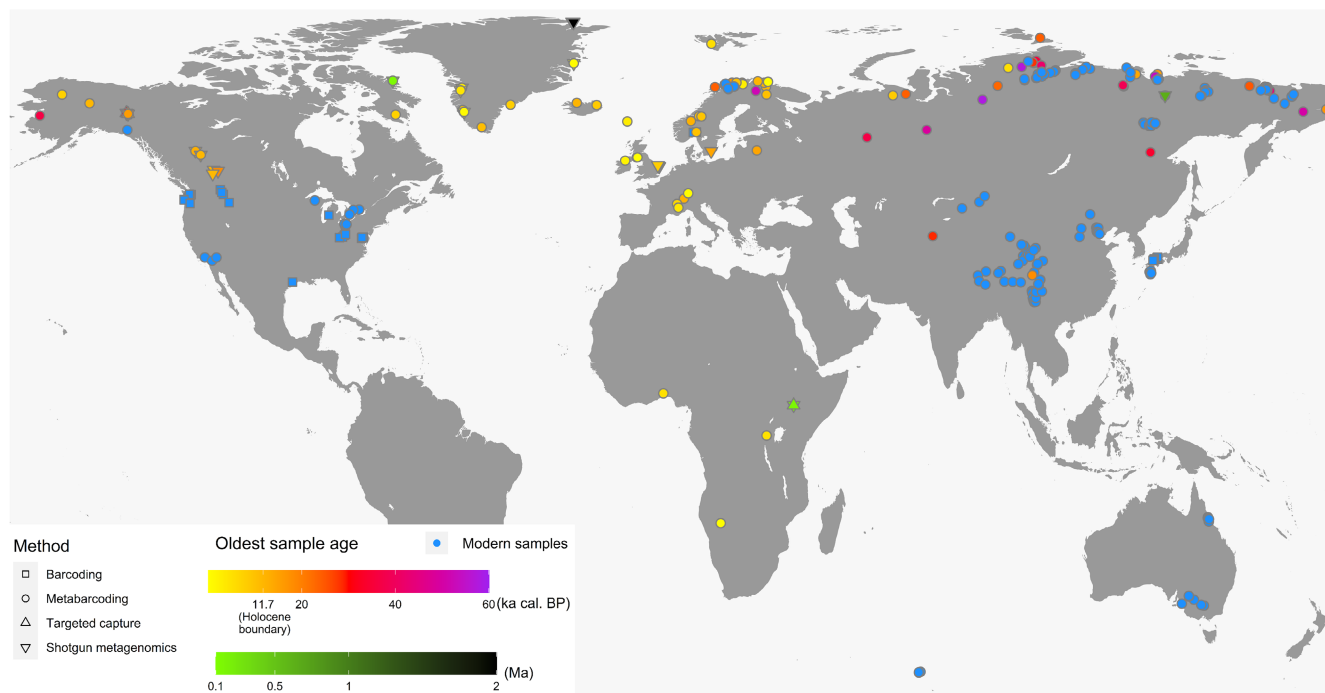


FIGURE 1 World map showing the location and age for environmental DNA studies reporting aquatic macrophytes. Sites in blue account for modern samples, i.e. water, surface sediment, and other undated material. All studies sampled freshwater or terrestrial material, except for two that retrieved marine sediment.

TABLE 1 Number of reviewed publications by method used and locus targeted, depending on their sample age.

	Barcoding and metabarcoding									Targeted capture		
	P6 loop	<i>trnL-trnF</i>	<i>rbcL</i>	<i>atpB-rbcL</i>	<i>matK</i>	ITS	18S	26S	Shotgun metagenomics	<i>trnL</i>	<i>rbcL</i>	<i>matK</i>
Modern	7	4	3	1	3	7	1	1	0	0	0	0
Ancient	36	0	1	0	0	1	1	0	10	1	2	1
Total	43	4	4	1	3	8	2	1	10	1	2	1

Note: Studies using several methods or targeting several markers are counted multiple times.

been used to detect single species in modern water samples, for the monitoring of invasive or threatened species. Only two studies used targeted capture (Table 2); and this was to investigate plant and animal diversity in permafrost between 10,000 and 30,000 years old (Murchie et al., 2020), and in Late Pleistocene sediment from an arid palaeolake basin (Krueger et al., 2021). Metabarcoding has been applied to both modern and ancient samples, using multiple markers. The *trnL* P6 loop is by far the most used marker for ancient samples, having been used in 92% of all aDNA metabarcoding studies reviewed (Tables 1 and 2). For modern samples, a much wider array of markers is used including the standard barcodes *rbcL* and *matK* (Table 1).

The earliest study to report aquatic macrophytes detected in eDNA was published in Jørgensen et al. (2012), which found *Caltha* sp. and *Pleuropogon sabinei* in Weichselian sediments from Lake Taimyr, Siberia. Indeed, the occurrences of macrophyte eDNA were exclusively from metabarcoding of ancient material, until 2016 (Figure 2). The first studies to use multiple eDNA detection methods

simultaneously were published in 2016, introducing metagenomics as a new tool to study macrophyte palaeodiversity and inaugurating a more active period in this field (Figure 2). Between 2016 and 2022, barcoding assays have been implemented to monitor the invasive species *Egeria densa*, *Hydrilla verticillata*, *Myriophyllum spicatum*, *Elodea canadensis*, and *Myriophyllum sibiricum*. A second increase in publications is observed from 2020 onwards. Recent publications include the detection of ancient macrophyte DNA in 2-million-year-old samples (Figure 2).

Sedimentary DNA has been successfully used to unravel past changes in macrophyte communities at various timescales. For instance, Bremond et al. (2017) reconstructed plant palaeocommunities in a tropical lake over the last 5,000 years and found aquatic taxa that ecologically corresponded to the seasonal flooding of the shores. They also detected the common water-hyacinth *Pontederia crassipes*, a recently introduced plant now dominating the vegetation of the shallows. On a longer time scale, Alsos et al. (2022) reconstructed the postglacial arrival of aquatic macrophytes in northern

TABLE 2 Summary of publications which reported detection of aquatic macrophytes in environmental DNA, their molecular methodologies, aimed taxonomic breadth (within Viridiplantae), and stadial/interstadial corresponding to the oldest sample.

Approach	Marker	Submarker	Publication	Targeted taxon	Sample age
Shotgun metagenomics	(untargeted approach)		Pedersen et al. (2016)	(untargeted approach)	Younger Dryas
			Seersholm et al. (2016)		Late Holocene
			Parducci et al. (2019)		Bølling–Allerød
			Gaffney et al. (2020)		Early Holocene
			Murchie et al. (2020)		Weichselian
			Crump et al. (2021)		Last Interglacial
			Krueger et al. (2021)		Last Interglacial
			Wang et al. (2021)		Weichselian
			Courtin et al. (2022)		Günz Complex
Kjær et al. (2022)		Biber Complex			
Targeted capture	<i>trnL</i>		Murchie et al. (2020)	2,090 plant taxa	Weichselian
	<i>rbcL</i>		Murchie et al. (2020)	1,016 plant taxa	Weichselian
			Krueger et al. (2021)	20 plant species	Last Interglacial
	<i>matK</i>		Murchie et al. (2020)	921 plant taxa	Weichselian
Barcoding	<i>trnL</i>	<i>trnL-trnF</i> (spacer)	Fujiwara et al. (2016)	<i>Egeria densa</i>	modern
			Anglès d'Auriac et al. (2019)	<i>Elodea canadensis</i>	modern
			Doi et al. (2021)	<i>Egeria densa</i>	modern
			Kodama et al. (2022)	<i>Egeria densa</i>	modern
	<i>matK</i>		Matsuhashi et al. (2016)	<i>Hydrilla verticillata</i>	modern
			Gantz et al. (2018)	<i>Hydrilla verticillata</i>	modern
	ITS	ITS 1	Newton et al. (2016)	<i>Myriophyllum spicatum</i>	modern
			Kuehne et al. (2020)	<i>Egeria densa</i> , <i>Myriophyllum spicatum</i>	modern
			Gantz et al. (2018)	<i>Hydrilla verticillata</i>	modern
	26S		Schabacker et al. (2020)	<i>Myriophyllum sibiricum</i>	modern
Metabarcoding	<i>trnL</i>	P6 loop	Jørgensen et al. (2012)	vascular plants	Weichselian
			Parducci et al. (2013)	vascular plants	Early Holocene
			Pedersen et al. (2013)	vascular plants	Early Holocene
			Boessenkool et al. (2014)	vascular plants	Middle Holocene
			Willerslev et al. (2014)	vascular plants	Weichselian
			Pansu et al. (2015)	vascular plants	modern
			Parducci et al. (2015)	vascular plants	Weichselian
			Cannon et al. (2016)	vascular plants	modern
			Pedersen et al. (2016)	vascular plants	Younger Dryas
			Seersholm et al. (2016)	vascular plants	Late Holocene
			Bremond et al. (2017)	vascular plants	Middle Holocene
			Heinecke et al. (2017)	aquatic macrophytes	LGM
			Niemeyer et al. (2017)	vascular plants	modern
			Sjögren et al. (2017)	vascular plants	Late Holocene
			Zimmermann et al. (2017)	vascular plants	Weichselian
			Alsos et al. (2018)	vascular plants	modern
			Epp et al. (2018)	vascular plants	Early Holocene
			Clarke et al. (2019)	vascular plants	Early Holocene
			Crump et al. (2019)	vascular plants	Middle Holocene

(Continues)

TABLE 2 (Continued)

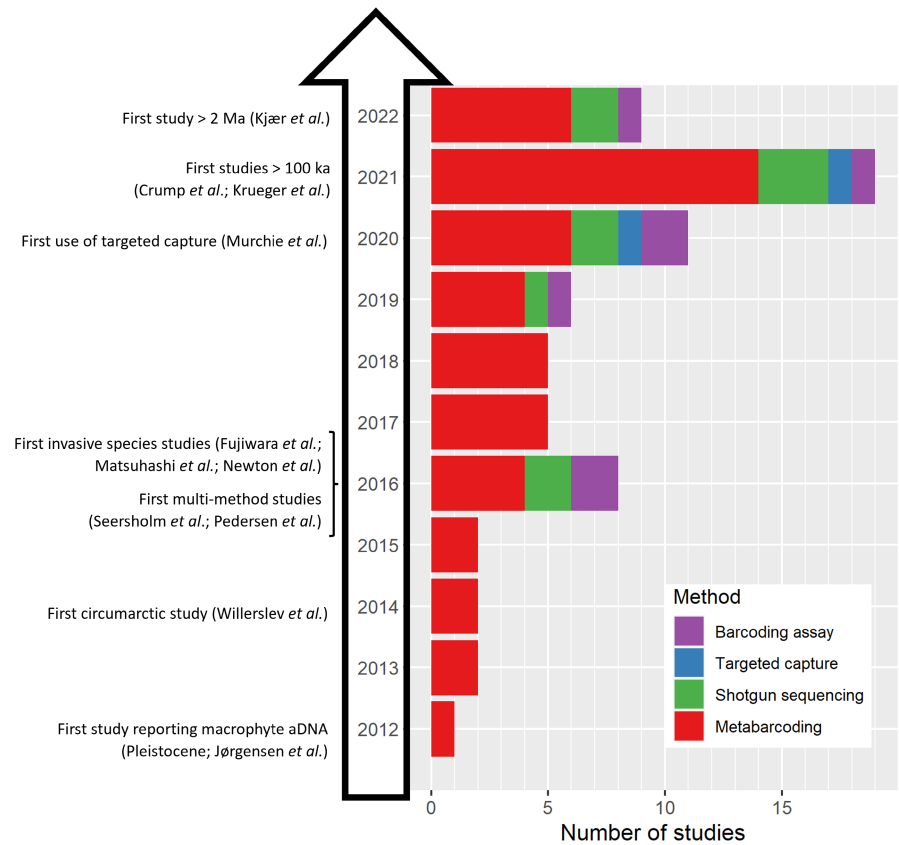
Approach	Marker	Submarker	Publication	Targeted taxon	Sample age
			Giguet-Covex et al. (2019)	vascular plants	Middle Holocene
			Shackleton et al. (2019)	vascular plants	modern
			Alsos, Sjögren, et al. (2020)	vascular plants	LGM
			Clarke et al. (2020)	vascular plants	LGM
			Liu et al. (2020)	vascular plants	Middle Holocene
			Murchie et al. (2020)	vascular plants	Weichselian
			Tabares et al. (2020)	vascular plants	Late Holocene
			Alsos et al. (2021)	vascular plants	Younger Dryas
			Brown et al. (2021)	vascular plants	Late Holocene
			Coghlan et al. (2021)	aquatic macrophytes	modern
			Courtin et al. (2021)	vascular plants	Weichselian
			Crump et al. (2021)	vascular plants	Last Interglacial
			Curtin et al. (2021)	vascular plants	Late Holocene
			Huang et al. (2021)	vascular plants	LGM
			Liu et al. (2021)	vascular plants	LGM
			ter Schure et al. (2021)	vascular plants	Early Holocene
			Tsukamoto et al. (2021)	Podostemaceae	modern
			Wang et al. (2021)	vascular plants	Weichselian
			Alsos et al. (2022)	vascular plants	LGM
			Courtin et al. (2022)	vascular plants	Günz Complex
			Garcés-Pastor et al. (2022)	vascular plants	Younger Dryas
			Ibrahim et al. (2022)	vascular plants	Late Holocene
			Stoof-Leichsenring et al. (2022)	aquatic macrophytes	LGM
			von Hippel et al. (2022)	vascular plants	Weichselian
	<i>rbcL</i>	<i>atpB-rbcL</i> (spacer)	Kuzmina et al. (2018)	aquatic macrophytes	modern
			Crump et al. (2019)	vascular plants	Middle Holocene
			Adame & Reef, 2020	vascular plants	modern
			Coghlan et al. (2021)	aquatic macrophytes	modern
			Ji et al. (2021)	aquatic macrophytes	modern
	<i>matK</i>		Coghlan et al. (2021)	aquatic macrophytes	modern
	ITS	ITS 1	Willerslev et al. (2014)	Asteraceae, Cyperaceae, Poaceae	Weichselian
		ITS 2	Drummond et al. (2021)	vascular plants & algae	modern
			Kuzmina et al. (2018)	aquatic macrophytes	modern
			Coghlan et al. (2021)	aquatic macrophytes	modern
			Palacios Mejia et al. (2021)	vascular plants	modern
	18S		Shackleton et al. (2019)	vascular plants	modern
			Kisand et al. (2018)	eukaryotes	Bølling-Allerød

Note: LGM is Last Glacial Maximum (c. 30–14.7 ka), Bølling-Allerød (14.7–12.9 ka), Younger Dryas (12.9–11.7 ka), Early Holocene (11.7–8.3 ka), Middle Holocene (8.3–4.25 ka), and Late Holocene (4.25–0.0 ka).

Fennoscandia and, although it was the last growth form to appear last (c. 12,900 calibrated years before the present [cal BP]), nearly all taxa appeared at once, highlighting their capacity to immigrate rapidly once suitable environmental conditions are established. Ibrahim et al. (2022) detected the presence of the alien Canadian waterweed

Elodea canadensis in *sedaDNA* over the last centuries, and Anglès d'Auriac et al. (2019) tracked it from water samples over a narrow spatial scale. To date, no study has addressed evolutionary questions for macrophytes with *sedaDNA*, but examples on other plant taxa have showcased this potential.

FIGURE 2 Number of reviewed studies per publication date and approach used. Publications featuring several methods are shown as multiple studies.



Many studies referred to one, or more, other proxies in order to confirm their DNA findings, but only few carried out an in-study comparison with their DNA samples while the rest referred to previous work from the site. Papers investigating modern communities often chose to carry out vegetation surveys. Such surveys often only target a specific taxon of interest in the vicinity (e.g. Tsukamoto et al., 2021), a list of species detected at the spring (Palacios Mejia et al., 2021), or in a given area (e.g. Drummond et al., 2021), or a more complete version providing abundance and biomass estimates (e.g. Ji et al., 2021). The latter showed that eDNA metabarcoding of a c. 300-bp barcode was better than a visual survey for submerged vegetation, which is often hampered by factors such as waterbody area, flow speed, turbidity, and depth (Hughes et al., 2018). Moreover, Alsos et al. (2018) demonstrated that lake sediment eDNA from recent samples largely matches the extant vegetation with 88% of dominant and common taxa and 60% of rare taxa detected, and additionally records taxa missing in the survey that are likely true positives such as plants growing in deep water. Detection is also highly related to environmental conditions, especially temperature and water conductivity (Stoof-Leichsenring et al., 2022). Macrophyte communities constitute a good indicator of environmental conditions, especially in regions with high aquatic plant diversity, which allows sensitivity to the spatial heterogeneity of the ecosystem, and can distinguish land use in river sections (Ji et al., 2021). Focusing on this group in *sedDNA* could support a temporal reconstruction of water quality and trophic status, for instance where human-induced eutrophication is a major stake. If multiple sedimentation

basins are encountered, a spatial approach could even be added, as showcased by Ibrahim et al. (2022) with the overall vegetation in Lake Constance. Pollen remains a widely used proxy for comparison to plant *sedDNA*, but no comparison has focused on aquatic taxa that, in general, have more locally dispersed pollen (by water or insects) but poorer taxonomic resolution than the P6 loop (Sønstebø et al., 2010). Macrofossils only identify a small fraction of the plant community but are particularly suited for aquatics (Parducci et al., 2019).

We found taxonomic resolution to vary greatly between methodological approaches (Figure 3). Metabarcoding has contrasting results that are dependent on the selected marker. The P6 loop identified on average 55% of all aquatic taxa detected to species-level (Figure 3, Table S1). ITS 1, ITS 2, and *rbcL* have higher discriminative power, and respectively assigned 74%, 79%, and 80% of aquatics to species, although each of them was used in only two to four studies (Table S1). Shotgun sequencing identifies 12% and 45% of aquatic taxa respectively at family and genus level, but only 38% at the species level (Table S1). For metabarcoding, the taxonomic resolution was generally higher for aquatics than for other vascular plants (Table S5). Regarding targeted capture, Murchie et al. (2020) created a bait-set targeting the three chloroplast loci *trnL*, *rbcL*, and *matK* based on sequences from 921 to 2,090 arctic and subarctic taxa (Table 2), but the resulting sequences were not identified to the genus level (Figure 3). On the other hand, Krueger et al. (2021) targeted *rbcL* only with a bait-set designed from just 20 vascular plant species including five aquatics (Table 2), and reported six aquatic

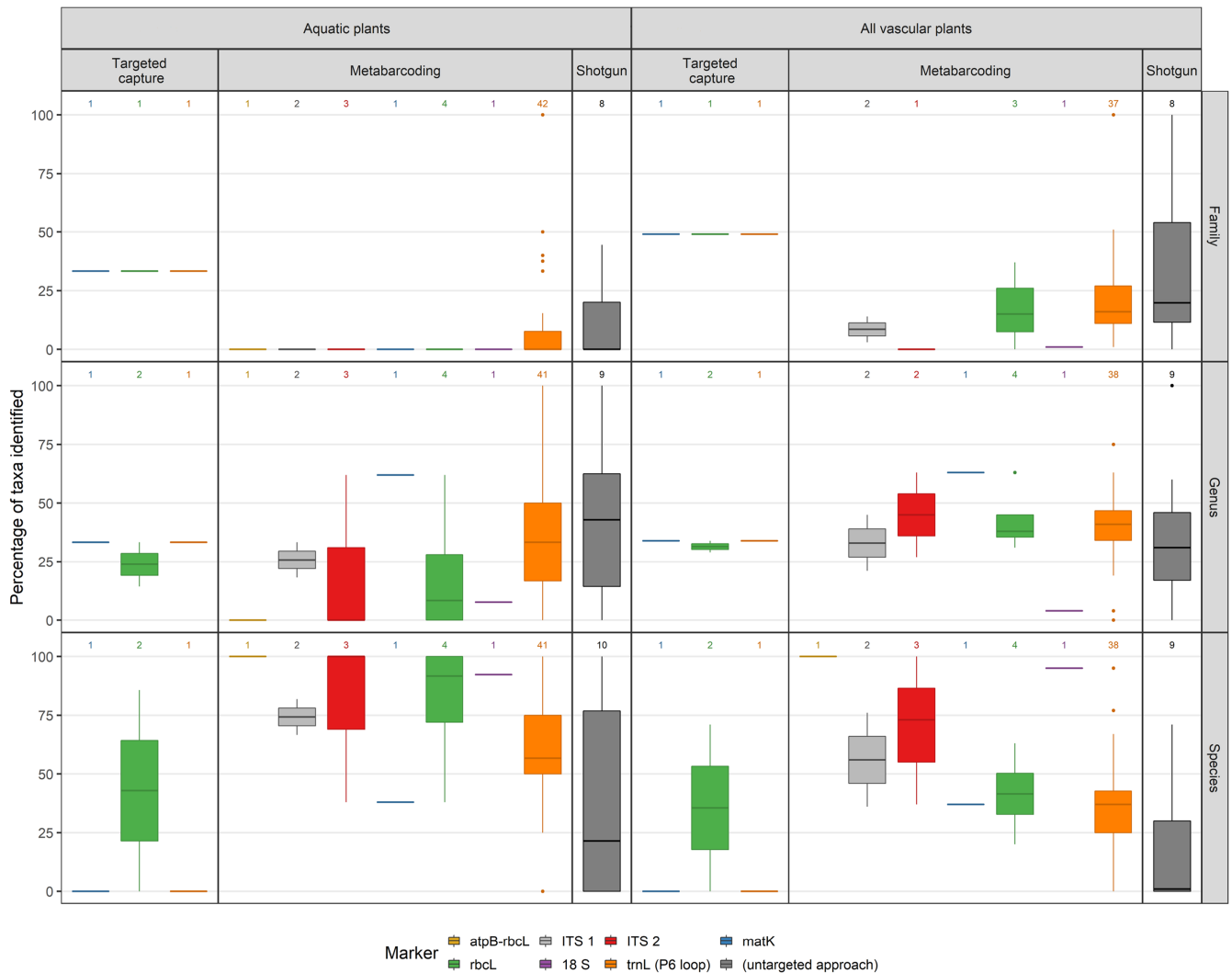


FIGURE 3 Taxonomic resolution in percent of taxa identified to the family, genus, or species level; by method and marker used, for aquatic macrophytes only (left) and all vascular plant taxa (right). Sums of taxa identified might not reach 100%, i.e. taxa were identified at a level higher than family. Number of studies is shown above each boxplot, it is inconsistent whenever a study gives partial results and mentions only a certain taxonomic level. We excluded datasets that used single-species approaches, because by definition their taxonomic resolution would be 100% at the species level.

species and one genus (Figure 3, Table S1). For all vascular plants, average taxonomic resolution at species-level is 41% with metabarcoding, 18% with targeted capture, and 18% with metagenomics. The P6 loop marker remains more accurate (35% of species-level identification) than metagenomics for all vascular plants; both are commonly used in palaeobotany.

The quality and exhaustiveness of the reference libraries affects the taxonomic resolution for all three methods (Box 1). The reviewed studies used a median of two reference libraries to assign their OTUs (Table S1): they usually used a global one, such as GenBank or EMBL, and a better curated one with focused regional coverage, which reduces the possibility of sequence sharing with geographically non-plausible species (e.g. PhyloNorway, ArcBorBryo).

Aquatic macrophyte richness is not correlated to their taxonomic resolution at the species level, implying that a method with higher taxonomic resolution does not result in a higher diversity captured

(Table 3). However, macrophyte richness detected depends in turn on other parameters: it is higher in more recent publications and may also decrease with (oldest) sample age although it is less clear for P6 loop and metagenomic studies (Table 3, Figure S1), but old (>60,000 cal BP) samples are too scarce to be considered representative. Furthermore, total richness detected is also largely affected by DNA preservation, which is itself dependent on sample age as well as environmental factors (Jia et al., 2021).

3.2 | Case study: Reconstruction of past aquatic conditions in 10 lakes from northern Fennoscandia

There were 28 aquatic macrophyte taxa in our northern Fennoscandia case study, 15 of which were unambiguously identified to the species level. In total, 19 taxa had trait values available

TABLE 3 Parameters and output of (a) correlation tests between macrophyte diversity detected and resolution at four taxonomic levels after a centred log ratio transformation, and (b) generalised linear models assessing the respective influences of the year of study and the age of samples on macrophyte diversity detected, and on its species-level resolution.

	Formula	Coefficient	p-value	Method
(a)	$\text{corr}(\text{Richness, Species})$	0.084	0.496	Pearson
	$\text{corr}(\text{Richness, Genus})$	0.114	0.350	
	$\text{corr}(\text{Richness, Family})$	-0.059	0.631	
	$\text{corr}(\text{Richness, higher})$	-0.181	0.136	
(b)	Richness ~ Study year	0.187	0.004 *	quasi-Poisson
	Species ~ Study year	-0.074	0.846	Gaussian
	Richness ~ Oldest sample age	-1.112×10^{-5}	0.058	quasi-Poisson
	Species ~ Oldest sample age	-2.041×10^{-5}	0.473	Gaussian
	Richness ~ Oldest sample age (P6 loop only)	-4.359×10^{-6}	0.431	quasi-Poisson
	Species ~ Oldest sample age	1.476×10^{-5}	0.160	Gaussian
	Richness ~ Oldest sample age (shotgun only)	-1.039×10^{-7}	0.778	quasi-Poisson
	Species ~ Oldest sample age	7.844×10^{-7}	0.868	Gaussian

Note: We used a quasi-Poisson distribution in the model when the response variable was a count data. We excluded Kjær et al. (2022) and Courtin et al. (2022) from the overall sample age models, because they studied material much older than the rest (>0.6 Ma). The asterisk indicates a significant ($p < 0.05$) relationship, the apostrophe indicates a near significant ($0.05 < p < 0.1$) relationship. Values were rounded to three decimal places, and outliers were removed.

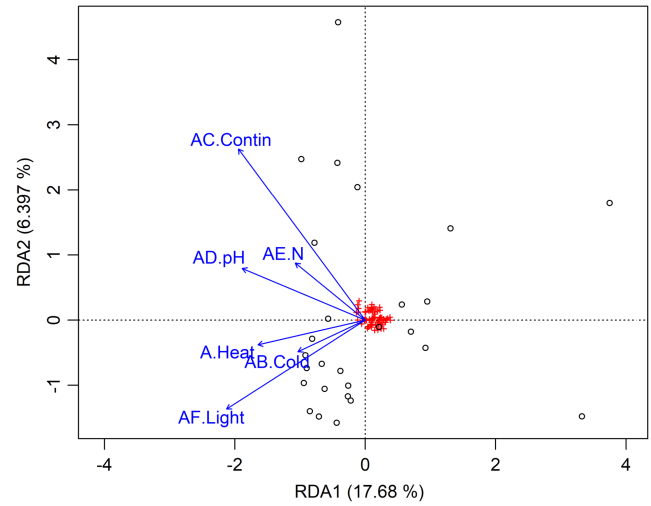


FIGURE 4 Redundancy analysis (RDA) of macrophyte community composition constrained by six selected environmental traits. Percentages of variance explained by each axis are given in brackets.

across all six reconstructed traits, i.e. heat- and cold requirements, continentality, pH, nitrogen availability, and light optimum. We found no significant correlation between the first recorded or estimated arrival dates of taxa and our selected traits, correlation coefficients being between -0.3 and 0.5 for the detected arrivals, and between -0.4 and 0.1 for the estimated arrivals. The first and second RDA axes respectively explained 17.7 and 6.4% of the variation in aquatic macrophyte community composition (Figure 4). Of the six traits included in the RDA, heat requirement had the highest correlation with axis 1 ($\rho = -0.974$), and continentality had the highest correlation with axis 2 ($\rho = 0.804$).

Our reconstruction of past environmental conditions across northern Fennoscandia shows the variability in the diversity of niches, and an overall stability since 9,000 cal BP (Figure 5). There is a weak, but steady acidification trend: the early record is largely dominated by circumneutral (pH c. 6.5) taxa, but modern communities are characterised by moderately acidic (pH c. 5) taxa. Since the end of the Early Holocene, heat requirement, continentality, nitrogen availability, and light optimum are dominated by a single trait value representing over half of weighted PCR replicates. Indeed, since c. 8,000 cal BP, the majority of niches realised by aquatic macrophyte communities has been characterised by a low heat requirement (i.e. value 3 = reaching as high as the low-alpine belt, sensu Tyler et al., 2021), with no effect of continentality on their geographical range (i.e. values 4.5–5 = distributed indifferently across Scandinavia, sensu Tyler et al., 2021), moderately nitrogen-poor water, and half-shade conditions. However, the pre-Holocene record is not a fully regional signal, as the Fennoscandian ice sheet still covered most of the region at 12,000 cal BP (Hughes et al., 2016). Only lakes Sandfjorddalen and Nordvivatnet have aquatic macrophyte records predating the Holocene ($>11,000$ cal BP). Although the Langfjordvannet core extends to 16,000 cal BP, no aquatics are detected before 10,000 cal BP. It is worth noting that this lake is the deepest (34.8 m) thus

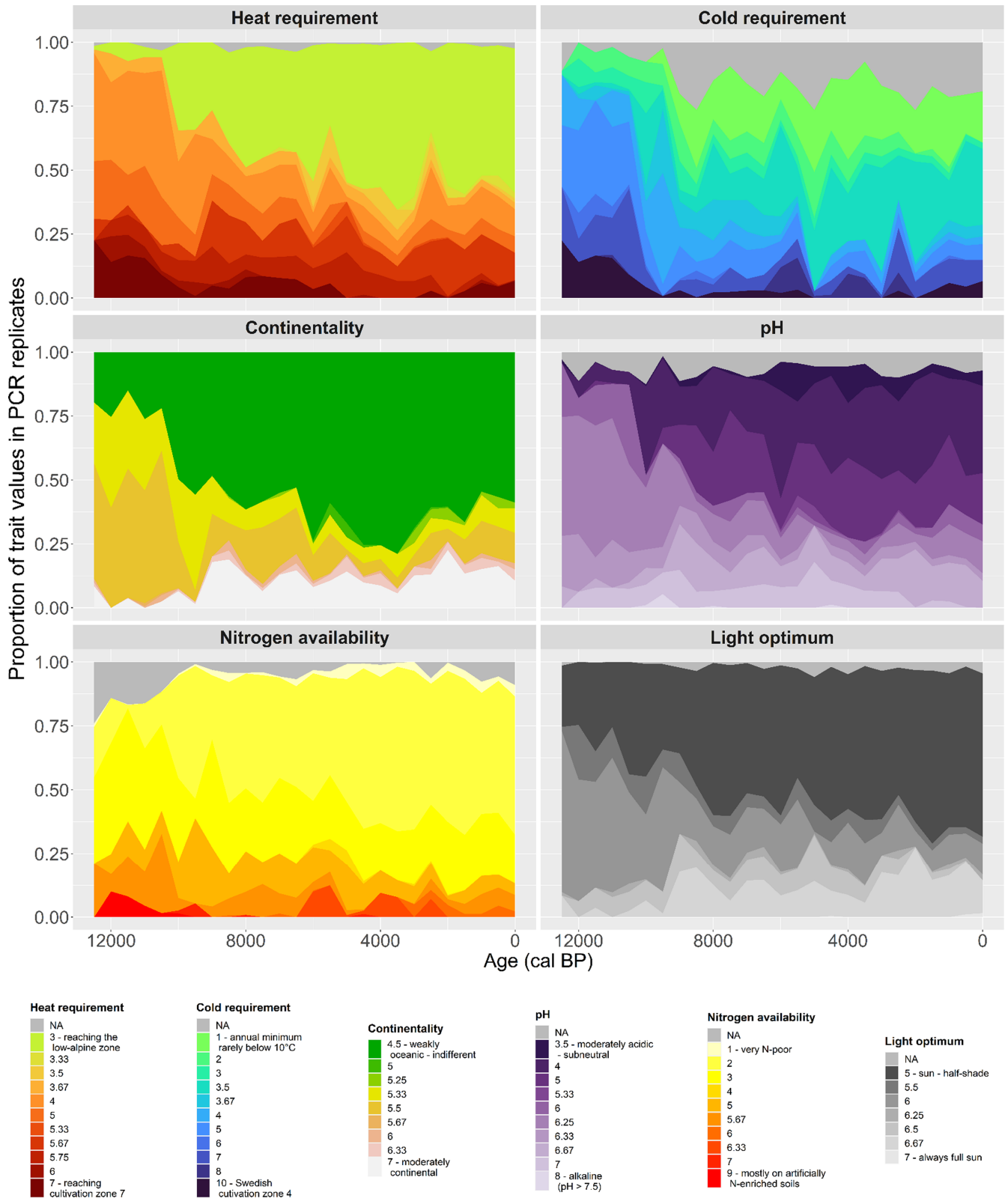


FIGURE 5 Proportion of traits values (see methods) in polymerase chain reaction replicates across northern Fennoscandia (all 10 lakes) through the end of Late Pleistocene (12,500 to 11,700 cal BP) and the Holocene (11,700 cal BP to present), in 500-years bins. A high trait value indicates a strong expression of the trait.

macrophytes cannot be found in its centre, as their maximum growth depth is about 12 m (Sheldon & Boylen, 1977). From 10,000 cal BP onwards, there are more than five lake records with aquatic macrophytes (Figure S3). Species requiring the warmest conditions, such as *Cicuta virosa* and *Stuckenia vaginata* (Figure S4), disappear between 5,000 and 3,000 cal BP (Figure 5), which can be interpreted as lower summer temperatures. This pattern is consistent with the long-term cooling trend after the mid-Holocene thermal maximum; but the late reappearance of *Stuckenia vaginata* in lakes Gaupstjern and Sandfjorddalen at c. 2,700 cal BP, then in lake Horntjernet at 100 cal BP indicates a recent warming (Figure S6a–c). Pioneer aquatic communities developed under intermediate light conditions, with most taxa requiring sun but enduring some shading, and there was a progressive shift towards increased heterogeneity between 9,000 and 4,000 cal BP. The modern presence of half-shade tolerant taxa as well as taxa requiring full sunlight exposure, reflects an increased habitat complexity and/or the development of closed-canopy riparian forest. The slow progress of light-dependent taxa could be linked to winter ice dynamics, and be indicative of winters becoming progressively shorter.

Individual lakes display different trends in environmental reconstructions, depending on their community composition and turnover. We retained only those lakes that have an average richness over time of five or more aquatic taxa (Sandfjorddalen=8.6, Nordvivatnet=6.2, Sierravannet=5.6, Horntjernet=5.6, Kuutsjärvi=5), so that the distribution of traits values is robust enough to support environmental inferences for a single site. For instance, lake Horntjernet sees a slow decrease of cold-tolerant taxa, followed by an absence around 4,000 cal BP then an increase (Figure S5a), with higher proportions of *Ranunculus cf. reptans* in PCR replicates (Figure S6c). Lake Sandfjorddalen, which has a record that spans the entire Holocene (except for a hiatus between 5,500 and 3,000 cal BP, Figure S6b), shows long-term stability in environmental conditions (Figure S5b), partly due to a low compositional turnover (Figure S6b). Such consistency suggests that the lake depth has probably remained stable and shallow (presently 1.23 m) throughout, because we would expect a complete drying or an increase in water level to cause significant changes in the macrophyte community. Conversely, lakes Horntjernet and Kuutsjärvi display more temporal variability in reconstructed environmental conditions (Figure S5a,c). There is a steady decrease in continental taxa over the long term in Kuutsjärvi; whereas Horntjernet is dominated by moderately continental taxa until c. 9,000 cal BP, and continentality increases again from 3,500 cal BP. Initial conditions in Horntjernet were circumneutral to slightly acidic until 8,500 cal BP, when pH drops below 6.5 concurrently with the arrival of *Pinus* forest (Rijal et al., 2021). The reappearance of *Stuckenia vaginata* in the most recent samples indicates another shift towards a more neutral pH, as this species has a pH tolerance of c. 7. *Cicuta virosa* appears in Kuutsjärvi as early as 9,000 cal BP and disappears 4 millennia later (Figure S6d); its presence indicates eutrophication and warmer temperatures.

4 | DISCUSSION

The general hypothesis that the DNA methodology used will affect the reconstructed aquatic plant assemblage, including estimated diversity, composition, and richness, is supported and the degree of variation is showcased by this study. We further demonstrate that the reconstruction of past environmental conditions based on the aquatic macrophyte *seda*DNA signal is possible at both local and regional scales.

4.1 | Global distribution of reviewed studies

Most sites concentrate on high-latitude or high-altitude regions. Polar regions are especially vulnerable to invasive species and so will be a priority for eDNA-based aquatic biodiversity monitoring, and there are also comprehensive DNA reference libraries available for this region for the P6 loop (Alsos et al., 2022; Sønstebo et al., 2010; Willerslev et al., 2014), standard barcodes (Alsos, Lavergne, et al., 2020; Kuzmina et al., 2017), and genome skims (Wang et al., 2021). However, genetic reference libraries are not evenly available across the globe, and their existence and implementation depend on the diversity and taxonomic specificities in each freshwater ecoregion. Aquatic macrophytes are less diverse and have larger ranges in northern Fennoscandia as compared to intertropical zones (Murphy et al., 2019), which contributes to the near-exhaustivity of the PhyloNorway reference library. In contrast, the large reference database used by Ji et al. (2021) to detect over 100 macrophyte species along the Chaobai river in China, was based on the global Nucleotide Sequence Database from NCBI's GenBank, which suffers from limited curation and may not be fully representative of the local flora. Additionally, using such libraries with global coverage can match genuine local sequences to their exotic counterparts, but this source of error can be avoided with a stringent cross-checking of the resulting taxa list (Table S3, see taxa excluded from the datasets of Cannon et al., 2016; Shackleton et al., 2019). Under similar ecological conditions, reference libraries could also be adapted to regional specificities, e.g. taxonomic differences between the Palearctic and Nearctic floras (Kuzmina et al., 2017).

The site map highlights the nearly exclusive distribution of old (>11,700 cal BP, i.e. Pleistocene) records in the Arctic, especially Siberia (e.g. meta-analysis by Wang et al., 2021). Indeed, current research suggests that a colder environment leads to a better preservation of *captive* eDNA, i.e. trapped in soil and sediment (Parducci et al., 2017; Taberlet et al., 2018). North America already has a comprehensive long-term macrofossil record for aquatic plants at the regional scale (Sawada et al., 2003), which foreshadows further reconstructions of freshwater postglacial conditions for that continent. However, the few existing studies for warmer regions, including one Eemian Ethiopian palaeolake, plus four Holocene records from Benin, Rwanda, and Namibia (Table S2), show the potential for tropical regions. Thus, the likely success of such studies depends

both on the completeness of a regional reference library adapted for the method, and the state of eDNA preservation, especially for ancient material.

Although we focused on freshwater ecosystems, Gaffney et al. (2020) also detected marine macrophytes (i.e. seagrasses), which represent entire families, e.g. Zosteraceae and Cymodoceaceae. Seagrasses play an important role in marine ecology, comparable in magnitude with coral reefs. Often growing in clonal colonies, they form large coastal habitats of paramount importance for the feeding and breeding of marine life, act as a carbon sink, and are a protection against coastal erosion (Arnaud-Haond et al., 2012; Christianen et al., 2013; Jiang et al., 2020; Lyimo, 2016; Zou et al., 2021). Seagrasses are well-detected in coastal sedimentary DNA (Foster et al., 2021; Ortega et al., 2020), and therefore their palaeorecord could also be used to reconstruct shallow marine environments in a similar way to our study. They may also be found in coastal lacustrine and fluvial environments, especially where there are large tidal ranges such as macrotidal estuaries.

4.2 | Taxonomic resolution of the different methodological approaches

Our review covers three molecular approaches for biodiversity detection, the use of which depends on the aim of the study as these methodologies have different advantages and disadvantages. Metagenomics and targeted capture are underrepresented in our dataset, as they have only recently been applied to eDNA and *seda*DNA and therefore few studies have used these approaches for vascular plants, which is a limiting factor for the comparison of their respective taxonomic resolutions in aquatic macrophytes. To our knowledge, no direct comparison has yet been made between metabarcoding, metagenomics, and targeted capture to investigate yields with their respective optimal settings. Although Murchie et al. (2020) used all three methods, they used reference libraries whose respective taxonomic and biogeographic breadths differed by orders of magnitude: most of the Canadian flora is covered by at least one of three barcodes (*rbcL*, *matK*, ITS 2; Kuzmina et al., 2017) which is ideal for metabarcoding and targeted capture, but the more incomplete and uneven coverage for metagenomics hampered identification for the shotgun dataset. The best way to compare methods would be to analyse the same samples with the optimised pipeline of each, while using adequate and complete reference libraries.

Modern eDNA metabarcoding studies have used a range of markers, mainly long ones (usually >400 bp); whereas the P6 loop is preferred in ancient studies. Indeed, the P6 loop is a short (51–135 bp) marker for vascular plants, and is thus especially suitable for degraded DNA (Taberlet et al., 2007). However, standard, longer (several hundred bp) barcodes are available for many more regions than P6 loop data, which provide opportunities for analyses of modern samples not limited by barcode length from more areas. Indeed, barcode taxonomic resolution usually increases with marker

length. Most metabarcoding studies have used a single marker, but the method has potential for improvement by targeting multiple loci. Multi-marker studies often have used a combination of a nuclear and a chloroplast barcode: Kuzmina et al. (2018) used ITS 2 and *atpB-rbcL*; while Shackleton et al. (2019) combined 18S with the *trnL* P6 loop. All these markers were chosen while targeting a broader group than aquatic macrophytes, but it may be possible to develop new markers specifically for aquatic macrophytes, which have improved taxonomic resolution (Scriver et al., 2015). Nevertheless, even when targeting aquatics only, some loci fail to distinguish closely related species (e.g. within the Potamogetonaceae) because intraspecific and interspecific variability are of comparable magnitude (Du et al., 2011).

Shotgun sequencing is considered a less biased approach than metabarcoding for estimating abundance because it omits the PCR step, which commonly causes biases (Pedersen et al., 2016). Metabarcoding and shotgun sequencing have both been used for nearly two decades (e.g. Tringe & Rubin, 2005; Willerslev et al., 2003), but the latter has greater sequencing costs. Most importantly, its efficiency relies on the availability of a considerably more complete reference library than a metabarcoding one, with entries from more target organisms and a broader genome coverage. As of today, genome skims are quite recent, and only available for the Arctic, part of the Chinese flora, and Western Australia (Alsos, Lavergne, et al., 2020; H.-T. Li et al., 2019; Nevill et al., 2020). Even genome skims cause unequal representation of taxa, and random match to closely related species may limit taxonomic resolution to the genus level (Wang et al., 2021). While full genome assemblies are not available for any regional flora, the global count approaches 1000 (Marks et al., 2021) and more will become available with time, enabling metagenomics to be used to its full potential. However, currently shotgun sequencing has a poor rate of raw sequence assignment compared to other methods, as its sequences originate from across entire genomes and any taxa, and most of them do not reliably correspond to any known genomic reference (Peabody et al., 2015). For example, most of the remainder are assigned to higher taxonomic ranks (e.g. kingdom, order), and only a minority gets identified to the genus or species level. Courtin et al. (2022) could only assign 12% of their shotgun sequencing reads, and Viridiplantae only accounted for 0.4% of these. This method requires comprehensive reference libraries, ideally entire genomes, as an incomplete reference library will cause many false positive matches as well as false negative misses.

The targeted capture approach consists of building a broad library of probes based on several hundreds to thousands of species, in order to cover the expected taxonomic breadth of broad groups such as vascular plants. It greatly increases the targeted region, at the cost of a diminished taxonomic resolution: Murchie et al. (2020) were not able to go much beyond genus level; and Krueger et al. (2021) only reported six macrophyte species and one genus, but did not report assignments at higher taxonomic levels. While it is in theory possible to identify more detected taxa to the species level, in practice most captured *seda*DNA fragments are too short to

be taxonomically informative at the species level, and DNA damage can lead to both false positives and negatives. However, detection of broad communities using targeted capture may have more promise for modern eDNA.

Our case study has a taxonomic resolution above average, with 68% of taxa identified to species level. However, some sequences remained unresolved between several species, sometimes even genera (e.g. *Nuphar* spp./*Nymphaea alba*). Because taxonomically unresolved sequences cannot always be assigned finite traits values, it in turn restricts the realised accuracy of our reconstruction. Taxonomic resolution in some aquatic taxa may be hampered by their close phylogeny, for instance within Potamogetonaceae. In our case study dataset, there were eight sequences matching this family represented by 1,316,980 reads and 2,082 PCR replicates in total. The P6 loop sequence of Potamogetonaceae is longer than average (86 bp), and so the high count of reads shows that even longer sequences can be well amplified, which is probably due to the fact that aquatic macrophyte eDNA is better detected because they grow and decay adjacent to the sediment. There are eight *Potamogeton* and three *Stuckenia* species native to northern Fennoscandia (Alsos et al., 2022; Elven et al., 2022). *Potamogeton praelongus* has intraspecific variability, and different haplotypes may explain why our metabarcoding data did not match to PhyloNorway even if it did match at 100% with the three other reference libraries. Hybridisation, which is common in the genus *Potamogeton*, probably explains this sequence variability that may complicate taxonomic assignment both in reference libraries and consequently in metabarcoding data. Indeed, that metabarcoding dataset contains intraspecific variations as well as interspecific sequence sharing, but also one homopolymer consisting of six adenine bases, which can cause problems during amplification and sequencing. For studies targeting these taxa alternative barcodes should be explored.

At the moment, metabarcoding stands out as the method with the highest taxonomic resolution, both for aquatic macrophytes and for the total flora. Recent advances have demonstrated that it is possible to assemble genome-scale reference libraries, including from herbarium collections (Alsos, Lavergne, et al., 2020). This can assist in unlocking the bottleneck to access the large potential of metagenomics for palaeoecology of aquatic macrophytes and other taxa (Kjær et al., 2022; Wang et al., 2021). We can expect to see a continued increase in eDNA publications (Capo et al., 2021), as methods are refined and reference libraries are supplemented. Metabarcoding is the least expensive of all three methods and requires smaller reference libraries. It is also analytically easier, as the genomic locus is constrained and full barcode sequences are generated, unlike either of the metagenomic-based methods; thus, it might remain popular for modern surveys of aquatic macrophyte diversity in the short-to-medium term. For palaeoecological studies, it is crucial to use a short marker in order to capture the diversity of taxa represented in ancient material, and in turn provide an overview of best estimate of past communities.

4.3 | Potential for ecological and environmental reconstruction

Environmental traits have different temporal trends: nitrogen availability appears to remain stable throughout the Holocene, while light optimum changes gradually and temperature shifts are more abrupt. We have reconstructed the continuous postglacial ecological history of northern Fennoscandia. This gives a general overview of regional conditions and follows the Fennoscandian ice sheet retreat as lakes become ice-free (Alsos et al., 2022). Here, the ecological succession of aquatic communities retraces the establishment of modern environmental conditions since the onset of the Holocene. Aquatic macrophytes appear later in lake records than any other plant functional group, but contrary to the terrestrial vegetation, their diversity soars rapidly after arrival (Alsos et al., 2022).

Environmental trends are also visible among lakes, and these site-specific conditions emphasise the local scale precision achieved by our reconstruction, precisely because aquatic macrophytes have restricted niches related to water level. The remarkable environmental stability in Sandfjorddalen throughout the Holocene demonstrates that niche persistence over time existed even in the early record. Conversely, other lakes displayed fluctuating conditions, offering evolving niches for aquatic plant communities. This suggests that even their early occurrences can be used confidently for environmental reconstruction. Indeed, the temporal compositional and richness changes could be observed across Siberia and Tibet for the Pleistocene and Holocene (Stoof-Leichsenring et al., 2022). Combining the modern dataset with the core metabarcoding data would allow use of the macrophyte community signal for the reconstruction of past environments, such as water conductivity and summer air temperature.

Beyond ecological and environmental reconstructions, the *se-da*DNA signal from macrophytes could in the future be leveraged to address evolutionary questions. High-resolution time series can be combined with other proxies in order to allow statistical analyses and inference of causal relationships among drivers of ecosystem changes, such as human land use and climate change (Garcés-Pastor et al., 2022). With the rapid ongoing methodological improvements, distinguishing closely related species and even haplotypes could support the fine-scale mapping of genetic diversity and its variation in time and space (Epp et al., 2018), and help retrace postglacial colonisation routes to resolve our understanding of the origin of modern boreal and arctic vegetation. Lastly, the recent retrieval of geologically ancient eDNA (Kjær et al., 2022) could give a direct insight on evolutionary genetic processes at million-year timescales, especially for organisms such as macrophytes which rarely persist as macrofossils.

In summary, macrophyte-based P6 loop metabarcoding supports reconstructions for a wide array of important freshwater metrics. From *se-da*DNA-derived macrophyte turnover and community composition, we can infer lake level changes (Heinecke et al., 2017) and lake water conductivity and summer air temperature changes

(Stoof-Leichsenring et al., 2022) on millennial time scales. Our study extends environmental reconstructions for northern Fennoscandian lakes over the last 12, 000 years to thermal range, continentality, nutrient availability, light conditions, and water pH. We show the potential that aquatic plants aDNA offers to reconstruct past environments when using a comprehensive trait database of the local flora.

5 | CONCLUSION

This review shows that aquatic macrophytes are often detected in both eDNA and *sedaDNA* studies, often being the most common and abundant taxa. Environmental DNA from aquatics is reported in a wide range of sample types, from stream water to permafrost, and in various bioregions although colder regions are overrepresented at present. *SedaDNA* of aquatic macrophytes can be detected from samples up to 2 million years old in favourable environments (Kjær et al., 2022), which indicates a considerable potential for long-term *sedaDNA*-based environmental reconstruction.

Shotgun sequencing appears to lack sufficient taxonomic resolution to identify environmentally indicative aquatic macrophyte taxa at present. Targeted capture has not been widely applied to plants, but aDNA results on mammals are encouraging for the development of bait-sets that could screen both past and present plant biodiversity. Barcoding can track a single species through space, working under the assumption of presence of the targeted taxon; yet its potential when applied to aDNA is limited by the length of barcode used. Metabarcoding is a more versatile tool, and there is a wide range of markers one can choose from. However, when applying this approach to ancient samples, we recommend the use of a short (<ca. 100 bp) marker in order to maximise the yield, but still able to capture and identify sequences assuming the availability of a corresponding reference library. In this regard, the *g-h* primer set targeting the P6 loop fulfils these requirements to a large extent and it has proven successful in a variety of studies, due to the availability of highly curated sequence libraries.

Using *sedaDNA* to reconstruct ecological and environmental conditions of the past can be supplemented by cross-disciplinary expertise on local and regional diversity, postglacial migration, vegetation cover and land use changes (Alsos et al., 2022; Brown et al., 2022). The paramount role of reference libraries is highlighted by the differences in taxonomic resolution between methods as the requirements for targeted capture largely vary depending on the taxonomic diversity targeted; metabarcoding often goes to the species level due to well-curated barcode databases; while metagenomics could potentially achieve comparable results but that will require increased availability of reference genomes.

AUTHOR CONTRIBUTIONS

Conceptualisation: Inger G. Alsos. Conducting the research: Aloïs Revéret, Peter D. Heintzman, Antony G. Brown, Dilli P. Rijal, Inger G. Alsos. Data analysis: Aloïs Revéret, Dilli P. Rijal. Preparation

of figures & tables: Aloïs Revéret. Data interpretation, writing: Aloïs Revéret, Dilli P. Rijal, Peter D. Heintzman, Antony G. Brown, Kathleen R. Stoof-Leichsenring, Inger G. Alsos.

ACKNOWLEDGEMENTS

This work was supported by the ECOGEN project (Ecosystem change and species persistence over time: a genome-based approach), Research Council of Norway grant 226134/F50 (to I.G.A.). We thank Pablo Raguet for his help with data analysis and visualisation.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

Full references for the reviewed papers, and data extracted from them, are found in Table S1 (by publication) and Table S2 (by site). The full list of aquatic taxa detected is given as Table S3. The *sedaDNA* dataset from Rijal et al. (2021) can be found in their supplementary information; the reanalysed version used here can be found in Alsos et al. (2022). The trait database is available as supplementary information in Tyler et al. (2021).

ORCID

Aloïs Revéret <https://orcid.org/0000-0002-3852-2598>

Dilli P. Rijal <https://orcid.org/0000-0002-6194-4414>

Peter D. Heintzman <https://orcid.org/0000-0002-6449-0219>

Antony G. Brown <https://orcid.org/0000-0002-1990-4654>

Kathleen R. Stoof-Leichsenring <https://orcid.org/0000-0002-6609-3217>

Inger G. Alsos <https://orcid.org/0000-0002-8610-1085>

REFERENCES

- Ackerfield, J. (2015). *Flora of Colorado* (Vol. 41). Botanical Research Institute of Texas. <https://www.nhbs.com/flora-of-colorado-book>
- Adame, M. F., & Reef, R. (2020). Potential pollution sources from agricultural activities on tropical forested floodplain wetlands revealed by soil eDNA. *Forests*, 11(8), 892. <https://doi.org/10.3390/f11080892>
- Alahuhta, J., Rosbakh, S., Chepinoga, V., & Heino, J. (2020). Environmental determinants of lake macrophyte communities in Baikal Siberia. *Aquatic Sciences*, 82(2), 39. <https://doi.org/10.1007/s00027-020-0710-8>
- Alsos, I. G., Lammers, Y., Kjellman, S. E., Merkel, M. K. F., Bender, E. M., Rouillard, A., Erlendsson, E., Guðmundsdóttir, E. R., Benediktsson, Í. Ö., Farnsworth, W. R., Brynjólfsson, S., Gísladóttir, G., Eddudóttir, S. D., & Schomacker, A. (2021). Ancient sedimentary DNA shows rapid post-glacial colonisation of Iceland followed by relatively stable vegetation until the Norse settlement (Landnám) AD 870. *Quaternary Science Reviews*, 259, 106903. <https://doi.org/10.1016/j.quascirev.2021.106903>
- Alsos, I. G., Lammers, Y., Yoccoz, N. G., Jørgensen, T., Sjögren, P., Gielly, L., & Edwards, M. E. (2018). Plant DNA metabarcoding of lake sediments: How does it represent the contemporary vegetation. *PLoS One*, 13(4), e0195403. <https://doi.org/10.1371/journal.pone.0195403>
- Alsos, I. G., Lavergne, S., Merkel, M. K. F., Boleda, M., Lammers, Y., Alberti, A., Pouchon, C., Denoëud, F., Pitelkova, I., Puşcaş, M., Roquet, C.,

- Hurdu, B.-I., Thuiller, W., Zimmermann, N. E., Hollingsworth, P. M., & Coissac, E. (2020). The treasure vault can be opened: Large-scale genome skimming works well using herbarium and silica gel dried material. *Plants*, 9(4), 432. <https://doi.org/10.3390/plants9040432>
- Alsos, I. G., Rijal, D. P., Ehrlich, D., Karger, D. N., Yoccoz, N. G., Heintzman, P. D., Brown, A. G., Lammers, Y., Pellissier, L., Alm, T., Bråthen, K. A., Coissac, E., Merkel, M. K. F., Alberti, A., Denoeud, F., Bakke, J., & PHYLLONORWAY CONSORTIUM. (2022). Postglacial species arrival and diversity buildup of northern ecosystems took millennia. *Science Advances*, 8(39), eabo7434. <https://doi.org/10.1126/sciadv.abo7434>
- Alsos, I. G., Sjögren, P., Brown, A. G., Gielly, L., Merkel, M. K. F., Paus, A., Lammers, Y., Edwards, M. E., Alm, T., Leng, M., Goslar, T., Langdon, C. T., Bakke, J., & van der Bilt, W. G. M. (2020). Last glacial maximum environmental conditions at Andøya, northern Norway; evidence for a northern ice-edge ecological "hotspot". *Quaternary Science Reviews*, 239, 106364. <https://doi.org/10.1016/j.quascirev.2020.106364>
- Alsos, I. G., Sjögren, P., Edwards, M. E., Landvik, J. Y., Gielly, L., Forwick, M., Coissac, E., Brown, A. G., Jakobsen, L. V., Føreid, M. K., & Pedersen, M. W. (2016). Sedimentary ancient DNA from Lake Skartjørna, Svalbard: Assessing the resilience of arctic flora to Holocene climate change. *The Holocene*, 26(4), 627–642. <https://doi.org/10.1177/0959683615612563>
- Anglès d'Auriac, M. B., Strand, D. A., Mjelde, M., Demars, B. O. L., & Thaulow, J. (2019). Detection of an invasive aquatic plant in natural water bodies using environmental DNA. *PLoS One*, 14(7), e0219700. <https://doi.org/10.1371/journal.pone.0219700>
- Arnaud-Haond, S., Duarte, C. M., Diaz-Almela, E., Marbà, N., Sintès, T., & Serrão, E. A. (2012). Implications of extreme life span in clonal organisms: Millenary clones in meadows of the threatened seagrass *Posidonia oceanica*. *PLoS One*, 7(2), e30454. <https://doi.org/10.1371/journal.pone.0030454>
- Australian Biological Resources Study. (2015). *Flora of Australia* (Vol. 1–59). CSIRO Publishing.
- Bjune, A. E., Greve Alsos, I., Brendryen, J., Edwards, M. E., Hafliðason, H., Johansen, M. S., Mangerud, J., Paus, A., Regnéll, C., Svendsen, J.-I., & Clarke, C. L. (2021). Rapid climate changes during the Lateglacial and the early Holocene as seen from plant community dynamics in the polar Urals, Russia. *Journal of Quaternary Science*, 37(5), 805–817. <https://doi.org/10.1002/jqs.3352>
- Boessenkool, S., McGlynn, G., Epp, L. S., Taylor, D., Pimentel, M., Gizaw, A., Nemomissa, S., Brochmann, C., & Popp, M. (2014). Use of ancient sedimentary DNA as a novel conservation tool for high-altitude tropical biodiversity. *Conservation Biology*, 28(2), 446–455. <https://doi.org/10.1111/cobi.12195>
- Bremond, L., Favier, C., Ficetola, G. F., Tossou, M. G., Akouégninou, A., Gielly, L., Giguët-Covex, C., Oslisly, R., & Salzmann, U. (2017). Five thousand years of tropical lake sediment DNA records from Benin. *Quaternary Science Reviews*, 170, 203–211. <https://doi.org/10.1016/j.quascirev.2017.06.025>
- Brooks, S. J., & Birks, H. J. B. (2000). Chironomid-inferred late-glacial and early-Holocene mean July air temperatures for Kråkenes Lake, western Norway. *Journal of Paleolimnology*, 23(1), 77–89. <https://doi.org/10.1023/A:1008044211484>
- Brown, A. G. (2002). Learning from the past: Palaeohydrology and palaeoecology. *Freshwater Biology*, 47(4), 817–829.
- Brown, A. G., Rijal, D. P., Heintzman, P. D., Clarke, C. L., Blankholm, H.-P., Høeg, H. I., Lammers, Y., Bråthen, K. A., Edwards, M., & Alsos, I. G. (2022). Paleoeconomy more than demography determined pre-historic human impact in Arctic Norway. *PNAS Nexus*, 1, pgac209. <https://doi.org/10.1093/pnasnexus/pgac209>
- Brown, A. G., Van Hardenbroek, M., Fonville, T., Davies, K., Mackay, H., Murray, E., Head, K., Barratt, P., McCormick, F., Ficetola, G. F., Gielly, L., Henderson, A. C. G., Crone, A., Cavers, G., Langdon, P. G., Whitehouse, N. J., Pirrie, D., & Alsos, I. G. (2021). Ancient DNA, lipid biomarkers and palaeoecological evidence reveals construction and life on early medieval lake settlements. *Scientific Reports*, 11(1), 11807. <https://doi.org/10.1038/s41598-021-91057-x>
- Buxton, A. S., Groombridge, J. J., & Griffiths, R. A. (2018). Seasonal variation in environmental DNA detection in sediment and water samples. *PLoS One*, 13(1), e0191737. <https://doi.org/10.1371/journal.pone.0191737>
- Cannon, M. V., Hester, J., Shalkhauser, A., Chan, E. R., Logue, K., Small, S. T., & Serre, D. (2016). In silico assessment of primers for eDNA studies using PrimerTree and application to characterize the biodiversity surrounding the Cuyahoga River. *Scientific Reports*, 6(1), 22908. <https://doi.org/10.1038/srep22908>
- Capo, E., Giguët-Covex, C., Rouillard, A., Nota, K., Heintzman, P. D., Vuillemin, A., Ariztegui, D., Arnaud, F., Belle, S., Bertilsson, S., Bigler, C., Bindler, R., Brown, A. G., Clarke, C. L., Crump, S. E., Debroas, D., Englund, G., Ficetola, G. F., Garner, R. E., ... Parducci, L. (2021). Lake sedimentary DNA research on past terrestrial and aquatic biodiversity: Overview and recommendations. *Quaternary*, 4(1), 6. <https://doi.org/10.3390/quat4010006>
- Christianen, M. J. A., van Belzen, J., Herman, P. M. J., van Katwijk, M. M., Lamers, L. P. M., van Leent, P. J. M., & Bouma, T. J. (2013). Low-canopy seagrass beds still provide important coastal protection services. *PLoS One*, 8(5), e62413. <https://doi.org/10.1371/journal.pone.0062413>
- Clarke, C. L., Alsos, I. G., Edwards, M. E., Paus, A., Gielly, L., Hafliðason, H., Mangerud, J., Regnéll, C., Hughes, P. D. M., Svendsen, J. I., & Bjune, A. E. (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. <https://doi.org/10.1016/j.quascirev.2020.106564>
- Clarke, C. L., Edwards, M. E., Brown, A. G., Gielly, L., Lammers, Y., Heintzman, P. D., Ancin-Murguzur, F. J., Bråthen, K.-A., Goslar, T., & Alsos, I. G. (2019). Holocene floristic diversity and richness in Northeast Norway revealed by sedimentary ancient DNA (sedaDNA) and pollen. *Boreas*, 48(2), 299–316. <https://doi.org/10.1111/bor.12357>
- Clayton, W. D. A., Phillips, S. M., & Renvoize, S. A. (1974). *Flora of tropical East Africa. Gramineae (Part 2)*. Royal Botanic Gardens. <https://www.cabdirect.org/cabdirect/abstract/19740724436>
- Coghlan, S. A., Shafer, A. B. A., & Freeland, J. R. (2021). Development of an environmental DNA metabarcoding assay for aquatic vascular plant communities. *Environmental DNA*, 3(2), 372–387. <https://doi.org/10.1002/edn3.120>
- Courtin, J., Andreev, A. A., Raschke, E., Bala, S., Biskaborn, B. K., Liu, S., Zimmermann, H., Diekmann, B., Stoof-Leichsenring, K. R., Pestryakova, L. A., & Herzschuh, U. (2021). Vegetation changes in southeastern Siberia during the late Pleistocene and the Holocene. *Frontiers in Ecology and Evolution*, 9, 18.
- Courtin, J., Perfumo, A., Andreev, A. A., Opel, T., Stoof-Leichsenring, K. R., Edwards, M. E., Murton, J. B., & Herzschuh, U. (2022). Pleistocene glacial and interglacial ecosystems inferred from ancient DNA analyses of permafrost sediments from Batagay megas-lump, East Siberia. *Environmental DNA*, 4(6), 1265–1283. <https://doi.org/10.1002/edn3.336>
- Crump, S. E., Fréchet, B., Power, M., Cutler, S., de Wet, G., Reynolds, M. K., Raberg, J. H., Briner, J. P., Thomas, E. K., Sepúlveda, J., Shapiro, B., Bunce, M., & Miller, G. H. (2021). Ancient plant DNA reveals high Arctic greening during the last interglacial. *Proceedings of the National Academy of Sciences*, 118(13), e2019069118. <https://doi.org/10.1073/pnas.2019069118>
- Crump, S. E., Miller, G. H., Power, M., Sepúlveda, J., Dildar, N., Coghlan, M., & Bunce, M. (2019). Arctic shrub colonization lagged peak post-glacial warmth: Molecular evidence in lake sediment from Arctic Canada. *Global Change Biology*, 25(12), 4244–4256. <https://doi.org/10.1111/gcb.14836>

- Curtin, L., D'Andrea, W. J., Balascio, N. L., Shirazi, S., Shapiro, B., de Wet, G. A., Bradley, R. S., & Bakke, J. (2021). Sedimentary DNA and molecular evidence for early human occupation of The Faroe Islands. *Communications Earth & Environment*, 2(1), 253. <https://doi.org/10.1038/s43247-021-00318-0>
- Dalla Vecchia, A., Villa, P., & Bolpagni, R. (2020). Functional traits in macrophyte studies: Current trends and future research agenda. *Aquatic Botany*, 167, 103290. <https://doi.org/10.1016/j.aquabot.2020.103290>
- Dan, Z., Chuan, W., Qiaohong, Z., & Xingzhong, Y. (2021). Sediments nitrogen cycling influenced by submerged macrophytes growing in winter. *Water Science and Technology*, 83(7), 1728–1738. <https://doi.org/10.2166/wst.2021.081>
- Dar, N. A., Pandit, A. K., & Ganai, B. A. (2014). Factors affecting the distribution patterns of aquatic macrophytes. *Limnological Review*, 14(2), 75–81. <https://doi.org/10.2478/limre-2014-0008>
- Doi, H., Akamatsu, Y., Goto, M., Inui, R., Komuro, T., Nagano, M., & Minamoto, T. (2021). Broad-scale detection of environmental DNA for an invasive macrophyte and the relationship between DNA concentration and coverage in rivers. *Biological Invasions*, 23(2), 507–520. <https://doi.org/10.1007/s10530-020-02380-9>
- Drummond, J. A., Larson, E. R., Li, Y., Lodge, D. M., Gantz, C. A., Pfrender, M. E., Renshaw, M. A., Correa, A. M. S., & Egan, S. P. (2021). Diversity metrics are robust to differences in sampling location and depth for environmental DNA of plants in small temperate lakes. *Frontiers in Environmental Science*, 9, 617924. <https://doi.org/10.3389/fenvs.2021.617924>
- Du, Z.-Y., Qimike, A., Yang, C.-F., Chen, J.-M., & Wang, Q.-F. (2011). Testing four barcoding markers for species identification of Potamogetonaceae. *Journal of Systematics and Evolution*, 49(3), 246–251. <https://doi.org/10.1111/j.1759-6831.2011.00131.x>
- Ellenberg, H., Weber, H. E., Düll, R., Wirth, V., Werner, W., & Paulißen, B. (1992). Zeigerwerte von Pflanzen in Mitteleuropa. *Scripta Geobotanica*, 18, 3–258.
- Elven, R., Arnesen, G., Alsos, I. G., & Sandbakk, B. (2020). SvalbardFlora. <https://svalbardflora.no>
- Elven, R., Sletten Bjarå, C., Fremstad, E., Hegre, H., & Solstad, H. (2022). *Norsk Flora* (8th ed.). Det Norske Samlaget. <https://www.naturgfrtid.no/860109/Norsk+Flora>
- Epp, L. S., Kruse, S., Kath, N. J., Stoof-Leichsenring, K. R., Tiedemann, R., Pestryakova, L. A., & Herzschuh, U. (2018). Temporal and spatial patterns of mitochondrial haplotype and species distributions in Siberian larches inferred from ancient environmental DNA and modeling. *Scientific Reports*, 8(1), 17436. <https://doi.org/10.1038/s41598-018-35550-w>
- Fellows Yates, J. A., Andrades Valtueña, A., Vågane, Å. J., Cribdon, B., Velsko, I. M., Borry, M., Bravo-Lopez, M. J., Fernandez-Guerra, A., Green, E. J., Ramachandran, S. L., Heintzman, P. D., Sproul, M. A., Hübner, A., Gancz, A. S., Hider, J., Allshouse, A. F., Zaro, V., & Warinner, C. (2021). Community-curated and standardised metadata of published ancient metagenomic samples with AncientMetagenomeDir. *Scientific Data*, 8(1), 31. <https://doi.org/10.1038/s41597-021-00816-y>
- Foster, N. R., van Dijk, K., Biffin, E., Young, J. M., Thomson, V. A., Gillanders, B. M., Jones, A. R., & Waycott, M. (2021). A multi-gene region targeted capture approach to detect plant DNA in environmental samples: A case study from coastal environments. *Frontiers in Ecology and Evolution*, 9, 735744. <https://doi.org/10.3389/fevo.2021.735744>
- Fujiwara, A., Matsuhashi, S., Doi, H., Yamamoto, S., & Minamoto, T. (2016). Use of environmental DNA to survey the distribution of an invasive submerged plant in ponds. *Freshwater Science*, 35(2), 748–754. <https://doi.org/10.1086/685882>
- Gaffney, V., Fitch, S., Bates, M., Ware, R. L., Kinnaird, T., Gearey, B., Hill, T., Telford, R., Batt, C., Stern, B., Whittaker, J., Davies, S., Sharada, M. B., Everrett, R., Cribdon, R., Kistler, L., Harris, S., Kearney, K., Walker, J., ... Allaby, R. G. (2020). Multi-proxy characterisation of the Storegga tsunami and its impact on the early Holocene landscapes of the southern North Sea. *Geosciences*, 10(7), 270. <https://doi.org/10.3390/geosciences10070270>
- Gantz, C. A., Renshaw, M. A., Erickson, D., Lodge, D. M., & Egan, S. P. (2018). Environmental DNA detection of aquatic invasive plants in lab mesocosm and natural field conditions. *Biological Invasions*, 20(9), 2535–2552. <https://doi.org/10.1007/s10530-018-1718-z>
- Garcés-Pastor, S., Coissac, E., Lavergne, S., Schwörer, C., Theurillat, J.-P., Heintzman, P. D., Wangensteen, O. S., Tinner, W., Rey, F., Heer, M., Rützer, A., Walsh, K., Lammers, Y., Brown, A. G., Goslar, T., Rijal, D. P., Karger, D. N., Pellissier, L., Heiri, O., & Alsos, I. G. (2022). High resolution ancient sedimentary DNA shows that alpine plant diversity is associated with human land use and climate change. *Nature Communications*, 13(1), 6559. <https://doi.org/10.1038/s41467-022-34010-4>
- Garcés-Pastor, S., Wangensteen, O. S., Pérez-Haase, A., Pèlachs, A., Pérez-Obiol, R., Cañellas-Boltà, N., Mariani, S., & Vegas-Vilarrúbia, T. (2019). DNA metabarcoding reveals modern and past eukaryotic communities in a high-mountain peat bog system. *Journal of Paleolimnology*, 62(4), 425–441. <https://doi.org/10.1007/s10933-019-00097-x>
- GBIF.org. (2022). *Global Biodiversity Information Facility*. <https://www.gbif.org>
- Giguet-Covex, C., Ficetola, G. F., Walsh, K., Poulenard, J., Bajard, M., Fouinat, L., Sabatier, P., Gielly, L., Messenger, E., Develle, A. L., David, F., Taberlet, P., Brisset, E., Guiter, F., Sinet, R., & Arnaud, F. (2019). New insights on lake sediment DNA from the catchment: Importance of taphonomic and analytical issues on the record quality. *Scientific Reports*, 9, 21.
- Haines, A., Farnsworth, E. J., & Morrison, G. (2011). & new England wild flower society. In *New England wild Flower Society's Flora novae Angliae A manual for the identification of native and naturalized higher vascular plants of New England*. Yale University Press.
- Harper, L. R., Buxton, A. S., Rees, H. C., Bruce, K., Brys, R., Halfmaerten, D., Read, D. S., Watson, H. V., Sayer, C. D., Jones, E. P., Priestley, V., Mächler, E., Múrria, C., Garcés-Pastor, S., Medupin, C., Burgess, K., Benson, G., Boonham, N., Griffiths, R. A., ... Hänfling, B. (2019). Prospects and challenges of environmental DNA (eDNA) monitoring in freshwater ponds. *Hydrobiologia*, 826(1), 25–41. <https://doi.org/10.1007/s10750-018-3750-5>
- Harrington, M. G., Jackes, B. R., Barrett, M. D., Craven, L. A., Barrett, R. L., Harrington, M. G., Jackes, B. R., Barrett, M. D., Craven, L. A., & Barrett, R. L. (2012). Phylogenetic revision of Backhousieae (Myrtaceae): Neogene divergence, a revised circumscription of Backhousia and two new species. *Australian Systematic Botany*, 25(6), 404–417. <https://doi.org/10.1071/SB12015>
- Heinecke, L., Epp, L. S., Reschke, M., Stoof-Leichsenring, K. R., Mischke, S., Plessen, B., & Herzschuh, U. (2017). Aquatic macrophyte dynamics in Lake karakul (eastern Pamir) over the last 29 cal ka revealed by sedimentary ancient DNA and geochemical analyses of macrofossil remains. *Journal of Paleolimnology*, 58(3), 403–417. <https://doi.org/10.1007/s10933-017-9986-7>
- Hollingsworth, P. M., Graham, S. W., & Little, D. P. (2011). Choosing and using a plant DNA barcode. *PLoS One*, 6(5), e19254. <https://doi.org/10.1371/journal.pone.0019254>
- Huang, S., Stoof-Leichsenring, K. R., Liu, S., Courtin, J., Andreev, A. A., Pestryakova, L. A., & Herzschuh, U. (2021). Plant sedimentary ancient DNA from Far East Russia covering the last 28,000 years reveals different assembly rules in cold and warm climates. *Frontiers in Ecology and Evolution*, 9, 873. <https://doi.org/10.3389/fevo.2021.763747>
- Hughes, A. L. C., Gyllencreutz, R., Lohne, Ø. S., Mangerud, J., & Svendsen, J. I. (2016). The last Eurasian ice sheets – A chronological database and time-slice reconstruction, DATED-1. *Boreas*, 45(1), 1–45. <https://doi.org/10.1111/bor.12142>

- Hughes, J. M. R., Clarkson, B. R., Castro-Castellon, A. T., & Hess, L. L. (2018). Wetland plants and aquatic macrophytes. In J. M. R. Hughes (Ed.), *Freshwater ecology and conservation: Approaches and techniques* (Vol. 480). Oxford University Press.
- Ibrahim, A., Höckendorff, S., Schleheck, D., Epp, L., van Kleunen, M., & Meyer, A. (2022). Vegetation changes over the last centuries in the lower Lake Constance region reconstructed from sediment-core environmental DNA. *Environmental DNA*, 4(4), 830–845. <https://doi.org/10.1002/edn3.292>
- Info Flora. (2022). Info flora. <https://www.infoflora.ch/en/>
- Ji, F., Yan, L., Yan, S., Qin, T., Shen, J., & Zha, J. (2021). Estimating aquatic plant diversity and distribution in rivers from Jingjinji region, China, using environmental DNA metabarcoding and a traditional survey method. *Environmental Research*, 199, 111348. <https://doi.org/10.1016/j.envres.2021.111348>
- Jia, W., Liu, X., Stoof-Leichsenring, K. R., Liu, S., Li, K., & Herzsuh, U. (2021). Preservation of sedimentary plant DNA is related to lake water chemistry. *Environmental DNA*, 4(2), 425–439. <https://doi.org/10.1002/edn3.259>
- Jiang, Z., Huang, D., Fang, Y., Cui, L., Zhao, C., Liu, S., Wu, Y., Chen, Q., Ranvilage, C. I. P. M., He, J., & Huang, X. (2020). Home for marine species: Seagrass leaves as vital spawning grounds and food source. *Frontiers in Marine Science*, 7, 194. <https://doi.org/10.3389/fmars.2020.00194>
- Johnson, R. K., & Toprak, V. (2021). Local habitat is a strong determinant of spatial and temporal patterns of macrophyte diversity and composition in boreal lakes. *Freshwater Biology*, 66(8), 1490–1501. <https://doi.org/10.1111/fwb.13733>
- Jørgensen, T., Haile, J., Möller, P., Andreev, A., Boessenkool, S., Rasmussen, M., Kienast, F., Coissac, E., Taberlet, P., Brochmann, C., Bigelow, N. H., Andersen, K., Orlando, L., Gilbert, M. T. P., & Willerslev, E. (2012). A comparative study of ancient sedimentary DNA, pollen and microfossils from permafrost sediments of northern Siberia reveals long-term vegetational stability. *Molecular Ecology*, 21(8), 1989–2003. <https://doi.org/10.1111/j.1365-294X.2011.05287.x>
- Kisand, V., Talas, L., Kisand, A., Stivirins, N., Reitalu, T., Alliksaar, T., Vassiljev, J., Liiv, M., Heinsalu, A., Seppä, H., & Veski, S. (2018). From microbial eukaryotes to metazoan vertebrates: Wide spectrum paleo-diversity in sedimentary ancient DNA over the last 14,500 years. *Geobiology*, 16(6), 628–639. <https://doi.org/10.1111/gbi.12307>
- Kjær, K. H., Pedersen, M. W., De Sanctis, B., De Cahsan, B., Korneliusen, T. S., Michelsen, C. S., Sand, K. K., Jelavić, S., Ruter, A. H., Schmidt, A. M. A., Kjeldsen, K. K., Tesakov, A. S., Snowball, I., Gosse, J. C., Alsos, I. G., Wang, Y., Dockter, C., Rasmussen, M., Jørgensen, M. E., ... Willerslev, E. (2022). A 2-million-year-old ecosystem in Greenland uncovered by environmental DNA. *Nature*, 612(7939), 283–291. <https://doi.org/10.1038/s41586-022-05453-y>
- Kodama, T., Miyazono, S., Akamatsu, Y., Tsuji, S., & Nakao, R. (2022). Abundance estimation of riverine macrophyte *Egeria densa* using environmental DNA: Effects of sampling season and location. *Limnology*, 23(2), 299–308. <https://doi.org/10.1007/s10201-021-00689-5>
- Krueger, J., Foerster, V., Trauth, M. H., Hofreiter, M., & Tiedemann, R. (2021). Exploring the past biosphere of chew Bahir/Southern Ethiopia: Cross-species hybridization capture of ancient sedimentary DNA from a deep drill Core. *Frontiers in Earth Science*, 9, 683010. <https://doi.org/10.3389/feart.2021.683010>
- Kuehne, L. M., Ostberg, C. O., Chase, D. M., Duda, J. J., & Olden, J. D. (2020). Use of environmental DNA to detect the invasive aquatic plants *Myriophyllum spicatum* and *Egeria densa* in lakes. *Freshwater Science*, 39(3), 521–533. <https://doi.org/10.1086/710106>
- Kuzmina, M. L., Braukmann, T. W. A., Fazekas, A. J., Graham, S. W., Dewaard, S. L., Rodrigues, A., Bennett, B. A., Dickinson, T. A., Saarela, J. M., Catling, P. M., Newmaster, S. G., Percy, D. M., Fenneman, E., Lauron-Moreau, A., Ford, B., Gillespie, L., Subramanyam, R., Whitton, J., Jennings, L., ... Hebert, P. D. N. (2017). Using herbarium-derived DNAs to assemble a large-scale DNA barcode library for the vascular plants of Canada. *Applications in Plant Sciences*, 5(12), 1700079. <https://doi.org/10.3732/apps.1700079>
- Kuzmina, M. L., Braukmann, T. W. A., & Zakharov, E. V. (2018). Finding the pond through the weeds: EDNA reveals underestimated diversity of pondweeds. *Applications in Plant Sciences*, 6(5), e01155. <https://doi.org/10.1002/aps3.1155>
- Li, H., Zhang, H., Chang, F., Liu, Q., Zhang, Y., Liu, F., & Zhang, X. (2023). Sedimentary DNA for tracking the long-term changes in biodiversity. *Environmental Science and Pollution Research*, 30, 17039–17050. <https://doi.org/10.1007/s11356-023-25130-5>
- Li, H.-T., Yi, T.-S., Gao, L.-M., Ma, P.-F., Zhang, T., Yang, J.-B., Gitzendanner, M. A., Fritsch, P. W., Cai, J., Luo, Y., Wang, H., van der Bank, M., Zhang, S.-D., Wang, Q.-F., Wang, J., Zhang, Z.-R., Fu, C.-N., Yang, J., Hollingsworth, P. M., ... Li, D.-Z. (2019). Origin of angiosperms and the puzzle of the Jurassic gap. *Nature Plants*, 5(5), 461–470. <https://doi.org/10.1038/s41477-019-0421-0>
- Liu, S., Kruse, S., Scherler, D., Ree, R. H., Zimmermann, H. H., Stoof-Leichsenring, K. R., Epp, L. S., Mischke, S., & Herzsuh, U. (2021). Sedimentary ancient DNA reveals a threat of warming-induced alpine habitat loss to Tibetan plateau plant diversity. *Nature Communications*, 12(1), 2995. <https://doi.org/10.1038/s41467-021-22986-4>
- Liu, S., Stoof-Leichsenring, K. R., Kruse, S., Pestyakova, L. A., & Herzsuh, 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. <https://doi.org/10.3389/fevo.2020.560243>
- Lyimo, L. D. (2016). *Carbon sequestration processes in tropical seagrass beds* [Stockholm University, Faculty of Science, Department of Ecology, Environment and Plant Sciences. University of Dodoma.]. <http://urn.kb.se/resolve?urn=urn:nbn:se:su:diva-128201>
- Maasri, A., Jähniq, S. C., Adamescu, M. C., Adrian, R., Baigun, C., Baird, D. J., Batista-Morales, A., Bonada, N., Brown, L. E., Cai, Q., Campos-Silva, J. V., Clausnitzer, V., Contreras-MacBeath, T., Cooke, S. J., Datry, T., Delacámara, G., De Meester, L., Dijkstra, K.-D. B., Do, V. T., ... Worischka, S. (2022). A global agenda for advancing freshwater biodiversity research. *Ecology Letters*, 25(2), 255–263. <https://doi.org/10.1111/ele.13931>
- Maddison, D. R., & Schulz, K.-S. (2007). The tree of life web project. <http://tolweb.org>
- Marks, R. A., Hotaling, S., Frandsen, P. B., & VanBuren, R. (2021). Representation and participation across 20 years of plant genome sequencing. *Nature Plants*, 7(12), 1571–1578. <https://doi.org/10.1038/s41477-021-01031-8>
- Matsushashi, S., Doi, H., Fujiwara, A., Watanabe, S., & Minamoto, T. (2016). Evaluation of the environmental DNA method for estimating distribution and biomass of submerged aquatic plants. *PLoS One*, 11(6), e0156217. <https://doi.org/10.1371/journal.pone.0156217>
- Murchie, T. J., Kuch, M., Duggan, A. T., Ledger, M. L., Roche, K., Klunk, J., Karpinski, E., Hackenberger, D., Sadoway, T., MacPhee, R., Froese, D., & Poinar, H. (2020). Optimizing extraction and targeted capture of ancient environmental DNA for reconstructing past environments using the PalaeoChip Arctic-1.0 bait-set. *Quaternary Research*, 99, 305–328. <https://doi.org/10.1017/qua.2020.59>
- Murphy, K., Efremov, A., Davidson, T. A., Molina-Navarro, E., Fidanza, K., Crivelari Betiol, T. C., Chambers, P., Tapia Grimaldo, J., Varandas Martins, S., Springuel, I., Kennedy, M., Mormul, R. P., Dibble, E., Hofstra, D., Lukács, B. A., Gebler, D., Baastrup-Spohr, L., & Urrutia-Estrada, J. (2019). World distribution, diversity and endemism of aquatic macrophytes. *Aquatic Botany*, 158, 103127. <https://doi.org/10.1016/j.aquabot.2019.06.006>
- Nevill, P. G., Zhong, X., Tonti-Filippini, J., Byrne, M., Hislop, M., Thiele, K., van Leeuwen, S., Boykin, L. M., & Small, I. (2020). Large scale genome skimming from herbarium material for accurate plant

- identification and phylogenomics. *Plant Methods*, 16(1), 1. <https://doi.org/10.1186/s13007-019-0534-5>
- Newton, J., Sepulveda, A., Sylvester, K., & Thum, R. A. (2016). Potential utility of environmental DNA for early detection of Eurasian water-milfoil. *Journal of Aquatic Plant Management*, 54, 46–49.
- Niemeyer, B., Epp, L. S., Stoof-Leichsenring, K. R., Pestryakova, L. A., & Herzschuh, U. (2017). A comparison of sedimentary DNA and pollen from lake sediments in recording vegetation composition at the Siberian treeline. *Molecular Ecology Resources*, 17(6), e46–e62. <https://doi.org/10.1111/1755-0998.12689>
- O'Hare, M. T., Baattrup-Pedersen, A., Baumgarte, I., Freeman, A., Gunn, I. D. M., Lázár, A. N., Sinclair, R., Wade, A. J., & Bowes, M. J. (2018). Responses of aquatic plants to eutrophication in rivers: A revised conceptual model. *Frontiers in Plant Science*, 9, 451. <https://doi.org/10.3389/fpls.2018.00451>
- Ortega, A., Geraldi, N. R., Díaz-Rúa, R., Ørberg, S. B., Wesselmann, M., Krause-Jensen, D., & Duarte, C. M. (2020). A DNA mini-barcode for marine macrophytes. *Molecular Ecology Resources*, 20(4), 920–935. <https://doi.org/10.1111/1755-0998.13164>
- Otoni, C., Borić, D., Cheronet, O., Sparacello, V., Dori, I., Coppa, A., Antonović, D., Vujević, D., Price, T. D., Pinhasi, R., & Cristiani, E. (2021). Tracking the transition to agriculture in southern Europe through ancient DNA analysis of dental calculus. *Proceedings of the National Academy of Sciences*, 118(32), e2102116118. <https://doi.org/10.1073/pnas.2102116118>
- Palacios Mejia, M., Curd, E., Edalati, K., Renshaw, M. A., Dunn, R., Potter, D., Fraga, N., Moore, J., Saiz, J., Wayne, R., & Parker, S. S. (2021). The utility of environmental DNA from sediment and water samples for recovery of observed plant and animal species from four Mojave Desert springs. *Environmental DNA*, 3(1), 214–230. <https://doi.org/10.1002/edn3.161>
- Pansu, J., Winkworth, R. C., Hennion, F., Gielly, L., Taberlet, P., & Choler, P. (2015). Long-lasting modification of soil fungal diversity associated with the introduction of rabbits to a remote sub-Antarctic archipelago. *Biology Letters*, 11(9), 20150408. <https://doi.org/10.1098/rsbl.2015.0408>
- Parducci, L., Alsos, I. G., Unneberg, P., Pedersen, M. W., Han, L., Lammers, Y., Salonen, J. S., Väilänta, M. M., Slotte, T., & Wohlfarth, B. (2019). Shotgun environmental DNA, pollen, and macrofossil analysis of Lateglacial Lake sediments from southern Sweden. *Frontiers in Ecology and Evolution*, 7, 189. <https://doi.org/10.3389/fevo.2019.00189>
- Parducci, L., Bennett, K. D., Ficetola, G. F., Alsos, I. G., Suyama, Y., Wood, J. R., & Pedersen, M. W. (2017). Ancient plant DNA in lake sediments. *New Phytologist*, 214(3), 924–942. <https://doi.org/10.1111/nph.14470>
- Parducci, L., Matetovici, I., Fontana, S. L., Bennett, K. D., Suyama, Y., Haile, J., Kjær, K. H., Larsen, N. K., Drouzas, A. D., & Willerslev, E. (2013). Molecular- and pollen-based vegetation analysis in lake sediments from Central Scandinavia. *Molecular Ecology*, 22(13), 3511–3524. <https://doi.org/10.1111/mec.12298>
- Parducci, L., Väilänta, M., Salonen, J. S., Ronkainen, T., Matetovici, I., Fontana, S. L., Eskola, T., Sarala, P., & Suyama, Y. (2015). Proxy comparison in ancient peat sediments: Pollen, macrofossil and plant DNA. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 370(1660), 20130382. <https://doi.org/10.1098/rstb.2013.0382>
- Peabody, M. A., Van Rossum, T., Lo, R., & Brinkman, F. S. L. (2015). Evaluation of shotgun metagenomics sequence classification methods using in silico and in vitro simulated communities. *BMC Bioinformatics*, 16(1), 362. <https://doi.org/10.1186/s12859-015-0788-5>
- Pedersen, M. W., Ginolhac, A., Orlando, L., Olsen, J., Andersen, K., Holm, J., Funder, S., Willerslev, E., & Kjær, K. H. (2013). A comparative study of ancient environmental DNA to pollen and macrofossils from lake sediments reveals taxonomic overlap and additional plant taxa. *Quaternary Science Reviews*, 75, 161–168. <https://doi.org/10.1016/j.quascirev.2013.06.006>
- Pedersen, M. W., Ruter, A., Schweger, C., Friebe, H., Staff, R. A., Kjeldsen, K. K., Mendoza, M. L. Z., Beaudoin, A. B., Zutter, C., Larsen, N. K., Potter, B. A., Nielsen, R., Rainville, R. A., Orlando, L., Meltzer, D. J., Kjær, K. H., & Willerslev, E. (2016). Postglacial viability and colonization in North America's ice-free corridor. *Nature*, 537(7618), 45–49. <https://doi.org/10.1038/nature19085>
- Penning, W. E., Mjelde, M., Dudley, B., Hellsten, S., Hanganu, J., Kolada, A., van den Berg, M., Poikane, S., Phillips, G., Willby, N., & Ecke, F. (2008). Classifying aquatic macrophytes as indicators of eutrophication in European lakes. *Aquatic Ecology*, 42(2), 237–251. <https://doi.org/10.1007/s10452-008-9182-y>
- Poikane, S., Portielje, R., Denys, L., Elferts, D., Kelly, M., Kolada, A., Mäemets, H., Phillips, G., Søndergaard, M., Willby, N., & van den Berg, M. S. (2018). Macrophyte assessment in European lakes: Diverse approaches but convergent views of 'good' ecological status. *Ecological Indicators*, 94, 185–197. <https://doi.org/10.1016/j.ecolind.2018.06.056>
- Powell, C., Malpas, J., Tollett, M., Anderson, D., Dorrington, E., Frye, P., Sieck-Hill, F., & Kunz, K. (2022). CalFlora. <https://www.calflora.org/>
- R Core Team. (2022). R: A Language and Environment for Statistical Computing (4.1.1). R Foundation for Statistical Computing. <https://www.R-project.org/>
- Reitsemä, R. E., Meire, P., & Schoelynck, J. (2018). The future of freshwater Macrophytes in a changing world: Dissolved organic carbon quantity and quality and its interactions with Macrophytes. *Frontiers in Plant Science*, 9, 629. <https://doi.org/10.3389/fpls.2018.00629>
- Rijal, D. P., Heintzman, P. D., Lammers, Y., Yoccoz, N. G., Lorberau, K. E., Pitelkova, I., Goslar, T., Murguzur, F. J. A., Salonen, J. S., Helmens, K. F., Bakke, J., Edwards, M. E., Alm, T., Bräthen, K. A., Brown, A. G., & Alsos, I. G. (2021). Sedimentary ancient DNA shows terrestrial plant richness continuously increased over the Holocene in northern Fennoscandia. *Science Advances*, 7(eabf9557), 16.
- Sawada, M., Viau, A. E., & Gajewski, K. (2003). The biogeography of aquatic macrophytes in North America since the last glacial maximum. *Journal of Biogeography*, 30(7), 999–1017. <https://doi.org/10.1046/j.1365-2699.2003.00866.x>
- Sawafuji, R., Saso, A., Suda, W., Hattori, M., & Ueda, S. (2020). Ancient DNA analysis of food remains in human dental calculus from the Edo period, Japan. *PLoS One*, 15(3), e0226654. <https://doi.org/10.1371/journal.pone.0226654>
- Schabacker, J. C., Amish, S. J., Ellis, B. K., Gardner, B., Miller, D. L., Rutledge, E. A., Sepulveda, A. J., & Luikart, G. (2020). Increased eDNA detection sensitivity using a novel high-volume water sampling method. *Environmental DNA*, 2(2), 244–251. <https://doi.org/10.1002/edn3.63>
- Scriver, M., Marinich, A., Wilson, C., & Freeland, J. (2015). Development of species-specific environmental DNA (eDNA) markers for invasive aquatic plants. *Aquatic Botany*, 122, 27–31. <https://doi.org/10.1016/j.aquabot.2015.01.003>
- Seersholm, F. V., Pedersen, M. W., Søm, M. J., Shokry, H., Mak, S. S. T., Ruter, A., Raghavan, M., Fitzhugh, W., Kjær, K. H., Willerslev, E., Meldgaard, M., Kapel, C. M. O., & Hansen, A. J. (2016). DNA evidence of bowhead whale exploitation by Greenlandic paleo-Inuit 4,000 years ago. *Nature Communications*, 7(1), 13389. <https://doi.org/10.1038/ncomms13389>
- Shackleton, M. E., Rees, G. N., Watson, G., Campbell, C., & Nielsen, D. (2019). Environmental DNA reveals landscape mosaic of wetland plant communities. *Global Ecology and Conservation*, 19, e00689. <https://doi.org/10.1016/j.gecco.2019.e00689>
- Sheldon, R. B., & Boylen, C. W. (1977). Maximum depth inhabited by aquatic vascular plants. *The American Midland Naturalist*, 97(1), 248–254. <https://doi.org/10.2307/2424706>
- Sjögren, P., Edwards, M. E., Gielly, L., Langdon, C. T., Croudace, I. W., Merkel, M. K. F., Fonville, T., & Alsos, I. G. (2017). Lake sedimentary

- DNA accurately records 20th century introductions of exotic conifers in Scotland. *New Phytologist*, 213(2), 929–941. <https://doi.org/10.1111/nph.14199>
- Sønsteby, J. H., Gielly, L., Brysting, A. K., Elven, R., Edwards, M., Haile, J., Willerslev, E., Coissac, E., Rioux, D., Sannier, J., Taberlet, P., & Brochmann, C. (2010). Using next-generation sequencing for molecular reconstruction of past Arctic vegetation and climate. *Molecular Ecology Resources*, 10(6), 1009–1018. <https://doi.org/10.1111/j.1755-0998.2010.02855.x>
- South African National Biodiversity Institute. (2022). PlantZAfrica. <http://pza.sanbi.org/>
- Stoof-Leichsenring, K. R., Huang, S., Liu, S., Jia, W., Li, K., Liu, X., Pestryakova, L. A., & Herzschuh, U. (2022). Sedimentary DNA identifies modern and past macrophyte diversity and its environmental drivers in high-latitude and high-elevation lakes in Siberia and China. *Limnology and Oceanography*, 67(5), 126–1141. <https://doi.org/10.1002/lno.12061>
- Tabares, X., Zimmermann, H., Dietze, E., Ratzmann, G., Belz, L., Vieth-Hillebrand, A., Dupont, L., Wilkes, H., Mapani, B., & Herzschuh, U. (2020). Vegetation state changes in the course of shrub encroachment in an African savanna since about 1850 CE and their potential drivers. *Ecology and Evolution*, 10(2), 962–979. <https://doi.org/10.1002/ece3.5955>
- Taberlet, P., Bonin, A., Zinger, L., & Coissac, E. (2018). *Environmental DNA: For Biodiversity Research and Monitoring*. Oxford University Press. <https://doi.org/10.1093/oso/9780198767220.001.0001>
- Taberlet, P., Coissac, E., Pompanon, F., Gielly, L., Miquel, C., Valentini, A., Vermet, T., Corthier, G., Brochmann, C., & Willerslev, E. (2007). Power and limitations of the chloroplast trn L (UAA) intron for plant DNA barcoding. *Nucleic Acids Research*, 35(3), e14. <https://doi.org/10.1093/nar/gkl938>
- Tela Botanica. (2022). EFlore. <https://www.tela-botanica.org/flore/>
- ter Schure, A. T. M., Bajard, M., Loftsgarden, K., Høeg, H. I., Ballo, E., Bakke, J., Støren, E. W. N., Iversen, F., Kool, A., Brysting, A. K., Krüger, K., & Boessenkool, S. (2021). Anthropogenic and environmental drivers of vegetation change in southeastern Norway during the Holocene. *Quaternary Science Reviews*, 270, 107175. <https://doi.org/10.1016/j.quascirev.2021.107175>
- Tringe, S. G., & Rubin, E. M. (2005). Metagenomics: DNA sequencing of environmental samples. *Nature Reviews Genetics*, 6(11), 805–814. <https://doi.org/10.1038/nrg1709>
- Tsukamoto, Y., Yonezawa, S., Katayama, N., & Isagi, Y. (2021). Detection of endangered aquatic plants in rapid streams using environmental DNA. *Frontiers in Ecology and Evolution*, 8, 622291. <https://doi.org/10.3389/fevo.2020.622291>
- Tyler, T., Herbertsson, L., Olofsson, J., & Olsson, P. A. (2021). Ecological indicator and traits values for Swedish vascular plants. *Ecological Indicators*, 120, 106923. <https://doi.org/10.1016/j.ecoli.2020.106923>
- Tyler, T., & Olsson, P. A. (2016). Substrate pH ranges of south Swedish bryophytes—Identifying critical pH values and richness patterns. *Flora*, 223, 74–82. <https://doi.org/10.1016/j.flora.2016.05.006>
- Tyrrell, C. D., Chambers, P. A., & Culp, J. M. (2022). Harnessing aquatic plant growth forms to apply European nutrient-enrichment bioindicators to Canadian waters. *Applications in Plant Sciences*, 10(4), e11487. <https://doi.org/10.1002/aps.3.11487>
- United States Department of Agriculture - National Wetland Plant List. (2022). *USDA Plant Database—Wetland Status*. <https://plants.usda.gov/home/wetlandSearch>
- Väliranta, M., Kultti, S., Nyman, M., & Sarmaja-Korjonen, K. (2005). Holocene development of aquatic vegetation in shallow Lake Njargajavri, Finnish Lapland, with evidence of water-level fluctuations and drying. *Journal of Paleolimnology*, 34(2), 203–215. <https://doi.org/10.1007/s10933-005-1840-7>
- Väliranta, M., Salonen, J. S., Heikkilä, M., Amon, L., Helmens, K., Klimaschewski, A., Kuhry, P., Kultti, S., Poska, A., Shala, S., Veski, S., & Birks, H. H. (2015). Plant macrofossil evidence for an early onset of the Holocene summer thermal maximum in northernmost Europe. *Nature Communications*, 6(1), 6809. <https://doi.org/10.1038/ncomms7809>
- von Hippel, B., Stoof-Leichsenring, K. R., Schulte, L., Seeber, P., Epp, L. S., Biskaborn, B. K., Diekmann, B., Melles, M., Pestryakova, L., & Herzschuh, U. (2022). Long-term fungus-plant covariation from multi-site sedimentary ancient DNA metabarcoding. *Quaternary Science Reviews*, 295, 107758. <https://doi.org/10.1016/j.quascirev.2022.107758>
- Voss, E. G., & Reznicek, A. A. (2012). *Field manual of Michigan Flora*. University of Michigan Press.
- Walsh, N. G., & Entwisle, T. J. (1994). *Flora of Victoria* (Vol. 2–4). Inkata Press.
- Wang, L., Yang, T., Hei, P., Zhang, J., Yang, J., Luo, T., Zhou, G., Liu, C., Wang, R., & Chen, F. (2022). Internal phosphorus cycling in macrophyte-dominated eutrophic lakes and its implications. *Journal of Environmental Management*, 306, 114424. <https://doi.org/10.1016/j.jenvman.2021.114424>
- Wang, Y., Pedersen, M. W., Alsos, I. G., De Sanctis, B., Racimo, F., Prohaska, A., Coissac, E., Owens, H. L., Merkel, M. K. F., Fernandez-Guerra, A., Rouillard, A., Lammers, Y., Alberti, A., Denoeud, F., Money, D., Ruter, A. H., McColl, H., Larsen, N. K., Cherezova, A. A., ... Willerslev, E. (2021). Late quaternary dynamics of Arctic biota from ancient environmental genomics. *Nature*, 600(7887), 86–92. <https://doi.org/10.1038/s41586-021-04016-x>
- Weigand, H., Beermann, A. J., Čiampor, F., Costa, F. O., Csabai, Z., Duarte, S., Geiger, M. F., Grabowski, M., Rimet, F., Rulik, B., Strand, M., Szucsich, N., Weigand, A. M., Willassen, E., Wyler, S. A., Bouchez, A., Borja, A., Čiamporová-Zatovičová, Z., Ferreira, S., ... Ekrem, T. (2019). DNA barcode reference libraries for the monitoring of aquatic biota in Europe: Gap-analysis and recommendations for future work. *Science of the Total Environment*, 678, 499–524. <https://doi.org/10.1016/j.scitotenv.2019.04.247>
- WFO: World Flora Online. (2022). *World Flora Online*. World Flora Online <http://www.worldfloraonline.org>
- Willerslev, E., Davison, J., Moora, M., Zobel, M., Coissac, E., Edwards, M. E., Lorenzen, E. D., Vestergård, M., Gussarova, G., Haile, J., Craine, J., Gielly, L., Boessenkool, S., Epp, L. S., Pearman, P. B., Cheddadi, R., Murray, D., Bräthen, K. A., Yoccoz, N. G., ... Taberlet, P. (2014). Fifty thousand years of Arctic vegetation and megafaunal diet. *Nature*, 506, 47–51.
- Willerslev, E., Hansen, A. J., Binladen, J., Brand, T. B., Gilbert, M. T. P., Shapiro, B., Bunce, M., Wiuf, C., Gilichinsky, D. A., & Cooper, A. (2003). Diverse plant and animal genetic records from Holocene and Pleistocene sediments. *Science*, 300, 791–795.
- Wisconsin State Herbarium. (2022). Online virtual Flora of Wisconsin. <https://wisflora.herbarium.wisc.edu/index.php>
- Wood, J. R., Crown, A., Cole, T. L., & Wilmshurst, J. M. (2016). Microscopic and ancient DNA profiling of Polynesian dog (kuri) coprolites from northern New Zealand. *Journal of Archaeological Science: Reports*, 6, 496–505. <https://doi.org/10.1016/j.jasrep.2016.03.020>
- Zhang, J., Hei, P., Shang, Y., Yang, J., Wang, L., Yang, T., Zhou, G., & Chen, F. (2021). Internal nitrogen cycle in Macrophyte-dominated Eutrophic Lakes: Mechanisms and implications for ecological restoration. *ACS ES&T Water*, 1(11), 2359–2369. <https://doi.org/10.1021/acsestwater.1c00203>
- Zimmermann, H. H., Raschke, E., Epp, L. S., Stoof-Leichsenring, K. R., Schwamborn, G., Schirrmeister, L., Overduin, P. P., & Herzschuh, U. (2017). 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(3), 575–596. <https://doi.org/10.5194/bg-14-575-2017>

Zou, Y.-F., Chen, K.-Y., & Lin, H.-J. (2021). Significance of belowground production to the long-term carbon sequestration of intertidal sea-grass beds. *Science of the Total Environment*, 800, 149579. <https://doi.org/10.1016/j.scitotenv.2021.149579>

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Revéret, A., Rijal, D. P., Heintzman, P. D., Brown, A. G., Stooft-Leichsenring, K. R., & Alsos, I. G. (2023). Environmental DNA of aquatic macrophytes: The potential for reconstructing past and present vegetation and environments. *Freshwater Biology*, 68, 1929–1950. <https://doi.org/10.1111/fwb.14158>