Ancient plant DNA in lake sediments

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Summary

Recent advances in sequencing technologies now permit analyses of plant DNA from fossil samples (ancient plant DNA, plant aDNA), and thus enable molecular reconstruction of palaeofloras. Hitherto, ancient frozen soils have proved excellent in preserving DNA molecules, and have thus been the most commonly used source of plant aDNA. However, DNA from soil mainly represents taxa growing few meters from the sampling point. Lakes have larger catchment areas and recent studies suggest that plant aDNA from lake sediments is a more powerful tool for palaeofloristic reconstruction. Furthermore, lakes can be found globally in nearly all environments and are therefore not limited to perennially frozen areas. Here we review the latest approaches and methods for studying plant aDNA from lake sediments and discuss the progress made up to present. We argue that aDNA analyses add new and additional perspectives for studying ancient plant populations and in time will provide higher taxonomic resolution and more precise estimation of abundance. Despite this, key questions and challenges remain for such plant aDNA studies. Finally, we provide guidelines on technical issues, including lake selection and we suggest directions for future research on plant aDNA studies in lake sediments.

Keywords: ancient plant DNA, bioinformatics, environmental DNA, high-throughput DNA sequencing, lake sediments, metabarcoding, shotgun sequencing, pollen, taphonomy

Introduction

Ancient DNA (aDNA) analysis is a young, but rapidly developing research field. Since the pioneering work in the 1980s (Higuchi *et al*., 1984; Pääbo, 1984) there has been an exponential increase in aDNA studies investigating evolution and ecology of the last 800 thousand years before present (kyr BP).

A Web of Science search on aDNA studies (January 1984-August 2016, keyword 'ancient DNA'), detected 2104 papers. Most focus on vertebrates, especially humans (50%), while only 5% focus on plants and ecology (Fig. 1). Ancient DNA from skeletal remains has been so much easier to study than plant macrofossils, and the difficulty in getting aDNA from charred specimens that represent 95% of the plant archaeological record, may partially explain the lack of plant aDNA papers. Plant aDNA studies have been also delayed by the difficulty in finding standard barcode regions useful for all species (universal) and sufficiently variable to discriminate among taxa. Three important trends are evident from Fig. 1: (i) the total number of studies has increased steadily through the last three decades; (ii) the number of opinion and methodological papers accounts for most of the increase since ca 2004; (iii) after three decades of aDNA research, plants continue to receive limited attention in relation to their importance in the landscape.

Despite this, plant aDNA research has made dramatic progress with: (i) the recent adoption of high-throughput DNA sequencing (HTS) technology; (ii) the discovery that DNA can be isolated from fossil pollen (Suyama *et al*., 1996; Parducci *et al*., 2005); and (iii) the finding that plant aDNA can be extracted from ice-cores, permafrost soil, lake sediments, coprolites and peat cores (see Rawlence *et al*., 2014). Since the first research (Willerslev *et al*., 2003), a number of studies on past biodiversity have been published using aDNA from a variety of palaeoenvironments (ref. 3-18 in Pedersen *et al*. 2015). These studies (see also Table 1) show how DNA can often identify more species and at higher taxonomical resolution than those identified by pollen and macrofossil analyses, thus providing important ecological and climatic information on the investigated sites otherwise difficult or impossible to infer (e.g. minimum July temperature or nutrient conditions for taxa identified at species levels; Sønstebø *et al*. 2010, Parducci *et al*. 2015). DNA studies generally identify more herbs (Willerslev *et al*. 2014, Alsos *et al*. 2016) and have greater taxonomic resolution for grasses, thus providing better information on local biodiversity and for reconstruction of palaeoenvironments. The latter is an advantage compared to pollen especially at high latitudes/altitudes where local pollen productivity is low and long-distance pollen dispersal is more common.

The DNA field however, is not without technical challenges, for which specialised techniques and protocols have been developed (Hofreiter *et al*., 2001; Chan *et al*., 2005; Shapiro & Hofreiter, 2012). It is therefore timely to review recent progress on plant aDNA studies and to suggest new directions for the future. We focus our review on lake sediments, because their geological context provides a robust archive for retrieval of plant aDNA through time, and because lakes can be found in all environments around the world. We synthesize and discuss recent key findings on DNA from ancient pollen and sediment samples (see Table 1 form a complete list of studies) and in particular the progress achieved using HTS technologies. Finally we discuss issues relating to false positives and the need for authentication (i.e. replication, use of controls), taphonomic processes, and bioinformatic challenges relating to the newest taxonomic identification and authentication of aDNA sequences.

Environmental and temporal limits for survival of aDNA

The temporal limit of DNA preservation is dictated by the rate of degradation, which varies depending on the source material and micro-environmental conditions. The most favourable conditions (anoxic and frozen/cold) occur in permafrost and ice; here DNA can persist in biotic remains and environmental samples (e.g. soils) for hundreds of thousands of years (Lindhal, 1993; Hofreiter *et al*., 2001; Allentoft *et al*., 2012; Dabney *et al*., 2013; Orlando *et al*., 2014). Currently, the oldest authenticated plant aDNA sequences are from frozen environments dated between 450 and 800 kyr (Willerslev *et al*., 2007). Such favourable conditions however, are restricted to polar regions and high alpine environments. Plant aDNA has also been successfully extracted and analysed from arid and hot environments (Hofreiter *et al*., 2003; da Fonseca *et al*. 2015; Mascher *et al*. 2016), temperate middens and coprolites (see Rawlence *et al*., 2014), suggesting that warm temperatures are not necessarily a barrier for preservation of DNA molecules.

Several studies have investigated the post mortem processes affecting DNA molecules in different tissues. We know that after the death of an organism, several intra and extra-cellular processes (e.g. enzymatic, hydrolytic and oxidative processes) cause DNA damage (seen as misincorporation of C to T and G to A transitions primarily toward the ends of the DNA molecules) (Briggs *et al*., 2007; Jónsson *et al*., 2013), eventually leading to fragmentation of the DNA molecules. The highest success rate for aDNA isolation is normally obtained from

frozen, anoxic or arid areas, environments with limited bacterial abundance and therefore presence of nucleases, which reduces longer nucleic acids to short molecules (Hofreiter *et al.*, 2001). Nevertheless, exogenous processes will inevitably lead eventually to the destabilization, fragmentation and damage of DNA, even in good preservation conditions.

To determine the rate of DNA decay, Allentoft *et al.* (2012) quantified the temporal survival of DNA molecules in bones. They found a half-life of 521 years for short [242 base pairs (bp)] mitochondrial DNA (mtDNA) fragments preserved at ca. 13 °C. The fragmentation rate however was strongly reduced at lower temperatures, and it was assumed that under very cold and dry conditions short fragments should be retrievable from bone more than 1 million years old. No similar studies exist for ancient plant tissues, and there are currently no indications of an age limit for plant aDNA. Nevertheless, damage for plant DNA also accumulates with age (Pedersen *et al.*, 2016). So far, plant DNA has been recovered and analysed from sediments >55 ka BP (Willerslev *et al.*, 2014), from ice cores dated between 450 and 800 kya BP (Willerslev *et al.*, 2007) and from pollen ca. ~150 ka BP (Suyama *et al.*, 1996), which suggests that DNA studies from lake sediments can potentially span the Late Pleistocene and Holocene.

Lake sediments

During the last decade, molecular ecologists have increasingly begun investigating Late Quaternary floristic history using DNA isolated from pollen and DNA extracted from lake sediments (pollen DNA and *sed*aDNA, respectively) for a number of reasons. We review these below.

Lake sediment characteristics

Lakes are excellent sources of sediments containing aquatic and terrestrial environmental components accumulated over time and preserved in robust stratigraphic contexts and anoxic conditions. They offer the best settings for preservation of plant aDNA, as the sediments accumulate continuously; allowing establishment of high-resolution molecular records using available geo-chronological methods (Fig. 2a, b, c) and thus providing a robust record for interpretation of the molecular history. In particular, sediment records from small lakes (Fig. 3) are excellent archives for molecular studies, as the effects of disturbances are

low and seem to represent the surrounding terrestrial environment well. Lake sediments consist of variable proportions of autochthonous/allochthonous organic material (OM) and in-washed inorganic material. Microbial degradation of OM frequently creates anoxic conditions in the bottom water and below 1-2 cm sediment depth (Sobek *et al*., 2009). Such conditions preclude the presence of burrowing animals and thus minimise bioturbation, water percolation and sediment reworking (Pansu *et al*., 2015), all of which are major concerns for palaeoecological studies. Water has maximum density at 4°C, so water less dense than this, both warmer and colder, floats. The net result is that bottom waters become insulated from the atmosphere, thereby favouring the development of anoxia and temperature stability. However, the temperature of lake water also depends upon geography and depth (Hutchinson, 1957; Wetzel, 2001). If lakes are deep enough, the water column becomes thermally stratified. During the summer, surface water warms and establishes a gradient down to cooler lower water. During the winter, surface water cools until it reaches 4°C (maximum density), at which point no more cooling occurs until the whole lake is mixed at 4°C. Tropical lakes with little seasonal variation of temperature may have more or less constant temperatures year-round, at a level similar to ambient temperatures. In temperate and cold regions, the bottom waters will normally be colder than surface water in the summer, and at a similar temperature in the winter, when the whole lake is cold (and may be ice covered). Sediments are thus in contact with the coldest water, and become insulated from the atmosphere, favouring the development of anoxia, and have greater temperature stability, increasing the probability of DNA survival.

The sediments of lakes where anoxic conditions dominate can also be 'laminated' (i.e. layers of different composition reflecting seasonal environmental differences). In some cases, laminated sediments are sufficiently continuous to provide a temporal record of layers with annual resolution (Larsen & Macdonald, 1993), similar to tree rings. While vertical migration (leaching) of DNA has been observed in cave sediments (Haile *et al*., 2007) and non-frozen soils (Andersen *et al*., 2011), leaching has not been observed in lake sediments (Anderson-Carpenter *et al*., 2011; Pansu *et al*., 2015; Sjögren *et al*., 2016). Once imbedded in the sediments, plant macrofossils and pollen grains, as well as silica, clay or organic matter, to which extracellular DNA attach (Pietramellara *et al*., 2009; Poté *et al*., 2009; Taberlet *et al*., 2012a), are unlikely to move vertically. On the other hand, re-deposition of sediments can occur in lakes, contaminating the micro and macrofossil record with older material. While Pedersen *et al*. (2016) recently found pre-Quaternary re-deposited microfossils in lake sediments from the Peace River drainage basin in North America, they also found that the

194 DNA record remained un-affected, probably due to the significant older age and smaller
195 quantities of the re-deposited material. Nevertheless, re-deposition of material should not be
196 neglected as a possible source of DNA.

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198 Coring techniques for aDNA work

199 The collection of sediment cores for plant aDNA can be done with the same coring devices
200 used for conventional palaeoecological work, but some special precautions and procedures
201 may be necessary to avoid contamination in the field as far as possible. Corers that enclose
202 the sediment, such as piston or percussion corers (eg. Nesje, 1992; Wright *et al*., 1984) are to
203 be preferred. Other frequently used coring systems for palaeolimnology, such as the Kajak
204 corer or HTH gravity corer (Renberg & Hansson, 2008) can also be used, and be essential for
205 collection of surface sediments. If the coring is done in winter, there is minimal risk of
206 contamination by airborne DNA (e.g. in pollen), but even this can be eliminated by sealing the
207 core tubes (both piston and gravity systems), for transport to the laboratory, directly in the
208 field. Additional procedures, including equipment sterilisation, are also available (Feek *et al*.,
209 2006; 2011). On extruding and opening the cores, however, it must be assumed that the
210 surface is contaminated, so subsamples must be taken from inside the undisturbed centre.
211 During sub-sampling, it is therefore important to remove or avoid the first 4-10 mm of outer
212 sediment in a clean laboratory setting, using sterile tools, full bodysuit and gloves to obtain an
213 uncontaminated sample from within the centre of the core (Fig. 4a,b,c). Hence the core
214 should have sufficient diameter to allow this. Extra precautions can also be taken by applying
215 a DNA tracer to the coring equipment, which allows testing for infiltration by DNA molecules
216 from the outer layers into the inner sampled sediments (Pedersen *et al*., 2016), or by having a
217 DNA-free water sample exposed to air in the laboratory during subsampling as a negative
218 control.

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221 ## Perspective for plant aDNA research

222 Traditionally, lake sediment records have been routinely analysed for pollen and other plant
223 fossils, which have been the basis for our understanding of landscape-scale distribution of
224 plants on millennial timescales. Plant aDNA now offers the potential of helping elucidation of
225 long-standing ecological questions that classical palaeoecological techniques may not be able

to retrieve. It has been through collaborative work between molecular ecologists and classical palaeoecologists that robust and reliable results have been produced recently (Willerslev *et al.*, 2014; Pedersen *et al.*, 2016; Alsos *et al.*, 2016), and this type of collaboration is strongly advocated (Hu *et al.*, 2009; Anderson-Carpenter *et al.*, 2011; Brown *et al.*, 2014). Examples of problems being elucidated by combining the two disciplines include Reid's paradox of rapid plant migration (Clark *et al.*, 1998) and the question of whether or not trees survived the Last Glacial Maximum (LGM) at high-latitudes in Europe (Parducci *et al.*, 2012). Here, combined information from fossils and molecular studies (modern and ancient) has changed our traditional view of post-glacial migration of trees from southern European refugia (Hewitt, 2000; Stewart *et al.*, 2010). Another example is the limited taxonomic resolution generally achieved by pollen analysis. Even if such analyses have recently reached better taxonomic resolution due to improved identification keys, the work is still time demanding, and identifications are often at genus or family level, rarely species (Faegri *et al.*, 1989). We thus have little understanding about specific diversity, and even less information about ancient plant populations. The new possibilities offered by the latest HTS technologies will likely increase our ability to resolve plant taxa at species level and additionally elucidate ancestry and genetic composition of ancient plant populations. Likewise, metabarcoding and metagenomic analyses of *sed*aDNA will provide more detailed insights on ancient ecosystems and link changes more tightly to past climate shifts (see section on HTS).

Sources of pollen, macrofossil and DNA

The majority of lake sediment deposits contain both plant remains and non-biological material originating from the lake catchment. In small boreal lakes it is estimated that ca 70% of the deposited pollen is from vegetation growing within few km of the lake (Jacobson & Bradshaw, 1981). While the majority of the pollen often derives from high-pollen producing wind-pollinating plants, which are distributed regionally through the air, the proportions of pollen from insect-pollinated plants may vary between sites. Furthermore, in areas of local low pollen production the pollen record may be highly affected by long distance exotic pollen that is not part of the regional environment (Hyvärinen, 1970). Despite this, pollen records generally represent the regional flora. Plant macrofossils, however, are of local origin, as they are large and have low dispersal and transport capacity (Allen & Huntley, 1999). The macrofossil record is often dominated by the aquatic and wetland plants growing in and

around the lake, and terrestrial taxa often under-represented (Birks, 2003). *Sed*aDNA seems to have a similar source of origin as macrofossils (Jørgensen *et al.*, 2012; Pedersen *et al.*, 2013; Alsos *et al.*, 2015; Parducci *et al.*, 2015). To date, only a few studies have focussed on the release and deposition of DNA in the environment (Poté *et al.*, 2007; Pietramellara *et al.*, 2009; Poté *et al.*, 2009; Barnes & Turner, 2016) and much therefore remains unknown about the ecology of the DNA – e.g. all processes occurring from source to deposition (taphonomy). Current *sed*aDNA results suggest that DNA extracted from sediments does not derive from actual pollen grains (Pedersen *et al.*, 2016; Sjögren *et al.*, 2016), but from other components imbedded in the sediment matrix, thus pollen DNA needs to be extracted directly from single or multiple isolated grains (see below).

Pollen DNA

Fossil pollen is often abundant and can be well preserved in lake sediments. Once deposited, pollen remains in situ in the sediments and there is therefore a high degree of certainty to its stratigraphic context. Further, aerial transport and rapid burial rates in sediment results in minimal physical damage to pollen grains and minimal exposure of the grains to biotic degradation. In addition the outer part of pollen grains (exine) is composed of cellulose and sporopollenin, an acid-resistant polymer that contains saturated and unsaturated hydrocarbons and phenolics (Southworth, 1974), and which protects the grains from physical and chemical attack, aiding pollen preservation in the sediments (Bennett & Willis, 2001). Inside the pollen, after maturation, there are 2-3 cells (a large vegetative cell and 1-2 generative cells) that comprise the male gametophyte (Fig. 5). The vegetative cell comprises the cytoplasm, and numerous plastids and mitochondria, which are responsible for the development of the pollen tube and delivery of the generative cells to the embryo sac together with the nuclear haploid DNA. Generative cells of pollen from the majority of plant species contain multiple organelles, including several copies of organelle DNA [chloroplast (cpDNA) and mtDNA], regardless of the type of inheritance of these genomes (maternal or paternal). Some nuclear repetitive regions, like ITS ribosomal repeats, are also present in multiple copies in the nucleus of both cell types. During pollen maturation however, there is a selective increase or decrease in the amount of organelle DNA in the generative cells (not in the vegetative) depending on the inheritance pattern (Nagata *et al.*, 1999; Zhang & Liu, 2003). For example, mature pollen from species with paternal cpDNA inheritance (the majority of conifers), contains a regular amount of mtDNA and cpDNA in the vegetative cell and an increased amount of cpDNA in the generative cell/s. On the other hand, pollen from species

with maternal inheritance of cpDNA and mtDNA (most of the angiosperms) contains at maturation a regular amount of both genomes in the vegetative cell and a decreased amount in the generative cell/s (Fig. 5). All three plant genomes are therefore present in both pollen types, but the cpDNA and the mtDNA may be present in different amount (in the generative cell/s) in different taxa.

Single-pollen genotyping

Petersen *et al*. (1996) were the first to amplify cpDNA from single pollen grains (*Hordeum* and *Secale*), while Suyama *et al.* (1996) were the first to amplify DNA from peat sediment pollen (fir, *Abies*) ca. 150 kyr old. Successively, Parducci *et al*. (2005; 2012) succeeded in retrieving short cpDNA and mtDNA fragments from Holocene *Pinus* and *Picea* pollen. A description of the techniques used for isolating and direct amplifying from single grains is presented in Box 1 and in Parducci *et al*. (2005). Later, the same technique was used to sequence cpDNA from angiosperm fossil pollen from the Venice Lagoon (Paffetti *et al*., 2007) and conifer pollen from glaciers (Nakazawa *et al*., 2013). Using multiplex PCR and single-pollen genotyping methods on fresh pollen (Isagi & Suyama, 2010) it is also possible to perform paternity analysis and infer pattern and distance of pollen dispersal in modern plant populations (Matsuki *et al*., 2007; 2008; Hasegawa *et al*., 2009; Hirota *et al*., 2013; Hasegawa *et al*., 2015). The potential of single-pollen analysis on fossil pollen however has not been explored further using traditional PCR-based Sanger sequencing technologies, because the PCR success-rate is low and the time required to handle and prepare the grains is high. With the advent of HTS technology, and in particular the recent availability of methodologies to directly construct HTS libraries from single cells (single cell sequencing technologies, SCS), it will now be possible to investigate more efficiently individual fossil pollen grains and hence conduct plant aDNA studies more effectively even at the population level (see section on HTS).

Sedimentary ancient DNA

Different sediment types show distinctive physical and chemical characteristics that will differentially affect DNA preservation, thus DNA survival will vary between different locations. Likewise, it seems that extraction of DNA and removal of inhibiting substrates requires strategies adapted to differences in the sediment content (Taberlet *et al*., 2012a; Pedersen *et*

al., 2016). Furthermore, extraction of aDNA molecules requires optimized protocols and special laboratory precautions.

Proxy overlapping or complementary

Before a robust inference of past vegetation (presence and abundance of taxa) can be based on *sed*aDNA, it is critical to consider the origin and taphonomy of plant fossil assemblages in the sediments and the influences of environmental, biological and physical factors affecting the presence and relative abundance of their DNA molecules (Jackson, 2012; Barnes & Turner, 2016).

Typically a low overlap has been found in lakes between pollen flora and DNA identifications, which has led to the inference that DNA in lake sediments originates locally (Jørgensen *et al*., 2012; Pedersen *et al*., 2013; Parducci *et al*., 2013; 2015; Sjögren *et al*., 2016) (Fig. 6).

Most plant macrofossils found in lakes are from the local vegetation (Birks, 2013). Despite this, most studies show a low to medium overlap (12-56%) between taxa recorded by *sed*aDNA and macrofossils (Jørgensen *et al*., 2012; Parducci *et al*., 2012; Boessenkool *et al*., 2013; Porter *et al*., 2013; Pedersen *et al*., 2013), while only one study has showed an overlap close to 100% (Alsos *et al*., 2016). These differences and the limited overlap found between proxies may be due to: (i) differences in taphonomic processes between sites; (ii) incompleteness of reference genomic databases; (iii) robustness of the experimental design (Ficetola *et al*., 2016); and (iv) number of pollen/macrofossils counted vs. sequencing depth (see sections below for further discussion of all points).

The most stringent test for identifying the origin of *sed*aDNA is by direct comparison with modern vegetation survey or with detailed historical vegetation maps. Yoccoz *et al*. (2012) were the first to demonstrate that plant diversity detected from environmental DNA extracted from boreal soil was consistent with plant taxonomic diversity estimated from conventional aboveground surveys. To date, we still do not know if such a good relationship holds with DNA in lake sediments. In a study from a high altitude crater lakes in Africa, Boessenkool *et al*. (2013) showed that *sed*aDNA largely reflected local flora. Similarly, Sjögren *et al*. (2016) compared DNA, pollen and historical vegetation maps in two Scottish lakes and found that *sed*aDNA was of local origin in contrast to regionally dispersed deciduous tree pollen. Finally, in a comparison of *sed*aDNA with vegetation surveys of 11 lakes in Northern Norway, the majority of taxa recorded in the *sed*aDNA were growing within 2 m of the lake (I.

G. Alsos, unpublished; Alsos *et al.*, 2015). Thus, we conclude that DNA deposition in lakes is more similar to that of macrofossils than pollen, and represents flora from within the catchment area. However with future improved DNA reference databases, methods and understanding, we expect increased information gained will lead to an almost complete overlap between DNA and macrofossils, but likely not between DNA and pollen (Fig. 6).

Taphonomic processes in lake sediments

Taphonomic processes (i.e. dispersal, transport, incorporation, and preservation of fossils and molecules in sediments) can affect assemblages recovered from sediments (Barnes & Turner, 2016). How organisms or parts of organisms preserve in sediments, and the fact that some preserve better than others can influence the range of taxa identified and therefore result in molecular and fossil indicators that are 'silent'. These factors influence pollen, macrofossil and DNA records in lake sediments (Fig. 2d, e). Moreover, taphonomic processes clearly vary in their impact for different indicators and the relative intensity of the suite of processes influencing pollen preservation may be different from those affecting plant DNA and macrofossils.

Lake sediments contain DNA from many different organisms, which include cellular DNA from tissues and intact cells, as well as extracellular DNA. When a plant tissue is degraded and a cell is lysed, it releases its content in the surrounding environment and extracellular DNA can bind to charged minerogenic and organic particles or remain unbound. Pietramellara *et al.* (2009) showed that in modern soils, once the DNA binds to a particle, it is immediately protected against nuclease degradation. This implies that that clay-rich soil can be highly suitable for protection of DNA against degradation, as clay particles have relatively large and charged surface area (Huang, 2014). Extracellular DNA molecules in soils can also be taken up by competent prokaryotic cells in a process called natural transformation. Although it is unlikely that this will be a large source of 'plant' DNA, it remains however a possibility (Pedersen *et al.*, 2015). Whether the plant DNA in lake sediments primarily are present as extracellular DNA or as small plant parts like leaves, root caps cells, stem or fruits, remains however still unclear.

Inferring taxa abundance from PCR-based *sed*aDNA analyses

As with macrofossils, aquatic plants are well represented in *sed*aDNA from lake sediments. Detection of a species in modern or semi-modern sediments depends on both distance to the lake shore and its abundance in the vegetation (Alsos *et al*., 2015; Sjögren *et al*., 2016). For example, the dominant species growing around lakes are found with a high proportion of DNA reads and present in more PCR repeats for most cases. This also seems to be the case for ancient samples, as all species represented from at least one macrofossil are also detected in more than one PCR replicate (Alsos *et al*., 2016). Nevertheless, for the time being, we should be very cautious about interpreting quantities of DNA beyond rough estimates when using PCR-based methods, as several metabarcoding processes may cause bias (e.g. primer binding site, amplicon length, taxonomy and diversity of extract) (Pornon *et al*., 2016).

Recent key findings and future methods using HTS techniques

HTS expands upon traditional PCR-based Sanger sequencing techniques and has facilitated a rapid development of aDNA research during the last decade. Ancient DNA molecules converted to sequencing libraries can now be parallel sequenced massively on HTS platforms like the Illumina HiSeq or the Complete Genomics platforms. HTS approaches have become increasingly affordable and are now routinely used by most aDNA laboratories, allowing screening of *sed*aDNA from a wide range of complex ancient substrates. With the newest generation of desktop HTS platforms, e.g. NextSeq, even small laboratories can now sequence their own datasets. There are, in principle, three HTS methodological strategies for analysing plant aDNA in lake sediments: metabarcoding or shotgun sequencing of *sed*aDNA and HTS of pollen DNA. Of the two latter methods, 'metabarcoding' is a relative established method, which relies on the information on one single locus, while the second is newer and relies on shotgun sequencing, i.e. sequencing a non-discriminated pool of aDNA. For clarity, it is important to define this new method. Here we suggest the term 'shotgun metabarcoding' when shotgun sequencing of environmental DNA is used to identify taxa, and 'metagenomics' when shotgun is used for functional analyses of the environments. This will allow researchers to discriminate between studies focusing on taxon identification and studies focusing on functional and attribute analysis. In the following paragraphs we review and discuss the progress made and future of these three methodologies.

Metabarcoding on *sed*aDNA

DNA metabarcoding has received enormous attention in the last decade in many ecological fields (Soininen *et al*., 2009; Valentini *et al*., 2009; Taberlet *et al*., 2012b). The ability of different barcodes to target groups of organisms has been investigated in different environments and under different conditions (in silico and in vitro PCR) leading to the conclusion that metabarcoding is highly applicable for biodiversity screening of modern samples, which implies a good potential in palaeoecological studies too (Thomsen & Willerslev, 2015).

Metabarcoding has already been used in a variety of palaeoenvironmental studies (Willerslev *et al*., 2003; Valentini *et al*., 2009; Taberlet *et al*., 2012b), including permafrost (Lydolph *et al*., 2005; Jørgensen *et al*., 2011; 2012; Willerslev *et al*., 2014), mid to high latitude/altitude lake sediments (Coolen & Gibson, 2009; Parducci *et al*., 2012; Alsos *et al*., 2015; Pansu *et al*., 2015; Epp *et al*., 2015; Paus *et al*., 2015; Alsos *et al*., 2016); tropical lake sediments (Boessenkool *et al*., 2013) (F. M. Ficetola, unpublished), and deposits preserved under ice-sheets, even in the absence of visible macrofossil remains (Willerslev *et al*., 1999; 2007).

A main advantage of metabarcoding is the possibility of simultaneously amplifying and identifying a large number of taxa for limited cost. Metabarcoding data on plant assemblages from *sed*aDNA in an Alpine catchment over the last 6.4 kyr years is comparable to those obtained using relevées performed on modern vegetation so it was possible to identify vegetation changes through time (e.g. shifts from shade-tolerant to heliophilous vegetation) (Pansu *et al*., 2015). Plant metabarcoding data can also be combined with other proxies of past environments, to identify potential drivers of such changes using approaches derived from community ecology (Giguet-Covex *et al*., 2014).

The performance of the metabarcoding approach is often limited if markers are not able to amplify all the present taxa (universality), and by their capacity to differentiate and identify also closely related species (resolution) (Ficetola *et al*., 2010; Sønstebø *et al*., 2010; Furlan *et al*., 2016). Therefore, the use of primers with appropriate features (i.e. "universal" primers amplifying with high specificity all plants, and achieving a high taxonomic resolution) (Ficetola *et al*., 2010; Furlan *et al*., 2016) is essential for the success of metabarcoding studies. In aDNA studies the choice of the marker is particularly difficult, as prerequisites like minimal bias in amplification of different taxa and short sequence length, drastically reduce the ability to resolve taxa (Taberlet *et al*., 2007). Plant metabarcoding studies can use either a single marker or a combination of multiple primers to resolve taxa. For instance, the *trn*L g/h

primers (Taberlet *et al*., 2007) can first be used to obtain an overall analysis of plant diversity with a good resolution for most plant families, and additional primers (e.g. ITS1-F/ITS1Poa-R for Poaceae and ITS1-F/TS1Ast-R for Asteraceae) may be later added to increase resolution within families (Baamrane *et al*., 2012). We should however remember that different primers might favour amplification of different taxa, which may lead to biases in the final results (Yoccoz, 2012).

The power of metabarcoding for vegetation reconstruction is also dependant on the availability of comprehensive taxonomic reference libraries needed to identify *sed*aDNA sequences (Taberlet *et al*., 2012b). Unfortunately, for many plant species there are at the moment no sequences deposited in publicly available databases such as GenBank. The number of such sequences however is now rapidly increasing, boosting the utility of this technique for investigating past plant history and population response to environmental change.

Metabarcoding thus provides a complementary tool to classical palaeoecological analysis and the three main proxies (DNA, pollen and macrofossils) can be used in combination or singularly depending on the aim of each study. If the aim is local vegetation reconstruction, we can either choose DNA or macrofossils, as the two overlap to a large degree. If the aim is to get a more regional signal from taxa, pollen analysis is probably appropriate if the taxonomic resolution is sufficient for the aim of the study. If we want to obtain a general view of plant communities through time, metabarcoding alone can also be sufficient because the ecological signal of metabarcoding is often good, and data generation fast and cheaper.

Shotgun metabarcoding on *sed*aDNA

Shotgun metabarcode analysis is a newer alternative to the traditional metabarcoding approach. It relies on shotgun sequencing DNA (Orlando *et al*., 2015) from a non-discriminated genetic pool obtained from environmental samples and then computational investigation, e.g. bioinformatic analysis, is used to decipher the taxonomic composition. While the shotgun sequencing approach traditionally was used for studying the fraction of un-cultivable microbes in modern environmental samples (Vos *et al*., 2013; Sharon & Banfield, 2013) by genome assembly and functional analysis of the microbial fraction (Mackelprang *et al*., 2011). More recently shotgun sequencing has been recognized as an important tool for bypassing DNA barcode amplification biases (Ziesemer *et al*., 2015; Pedersen *et al*., 2016), and also appear to give a more comprehensive insight into the community composition from

all trophic layers represented by the environmental sample (Pedersen *et al*., 2016). While this approach has played an expanding role in studying ancient prokaryotic communities in soil, sediments (>90% of DNA molecules in a sample are likely to be prokaryote), but also teeth, coprolites, gut and ice (e.g. Warinner *et al*., 2015; Wood & Wilmshurst, 2016), only one study so far has published results that are accepted as authentic using shotgun sequencing data for palaeoenvironmental reconstruction (Pedersen *et al*., 2016). However, there exists a considerable potential for applying low-coverage shotgun sequencing of genomic DNA (genome skimming) for studying plant aDNA (Coissac *et al*., 2016), but the absence of a consensus data processing and lack of experience interpreting such datasets makes it prone to misinterpretations and false positives (Bennett, 2015; Weiß et al., 2015) (see section on Bioinformatics). Species identification using genomic plant data outside chloroplast barcode regions can be particularly problematic. For instance, of ca. 391,000 vascular plant species existing on earth (number constantly changing through new discoveries and taxonomic revisions) only 1,092 are represented by complete chloroplast genomes (NCBI RefSeq database, ftp://ftp.ncbi.nlm.nih.gov/refseq/release/plastid/ - accessed 31 August 2016). In addition, taxonomic identifications outside the chloroplast genome are skewed towards overrepresented taxa with fully sequenced genomes, especially of commercially important species such as *Zea mays, Triticum aestivum, Solanum lycopersicum, Hordeum vulgare, Oryza sativa, Nicotiana tabacum* and the model organism *Arabidopsis thaliana*. The latter are all amongst the 20th most sequenced organisms (no. of bases, NCBI, ftp://ftp.ncbi.nih.gov/genbank/gbrel.txt - accessed 29 March 2016). While we can limit false positives and confirm aDNA authenticity using bioinformatic techniques (see section on Metagenomic bioinformatics), the lack of genomic references and the limited experience in interpreting such datasets makes proper contextualized biological interpretation a necessity. However, reference databases are quickly improving and on-going projects (e.g. PhyloAlps, https://www.france-genomique.org/spip/spip.php?article112&lang=fr and NorBol, http://norbol.org/) are currently assembling the entire chloroplast and nuclear ribosomal genomes of the whole floras of respectively the Alps, Norway, and parts of the Arctic (Coissac *et al*., 2016). Shotgun metabarcoding will therefore become an important tool in the future years, as it will allow for detecting organismal diversity and potentially differentiate population structures, and will further help bridge the gap between different scientific disciplines in palaeoecology.

An important advantage of shotgun sequencing is the possibility of quantifying the degree of DNA degradation exerted by the sample, e.g. fragmentation and hydrolysation of cytosines

520 (C), which results in accumulating misincorporation frequencies of thymine (T) instead of C at
521 the ends of the DNA molecules during sequencing (Jónsson *et al*., 2013). Lastly, it was shown
522 recently that DNA damage correlates with age of the lake sediments (Pedersen *et al*., 2016)
523 and should therefore be used as a independent tool for aDNA authentication.

524

525 HTS on pollen

526 Recently Suyama & Matsuki (2015) developed a method for constructing HTS libraries and
527 genotyping genome-wide single-nucleotide polymorphism (SNP) from low-quantity DNA
528 templates termed 'multiplexed ISSR (inter-simple sequence repeat) genotyping by sequencing
529 or 'MIG-seq'. Unlike standard methods based on restriction enzyme steps that require large
530 amounts of good quality DNA templates, the MIG-seq procedure is based on an initial PCR
531 step and can therefore discover and genotype de novo SNPs starting from reduced amounts
532 of DNA. The technique has been recently applied on modern pollen of *Hemerocallis* and
533 tested also on Pinus pollen a few years old collected from subsurface snow layers on a glacier
534 (Y. Suyama, unpublished). The MIG-seq technique on the glacier pollen was first combined
535 with a whole-genome amplification (WGA) step and successfully detected good-quality SNPs
536 in an ancient pine population.

537 A more efficient way of analysing ancient pollen is to construct HTS libraries directly from
538 single pollen grains employing the SCS technology. SCS is a cost- and time-effective method to
539 sequence in parallel hundreds of single-cells isolated and assayed in tiny (nl) reaction
540 chambers for PCR and sequencing (Wang & Navin, 2015). Limiting reactions to few nl offers
541 the advantages of increased throughputs, improved reaction sensitivity (higher percentages
542 of grains successfully genotyped) and increased degree of PCR specificity (reduced DNA
543 contamination). SCS methodology on fossil pollen seems at the moment the best available
544 approach to investigate efficiently a large number of pollen grains and a good alternative to
545 the more time-consuming single-pollen genotyping technique (Parducci *et al*., 2005). The
546 method offers the unprecedented opportunity of analysing in a time-effective way the
547 genetic structures of large number of single plant individuals on millennial time scales.

548 SCS protocols for pollen can be broken down into four main steps: (i) pollen isolation from
549 sediments and cleaning; (ii) pollen walls disruption; (iii) PCR amplification and HTS library
550 construction; (iv) DNA sequencing. For a description of the technique used for pollen isolation
551 and cleaning see Box 1 and Parducci & Suyama (2011). Before step 2, pollen can be screened
552 for DNA content by staining pollen suspensions with NST-DAPI buffer so that grains are gated

by total DNA content using fluorescence-based flow cytometry (L. Parducci pers. comm.). Prior to amplification, each grain is encapsulated in SCS micro reaction droplets and different microfluidic liquid handling techniques can be used to automatically trap the grains in such droplets and to dispense and analyse them efficiently (Kong *et al.*, 2012). The droplet-based microfluidic approach (Brouzes *et al.*, 2009) uses a 2-phase system, in which each assay is compartmentalized in an aqueous micro droplet of 1 pl to 10 nl surrounded by an immiscible oil. For disrupting pollen walls, two main physical lysis methods can be used: bead beating with glass or ceramic beads (Roberts, 2007) and ultrasonic homogenization (Dong *et al.*, 2015). These methods avoid the use of chemicals or enzymes that may later interfere during amplification, however they appear to be challenging with SCS methods, because it is easy to perturb monodispersed water-in-oil emulsions. Alternative methods to disrupt pollen walls and release genomic DNA involve the use of detergent-based or enzymatic lysis agents. The use of an extraction buffer containing proteinase K, sodium dodecyl sulfate (SDS), tris-HCl and EDTA has been previously used successfully to extract DNA from manually-crushed fossil pollen grains (Parducci & Suyama, 2011), and should also work well in micro reaction droplets. Alternatively, the use of chemicals like ethanolamine, which specifically disrupt pollen wall components, can also be tested (Southworth, 1974). After breaking the pollen wall, the SCS workflow involves library construction through direct PCR amplification of short (50-200 bp) regions of interest [e. g. the *trn*L g/h cpDNA region (Taberlet *et al.*, 2007)] or other mini-barcodes for plants (Little, 2014)] and amplicon tagging of each grain using unique ID tags with sequencing adaptors using (for example, the Drop-seq strategy). Sequence capture technique can also be used to specifically enrich for target cpDNA or mtDNA regions of interest prior to sequencing (Stull *et al.*, 2013). In this case custom self-made 'baits' are used to first to capture DNA regions of interests from ancient samples and successively to NGS sequence the hybridized fragments.

Challenges when studying aDNA from lake sediments

Contamination, laboratory analyses and experimental setup

Contamination of low-concentration aDNA samples with high-concentration modern DNA poses a challenge that should not be taken lightly and special precautions should always be taken in all steps of analyses (Fulton, 2012). There is not a single strategy valid for avoiding all possible contamination sources occurring from: (i) laboratory facilities; (ii) cross contamination; and (iii) reagents used during extraction and downstream preparation prior to

586 sequencing. In general, multi-strategy procedures should be employed (Champlot *et al.*,
587 2010) to avoid contamination. However, while contamination from laboratory facilities and
588 cross contamination are strictly related to facilities and experience/training of the workers
589 and can be more easily taken under control, contamination from reagents, which are known
590 to contain DNA especially of common food plants, is more difficult to control. Firstly, not all
591 reagents can be filtered, UV-lighted, bleached or DNase-treated (the preferred
592 decontaminating method). Secondly, even when treated, short DNA molecules can still persist
593 and be a source of genetic material during extraction and downstream handling. We
594 therefore stress the importance for having always experimental controls covering all reagents
595 and all steps during handling. Equally important is that the experimental setup is properly
596 designed thus enabling understanding of pre-analysis workflows and results for better and
597 future-proof data production. This involves steps from sample collection, laboratory work to
598 eventually multiplexing the DNA libraries for sequencing, and in which vital discoveries have
599 been made (Murray *et al.*, 2015, Schnell *et al.*, 2015, Ficetola *et al.*, 2016).

600 Several publications have addressed the issues of contamination and how to authenticate
601 aDNA (Hebsgaard *et al.*, 2005; Gilbert *et al.*, 2005; Sawyer *et al.*, 2012; Jónsson *et al.*, 2013)
602 and two important authentication methods are now used to make aDNA inferences highly
603 robust: (i) replication and use of controls; and (ii) DNA damage estimates.

604

605 Replication and use of controls

606 Extensive use of controls and replicated analyses is an important strategy ensuring the quality
607 of aDNA results. First, multiple extraction and PCR controls must be performed and
608 sequenced to detect sporadic contaminants. Taxa that are detected at a significant rate
609 within controls (usually from known food or exotic plants) are usually easy to recognise and
610 must be removed from analyses (Cooper & Poinar, 2000; Ficetola *et al.*, 2016). Alternatively,
611 if a taxon is sporadically present within controls, but is abundant in test samples, statistical
612 tests can assess whether the detection within HTS samples is significantly higher than the
613 contamination rate (Champlot *et al.*, 2010). The use of positive controls is a further strategy
614 to limit false positives and false negatives. De Barba *et al.* (2014) added to their analyses
615 positive controls made by mixing DNA of known concentration from four known plant
616 species. After HTS, they detected in positive controls several sequences at very low
617 frequencies, which did not belong to any of the species actually present. This result was then
618 used to identify a frequency threshold, which allowed detection and removal of sequences

representing low-frequency noise, without removing species that were actually present in the sample. The use of positive controls is however risky in aDNA analyses and should be avoided when possible, as it is itself a potential source of contamination (Cooper & Poinar, 2000). Species that are exotic to the study area (e.g. tropical species in studies focusing on the boreal flora) can be suitable positive controls, as their eventual contamination of the sample can be easily spotted.

Reproducibility is another key criterion to ensure the quality of results (Cooper & Poinar, 2000). Alsos *et al*. (2016) compared aDNA with macrofossils and concluded that all common species could be detected using one DNA extraction and one PCR per sample independently of sample age. However, increasing the number of extractions or PCR repeats increased the chances for detecting rare species. When the probability of detecting the species of interest is low due to either low biomass in the local environment or high degradation due to age or temperature, multiple PCR replicates are needed for a more complete description of communities. Simulation studies show that performing 6-12 PCR reaction from the same extract may produce robust results (Ficetola *et al*., 2015). Unfortunately, increasing repeats can have the drawback of increasing the probability of having false positives (Ficetola *et al*., 2015), but the benefit of detecting the species generally outweighs the problems of false positives. When multiple samples are analysed multiple times (e.g. in studies analysing time series), site-occupancy detection models allow estimation of the true frequency of the species, its detection probability, and the false positive rate (Ficetola *et al*., 2015; Lahoz Monfort *et al*., 2016). Bayesian models can therefore allow integration of prior information and the contamination rate of a specific taxon obtained for example from the analysis of controls (Lahoz Monfort *et al*., 2016).

Authentication of ancient origin (DNA damage)

In all aDNA sequences, an excess of C to T transitions is observed at the 5' and 3' end of molecules. This pattern of post-mortem damage increases over time (Sawyer *et al*., 2012; Pedersen *et al*., 2016), and the increased frequency of C to T transitions can be used as a tool for distinguishing aDNA sequences from modern contaminants (Briggs *et al*., 2007). The bioinformatic tool package MapDamage2.0 (Jónsson *et al*., 2013) provides a way to quantify the rate of DNA damage. This led Weiß *et al*. (2015) to develop a specific computational approach for comparing DNA damage patterns of putative aDNA with modern DNA and thus

confirming whether a sequences was of ancient origin or not. The method may be particularly useful for validation of future metagenomic studies.

Bioinformatic processing

Metabarcoding bioinformatics

HTS generates large DNA data sets that require dedicated programs for analyses. There are a number of bioinformatic steps to follow after DNA metabarcoding sequencing on Illumina platforms. The first three steps are assembling paired-end reads, assigning HTS reads to samples or demultiplexing (metabarcoding studies generally analyse multiple samples within each HTS run) (Coissac, 2012 and subsequent papers), and filtering erroneous sequences originated from non-specific amplification and PCR/sequencing errors. The latter task is often critical for ensuring the quality of metabarcoding data, and can be achieved by removing sequences that are too short/too long relative to the known features of the used barcodes and chimera sequences or sequences that likely represent punctual errors (e.g. nucleotide substitutions, small insertions/deletions) originating during PCR. Furthermore, sequences with just one read (singletons) or two reads in one sample can be artefacts (De Barba *et al.*, 2014; Elbrecht & Leese, 2015), and should generally be filtered. If sequencing depth is high, it is even possible to find thousands of reads which do not correspond to real barcodes. Therefore treatment of rare sequences remains a major challenge of metabarcoding data analysis (Ficetola *et al.*, 2016). The fourth bioinformatic step is clustering, which merges sequences belonging to the same molecular operational taxonomic units (MOTU). The fifth and final step is taxonomic assignment of sequences to currently known taxa. This is generally done by comparing the retrieved sequences with reference databases, which can be either broad databases like GenBank (Benson *et al.*, 2013) or ENA-EMBL (Leinonen *et al.*, 2011) or high-quality reference databases containing the verified and curated sequences of potentially present species, such as the arctic plant database (Sønstebø *et al.*, 2010), or the on-going PhyloAlps and NorBol projects (Coissac *et al.*, 2016). Multiple bioinformatics tools are currently available and a more detailed description can be found in Note S1 in the supporting information.

683 Shotgun metabarcode bioinformatics

684 The idea of processing shotgun metabarcode data is to take all DNA sequences present in a
685 sample dataset, align them against a reference database, parse the alignment information,
686 assign a taxonomic label to each read and hereby generate the taxonomic profile of the
687 metagenome/sample. For short-read alignment standard programmes such as *BWA* (Li &
688 Durbin, 2009) and *Bowtie2* (Langmead & Salzberg, 2012) are often used to align reads to
689 reference sequences, however they offer no tools to decipher between the alignments or
690 downstream handling, and additional tools and further analyses are therefore required. Over
691 recent years several tools for taxonomic profiling of shotgun sequence data have therefore
692 been developed. Overall these can be categorised as tools using all available sequences
693 (GenBank) such as *MEGAN* (Huson *et al*., 2007), which can also be used for metabarcoding,
694 the metagenomic *MG-RAST* server (Wilke *et al*., 2015), *EBI-metagenomics* (Mitchell, *et al*.,
695 2016) *CLARK* (Ounit *et al*., 2015), *Holi* (Pedersen *et al*. 2016), *Kraken* (Wood & Salzberg,
696 2014), *Kaiju* (Menzel *et al*., 2016), and then tools using selected marker genes such as
697 *MetaPhlAn* (Segata *et al*., 2012), *mOTU* (Sunagawa *et al*., 2013) and *metaBit* (Louvel *et al*.,
698 2016). Pipelines such as *MG-RAST* and *EBI-metagenomics* accept raw and untrimmed datasets
699 and are able to parse these through piped-programmes aligning against in-house databases
700 and eventually DNA doing sequence classification and graphical presentation. The majority of
701 the tools however, are designed and optimized for fast and accurate alignments (e.g. *BWA*
702 and *Bowtie2*) or for alignment and eventual DNA sequence classification (e.g. *Kraken, Holi,*
703 *CLARK* and *Kaiju*) against custom-build databases. Alternatively, unique marker-gene
704 databases are used to lower computational time and for robust abundance estimates and
705 taxa classification (e.g. *MetaPhlAn* and *mOTU*) and graphical presentation (*metaBit*).

706 While the accuracy and speed of these tools make shotgun metabarcode dataset analysis
707 faster and easy to perform [for more details see Lindgreen, *et al*. (2015)], a common issue is
708 that they are designed for modern sequencing datasets and for analysis of the microbial
709 fraction and only two of the tools (i.e. *MG-RAST* and *EBI-metagenomics*) contain plant
710 reference sequences within their databases unless the database is custom build. However,
711 none of these tools are designed for aDNA analysis and therefore lack the stringent and
712 robust criteria required for taxonomic consideration of short and damaged reads. Schubert *et*
713 *al*. (2012) compared different computational methods for improving accuracy and sensitivity
714 of aDNA sequence identification and showed that using reads ≥ 30 bp increase the quality of
715 alignments to modern reference genomes and lower the number of false positives. Currently,
716 from the limited number of studies of ancient plant shotgun metabarcoding, much indicates

717 that the sequence-to-reference similarity for taxonomic consideration should be as high as
718 100%. An in silico test, modelling shotgun metabarcode libraries inferred with sequencing
719 errors, found that errors or nucleotide substitutions can lead to false positives in ancient
720 metagenomic datasets (Pedersen *et al*., 2016). However, these false-positives appear as low
721 abundance random 'back-ground' noise, and can thus be avoided by setting a minimum
722 number of reads as a threshold. Although the size of this threshold seems to depend on the
723 number of reads sequenced, the exact size and nature for setting this threshold remains
724 unknown and likely varies with the genomic composition in the sample. No clear-cut choice
725 exists for metagenomic analysis tool but with above suggested criteria taxonomic inference
726 will become more robust. We furthermore urge that biological interpretation is always
727 accompanied by aDNA authentication (Jónsson *et al*., 2013).

728 One major bioinformatic challenge lies in the choice of reference database, which affects the
729 taxonomic profiling of a metagenome and therefore should be selected with care. Ideally, if
730 all organismal DNA was sequenced, we could rely on alignments against all reference
731 sequences and eventually use a lowest common ancestor algorithm to resolve reads with
732 alignment against multiple species. Such a method, with no 'a priori' assumption about
733 environment or species composition in a sample, is objective and would be the preferred
734 methodology. Pedersen *et al*. (2016) employed this methodology to data from ancient lake
735 sediments using NCBI's nucleotide database and showed that, by using stringent assignment
736 criteria even to a non-complete database, the plant metagenomic profile at genus level was in
737 line with taxa found by pollen, macrofossils and faunal record. However, the full effect of the
738 choice of database still remains unexplored and future studies are needed to investigate the
739 potential consequences of this.

740 Finally, it is important to emphasize that due to the nature of an environmental DNA sample,
741 the majority of the sequenced DNA obtained using a shotgun metabarcoding approach
742 cannot be usually identified with the databases currently available (Pedersen *et al*., 2016).
743 Often, more than 90 % of the reads produced cannot be aligned to a reference, and in most
744 cases less than 2% of the reads are unique to any taxa. However, as more genomes become
745 available these proportions will improve.

746

747

748 Conclusions and directions for future research

749

Despite early challenges, the field of aDNA has lately experienced a massive improvement methodologically, technologically, and in the understanding of the underlying processes by which environmental DNA information is transferred and transformed in lake sediments. This has resulted in new and better use of the technique in lakes and a refined understanding of important long-standing palaeoecological issues. We expect that the applicability of both shotgun and conventional metabarcode analysis of *sed*aDNA and SCS on pollen will continue to improve in the coming years, as HTS methods become more refined, less expensive, and the genomic reference databases improves. In Fig. 2 we show our interpretation of how the biotic palaeoenvironmental proxies in lake sediments originate, accumulate and develop through time. In Fig.7 we show our understanding of the chain of processes involved in the transformation of pollen, macrofossils and aDNA. Below we present a number of conclusions drawn from this review and on papers from Table 1 that we hope will be useful for plant aDNA researchers.

1. With the methodological, technological and experience improvements achieved over the past decade, plant DNA from lake sediments has now become an established tool for analysing past vegetation in combination with classical palaeoecological analyses. At the same time, as it provides a local proxy, it will play a key role for identifying 'fossil silent diversity' useful for understanding past vegetation change and for modelling vegetation response to future climate changes.

2. Lake sediments will continue to provide continuous archives with a fine temporal and spatial resolution, allowing establishment of good molecular records for past vegetation history and the possibility for distinguishing origin, dispersal and ancestry of plant species and populations through time.

3. Ancient plant DNA from lake sediments will in time be more precise at determining local vegetation relative to macrofossil and pollen analysis.

4. Improved understanding of DNA taphonomy from lake sediments now allow a better understanding of the origin and fate of plant aDNA molecules during and after deposition in lakes. Further understanding of these processes is crucial, particularly those involved in DNA preservation (temperature, pH, adsorption onto mineral surfaces, and oxygen availability) for improve determination of the power and limitations of the new tools presented in this review.

5. SCS profiling of pollen from lake sediments will likely grow in the coming years and become an important tool for investigating histories and dynamics of plants at the population level.

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References

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

Allentoft ME, Collins M, Harker D, Haile J, Oskam CL, Hale ML, Campos PF, Samaniego JA, Gilbert MTP, Willerslev E, *et al.* 2012. The half-life of DNA in bone: measuring decay kinetics in 158 dated fossils. Proceedings of the Royal Society B: Biological *Sciences* **279**: 4724–4733.

Alsos IG, Sjögren P, Edwards ME, Landvik JY, Gielly L, Forwick M, Coissac E, Brown AG, Jakobsen LV, Føreid MK, *et al.* 2016. Sedimentary ancient DNA from Lake Skartjørna, Svalbard: Assessing the resilience of arctic flora to Holocene climate change. *The Holocene* **26**: 1–16.

Alsos IG, Coissac E, Edwards ME, Merkel M, Gielly L. 2015. Plant DNA in sediments: to which degree do they represent the flora? *Genome* **58**: 163–303.

Andersen K, Bird KL, Rasmussen M, Haile J, Breuning-Madsen H, Kjær KH, Orlando L, Gilbert MTP, Willerslev E. 2011. Meta-barcoding of 'dirt' DNA from soil reflects vertebrate biodiversity. *Molecular Ecology* **21**: 1966–1979.

Anderson-Carpenter L, Mclachlan J, Jackson S, Kuch M, Lumibao C, Poinar H. 2011. Ancient DNA from lake sediments: Bridging the gap between paleoecology and genetics. *BMC Evolutionary Biology* **11**: 1–15.

Baamrane MAA, Shehzad W, Ouhammou A, Abbad A, Naimi M, Coissac E, Taberlet P, Znari M. 2012. Assessment of the Food Habits of the Moroccan Dorcas Gazelle in M'Sabih Talaa, West Central Morocco, Using the *trn*L Approach (LAN Amaral, Ed.). *PLOS ONE* **7**: e35643.

Barnes MA, Turner CR. 2016. The ecology of environmental DNA and implications for conservation genetics. *Conservation Genetics* **17**: 1–17.

Belle S, Parent C, Frossard V, Verneaux V, Millet L, Chronopoulou P-M, Sabatier P, Magny M. 2014. Temporal changes in the contribution of methane-oxidizing bacteria to the biomass of chironomid larvae determined using stable carbon isotopes and ancient DNA. *Journal of Paleolimnology* **52**: 215–228.

Bennett KD. 2015. Comment on 'Sedimentary DNA from a submerged site reveals wheat in the British Isles 8000 years ago'. *Science* **349**: 247–247.

Benson DA, Cavanaugh M, Clark K, Karsch-Mizrachi I, Lipman DJ, Ostell J, Sayers EW. 2013. GenBank. *Nucleic Acids Research* 41: D36–42.

Birks HH. 2003. The importance of plant macrofossils in the reconstruction of Lateglacial vegetation and climate: examples from Scotland, western Norway, and Minnesota, USA. *Quaternary Science Reviews* **22**: 453–473.

Birks HH. 2013. Plant macrofossil introduction. Mock HP, Elias SA eds. Encyclopedia of Quaternary Science. Amsterdam: Elsevier, 593–612.

Birks HJB. 2014. Challenges in the presentation and analysis of plant-macrofossil stratigraphical data. *Vegetation History and Archaeobotany* **23**: 309–330.

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

833 **Bissett A, Gibson J, Jarman SN. 2005.** Isolation, amplification, and identification of ancient
834 copepod DNA from lake sediments. *Limnology and Oceanography: Methods* **3**: 533–542.

835 **Boessenkool S, MCGlynn G, Epp LS, Taylor D, Pimentel M, Gizaw A, Memomissa S, Brochmann**
836 **C, Popp M. 2013.** Use of Ancient Sedimentary DNA as a Novel Conservation Tool for High-
837 Altitude Tropical Biodiversity. *Conservation Biology* **28**: 446–455.

838 **Briggs AW, Stenzel U, Johnson PLF, Green RE, Kelso J, Prufer K, Meyer M, Krause J, Ronan MT,**
839 **Lachmann M, *et al.* 2007.** Patterns of damage in genomic DNA sequences from a Neandertal.
840 *Proceedings of the National Academy of Sciences USA* **104**: 14616–14621.

841 **Brouzes E, Medkova M, Savenelli N, Marran D, Twardowski M, Hutchison JB, Rothberg JM, Link**
842 **DR, Perrimon N, Samuels ML. 2009.** Droplet microfluidic technology for single-cell high-
843 throughput screening. *Proceedings of the National Academy of Sciences USA* **106**: 14195–
844 14200.

845 **Brown TA, Cappellini E, Kistler L, Lister DL, Oliveira HR, Wales N, Schlumbaum A. 2014.** Recent
846 advances in ancient DNA research and their implications for archaeobotany. Vegetation
847 History and Archaeobotany 24: 207–214.

848 **Capo E, Debroas D, Arnaud F, Domaizon I. 2015.** Is Planktonic Diversity Well Recorded in
849 Sedimentary DNA? Toward the Reconstruction of Past Protistan Diversity. *Microbial Ecology*
850 **70**: 865–875.

851 **Champlot S, Berthelot C, Pruvost M, Bennett EA, Grange T, Geigl E-M. 2010.** An efficient
852 multistrategy DNA decontamination procedure of PCR reagents for hypersensitive PCR
853 applications. *PLOS ONE* **5**: e13042.

854 **Chan YL, Lacey EA, Pearson OP, HADLY EA. 2005.** Ancient DNA reveals Holocene loss of genetic
855 diversity in a South American rodent. *Biology Letters* **272**: 423–426.

856 **Clark JS, Fastie C, Hurtt G, Jackson ST, Johnson C, King GA, Lewis M, Lynch J, Pacala S, Prentice**
857 **C, *et al.* 1998.** Reid's Paradox of Rapid Plant Migration Dispersal theory and interpretation of
858 paleoecological records. *BioScience* **48**: 13–24.

859 **Coissac E. 2012.** OligoTag: a program for designing sets of tags for next-generation sequencing
860 of multiplexed samples. Pompanon F, Bonin A eds. Methods in Molecular Biology. Data
861 Production and Analysis in Population Genomics. New York, Dordrecht, Heidelberg, London,
862 13–31.

863 **Coissac E, Hollingsworth PM, Lavergne S, Taberlet P. 2016.** From barcodes to genomes:
864 extending the concept of DNA barcoding. Molecular Ecology 25: 1423–1428.

865 **Coolen M, Overmann J. 1998.** Analysis of subfossil molecular remains of purple sulfur bacteria
866 in a lake sediment. *Applied and Environmental Microbiology* **64**: 4513–4521.

867 **Coolen M, Muyzer G, Rijpstra W, Schouten S, Volkman JK, Sinninghe Damsté JS. 2004.**
868 Combined DNA and lipid analyses of sediments reveal changes in Holocene haptophyte and
869 diatom populations in an Antarctic lake. *Earth and Planetary Science Letters* **223**: 225–239.

870 Coolen M, Talbot HM, Abbas BA, Ward C, Schouten S, Volkman JK, Damsté JSS. 2008. Sources
871 for sedimentary bacteriohopanepolyols as revealed by 16S rDNA stratigraphy. *Environmental*
872 *Microbiology* **10**: 1783–1803.

873 Coolen M, Gibson J. 2009. Ancient DNA in lake sediment records. *PAGES news* **17**, 104-106.

874 Cooper A, Poinar HN. 2000. Ancient DNA: Do it Right or Not at All. *Science* **289**: 1139–1141.

875 Dabney J, Meyer M, Pääbo S. 2013. Ancient DNA Damage. Cold Spring Harbor Perspectives in
876 Biology: doi: 10.1101–cshperspect.a012567.

877 da Fonseca RR, Smith BD, Wales N, Cappellini E, Skoglund P, Fumagalli M, Samaniego JA, Carøe
878 C, Ávila-Arcos MAC, Hufnagel DE, et al. 2015. The origin and evolution of maize in the
879 Southwestern United States. Nature Plants 1: 14003–14008.

880 D'Andrea WJ, Lage M, Martiny J. 2006. Alkenone producers inferred from well-preserved 18S
881 rDNA in Greenland lake sediments. *Journal of Geographic Research* **111**: G03013.

882 De Barba M, Miquel C, Boyer F, Mercier C, Rioux D, Coissac E, Taberlet P. 2014. DNA
883 metabarcoding multiplexing and validation of data accuracy for diet assessment: application
884 to omnivorous diet. *Molecular Ecology Resources* **14**: 306–323.

885 Domaizon I, Savichtcheva O, Debroas D, Arnaud F, Villar C, Pignol C, Alric B, Perga ME. 2013.
886 DNA from lake sediments reveals the long-term dynamics and diversity of Synechococcus
887 assemblages. *Biogeosciences* **10**: 3817–3838.

888 Dong J, Gao K, Wang K, Xu X, Zhang H. 2015. Cell Wall Disruption of Rape Bee Pollen Treated
889 with Combination of Protamex Hydrolysis and Ultrasonication. *Food Research International*
890 **75**: 123–130.

891 Elbrecht V, Leese F. 2015. Can DNA-Based Ecosystem Assessments Quantify Species
892 Abundance? Testing Primer Bias and Biomass - Sequence Relationships with an Innovative
893 Metabarcoding Protocol. PLOS ONE 10: e0130324.

894 Epp LS, Stoof-Leichsenring KR, Trauth MH, Tiedemann R. 2010. Historical genetics on a
895 sediment core from a Kenyan lake: intraspecific genotype turnover in a tropical rotifer is
896 related to past environmental changes. *Journal of Paleolimnology* **43**: 939–954.

897 Epp LS, Stoof-Leichsenring KR, Trauth MH, Tiedelman R. 2011. Molecular profiling of diatom
898 assemblages in tropical lake sediments using taxon-specific PCR and Denaturing High-
899 Performance Liquid Chromatography (PCR-DHPLC). *Molecular Ecology Resources* **11**: 842–
900 853.

901 Epp LS, Gussarova G, Boessenkool S, Olsen J, Haile J, Schrøder-Nielsen A, Ludikova A, Hassel K,
902 Stenøien HK, Funder S, *et al.* 2015. Lake sediment multi-taxon DNA from North Greenland
903 records early post-glacial appearance of vascular plants and accurately tracks environmental
904 changes. *Quaternary Science Reviews* **117**: 152–163.

905 Faegri K, Iversen J, Kaland PE, Krzywinski K. 1989. Textbook of Pollen Analysis. 4th edition.
906 Wiley, Chichester.

907 Etienne D, Destas M, Lyautey E, Marti R, Ruffaldi P, Georges-Leroy M, Dambrine E, Topp E.
908 2015. Two thousand–year reconstruction of livestock production intensity in France using

sediment-archived fecal Bacteroidales and source-specific mitochondrial markers. *The Holocene* **25**: 1384–1393.

Feek DT, Flenley JR, Chester PI, Welikala N, Matisoo-Smith EA, Tannock GW. 2006. A modified sampler for uncontaminated DNA cores from soft sediments. *Journal of Archaeological Science* **33**: 573–574.

Feek DT, Horrocks M, Baisden WT, Flenley J. 2011. The Mk II sampler: a device to collect sediment cores for analysis of uncontaminated DNA. *Journal of Paleolimnology* **45**: 115–119.

Ficetola GF, Coissac E, Zundel S, Riaz T, Shehzad W, Bessière J, Taberlet P, Pompanon F. 2010. An in silico approach for the evaluation of DNA barcodes. *BMC Genomics* **11**: 434–1572.

Ficetola GF, Pansu J, Bonin A, Coissac E, Giguet-Covex C, De Barba M, Gielly L, Lopes CM, Boyer F, Pompanon F, *et al.* 2015. Replication levels, false presences and the estimation of the presence/absence from eDNA metabarcoding data. *Molecular Ecology Resources* **15**: 543–556.

Ficetola GF, Taberlet P, Coissac E. 2016. How to limit false positives in environmental DNA and metabarcoding? *Molecular Ecology Resources* **16**: 604–607.

Fulton TL. 2012. Setting Up an Ancient DNA Laboratory.Shapiro B, Hofreiter M eds. Methods in Molecular Biology. Ancient DNA: methods and protocols. New York, Dordrecht, Heidelberg, London: Springer, 1–12.

Furlan EM, Gleeson D, Hardy CM, Duncan RP. 2016. A framework for estimating the sensitivity of eDNA surveys. *Molecular Ecology Resources* **16**: 641–654.

Giguet-Covex C, Pansu J, Arnaud F, Rey P-J, Griggo C, Gielly L, Domaizon I, Coissac E, David F, Choler P, *et al.* 2014. Long livestock farming history and human landscape shaping revealed by lake sediment DNA. *Nature Communications* **5**: 3211.

Gilbert MTP, Bandelt H-J, Hofreiter M, Barnes I. 2005. Assessing ancient DNA studies. *Theoretical and Applied Genetics* **20**: 541–544.

Haile J, Holdaway R, Oliver K, Bunce M, Gilbert MTP, Nielsen R, Munch K, Ho SYW, Shapiro B, Willerslev E. 2007. Ancient DNA Chronology within Sediment Deposits: Are Paleobiological Reconstructions Possible and Is DNA Leaching a Factor? *Molecular Biology and Evolution* **24**: 982–989.

Hasegawa Y, Suyama Y, Seiwa K. 2009. Pollen donor composition during the early phases of reproduction revealed by DNA genotyping of pollen grains and seeds of Castanea crenata. New Phytologist **182**: 994–1002.

Hasegawa Y, Suyama Y, Seiwa K. 2015. Variation in pollen-donor composition among pollinators in an entomophilous tree species, Castanea crenata, revealed by single-pollen genotyping. *PLOS ONE* **10**: e0120393.

Hebsgaard MB, Phillips MJ, Willerslev E. 2005. Geologically ancient DNA: fact or artefact? *Trends in Microbiology* **13**: 212–220.

Hewitt G. 2000. The genetic legacy of the Quaternary ice ages. *Nature* **405**: 907–913.

947 Higuchi R, Bowman B, Freiberger M, Ryder O, Wilson A. 1984. DNA sequences from the
948 quagga, an extinct member of the horse family. *Nature* 312: 282–284.

949 Hirota SK, Nitta K, Suyama Y, Kawakubo N, Yasumoto AA, Yahara T. 2013. Pollinator-mediated
950 selection on flower color, flower scent and flower morphology of Hemerocallis: evidence
951 from genotyping individual pollen grains on the stigma. *PLOS ONE* 8: e85601.

952 Hofreiter M, Mead JI, Martin P, Poinar H. 2003. Molecular caving. *Current Biology* 13: R693–
953 R695.

954 Hofreiter M, Serre D, Poinar H, Kuch M, Pääbo S. 2001. Ancient DNA. *Nature Reviews Genetics*
955 2: 353–359.

956 Hou W, Dong H, Li G, Yang J, Coolen MJL, Liu X, Wang S, Jiang H, Wu X, Xiao H, et al. 2014.
957 Identification of Photosynthetic Plankton Communities Using Sedimentary Ancient DNA and
958 Their Response to late-Holocene Climate Change on the Tibetan Plateau. *Scientific Reports* 4:
959 6648–9.

960 Hu FS, Hampe A, Petit RJ. 2009. Paleoecology meets genetics: deciphering past vegetational
961 dynamics. F*rontiers in Ecology and the Environment* 7: 371–379.

962 Huang YT. 2014. Studies on carbon and DNA preservation in allophanic soils and paleosols on
963 Holocene tephras in New Zealand. PhD thesis, University of Waikato, NZ.

964 Huson DH, Auch AF, Qi J, Schuster SC. 2007. MEGAN analysis of metagenomic data. *Genome*
965 *Research* 17: 377–386.

966 Hutchinson, G. E. (1957). A Treatise on Limnology, Vol. 1. Geography, Physics and Chemistry.
967 Wiley, New York.

968 Isagi Y, Suyama Y. 2010. Single-Pollen Genotyping (Y Isagi and Y Suyama, Eds.). Tokyo: Springer
969 Science & Business Media.

970 Jackson ST. 2012. Representation of flora and vegetation in Quaternary fossil assemblages:
971 known and unknown knowns and unknowns. *Quaternary Science Reviews* 49: 1–15.

972 Jacobson GL, Bradshaw RHW. 1981. The selection of sites for paleovegetational studies.
973 Quaternary Research 16: 80–96.

974 Jiang H, Dong H, Yu B, Liu X, Li Y, Ji S. 2007. Microbial response to salinity change in Lake
975 Chaka, a hypersaline lake on Tibetan plateau. *Environmental Microbiology* 9: 2603–2621

976 Jónsson H, Ginolhac A, Schubert M, Johnson PLF, Orlando L. 2013. mapDamage2.0: fast
977 approximate Bayesian estimates of ancient DNA damage parameters. *Bioinformatics* 29:
978 1682–1684.

979 Jørgensen T, Haile J, Moller P, AndreevV A, Boessenkool S, Rasmussen M, Kienast F, Coissac E,
980 Taberlet P, Brochmann C, *et al.* 2012. A comparative study of ancient sedimentary DNA, pollen
981 and macrofossils from permafrost sediments of northern Siberia reveals long-term
982 vegetational stability. *Molecular Ecology* 21: 1989–2003.

983 Jørgensen T, Kjær KH, Haile J, Rasmussen M, Boessenkool S, Andersen K, Coissac E, Taberlet P,
984 Brochmann C, Orlando L, *et al.* 2011. Islands in the ice: detecting past vegetation on

985 Greenlandic nunataks using historical records and sedimentary ancient DNA Meta-barcoding.
986 *Molecular Ecology* **21**: 1980–1988.

987 **Kyle M, Haande S, Sønstebø JH, Rohrlack T. 2015.** Amplification of DNA in sediment cores to
988 detect historic *Planktothrix* occurrence in three Norwegian lakes. Journal of *Paleolimnology*
989 **53**: 61–72.

990 Kong F, Yuan L, Zheng YF, Chen W. 2012. Automatic liquid handling for life science: a critical
991 review of the current state of the art. Journal of Laboratory Automation 17: 169–185.

992 **Kojima H, Iwata T, Fikui M. 2009.** DNA-based analysis of planktonic methanotrophs in a
993 stratified lake. *Freshwater Biology* **54**: 1501–1509.

994 **Lahoz Monfort JJ, Guillera Arroita G, Tingley R. 2016.** Statistical approaches to account for
995 false-positive errors in environmental DNA samples. *Molecular Ecology Resources* **16**: 673–
996 685.

997 **Langmead B, Salzberg SL. 2012.** Fast gapped-read alignment with Bowtie 2. *Nature Methods* **9**:
998 357–359.

999 **Larsen CPS, Macdonald GM. 1993.** Lake morphometry, sediment mixing and the selection of
1000 sites for fine resolution palaeoecological studies. *Quaternary Science Reviews* **12**: 781–792.

1001 **Leinonen R, Akhtar R, Birney E, Bower L, Cerdeno-Tárraga A, Cheng Y, Cleland I, Faruque N,**
1002 **Goodgame N, Gibson R, *et al.* 2011.** The European Nucleotide Archive. *Nucleic Acids Research*
1003 **39**: D28–31.

1004 **Li H, Durbin R. 2009.** Fast and accurate short read alignment with Burrows-Wheeler
1005 transform. Bioinformatics 25: 1754–1760.

1006 **Li G, Dong H, Hou W, Wang S, Jiang H, Yang J, Wu G. 2016.** Temporal Succession of Ancient
1007 Phytoplankton Community in Qinghai Lake and Implication for Paleo-environmental Change.
1008 *Scientific Reports* **6**: 19769.

1009 **Lindgreen S, Adair KL, Gardner PP. 2016.** An evaluation of the accuracy and speed of
1010 metagenome analysis tools. *Scientific Reports* **6**: 19233.

1011 Lindhal T. 1993. Instability and decay of the primary structure of DNA. **362**: 709–715.

1012 **Limburg PA, Weider LJ. 2002.** 'Ancient' DNA in the resting egg bank of a microcrustacean can
1013 serve as a palaeolimnological database. *Proceedings of the Royal Society B: Biological Sciences*
1014 **269**: 281–287.

1015 Little DP. 2014. A DNA mini-barcode for land plants. Molecular Ecology Resources 14: 437–
1016 446.

1017 **Louvel G, Sarkissian Der C, Hanghøj K, Orlando L. 2016.** metaBIT, an integrative and automated
1018 metagenomic pipeline for analysing microbial profiles from high-throughput sequencing
1019 shotgun data. Molecular Ecology Resources 16: 1415–1427

1020 Lydolph MC, Jacobsen J, Arctander P, Gilbert MTP, Gilichinsky DA, Hansen AJ, Willerslev E,
1021 Lange L. 2005. Beringian Paleoecology Inferred from Permafrost-Preserved Fungal DNA.
1022 *Applied* and Environmental Microbiology 71: 1012–1017.

1023 Mackelprang R, Waldrop MP, DeAngelis KM, David MM, Chavarria KL, Blazewicz SJ, Rubin EM,
1024 Jansson JK. 2011. Metagenomic analysis of a permafrost microbial community reveals a rapid
1025 response to thaw. Nature 480: 368–371.

1026 Madeja J, Wacnik A, Zyga A, Stankiewicz E, Wypasek E, Guminski W, Harmata K. 2009. Bacterial
1027 ancient DNA as an indicator of human presence in the past: its correlation with palynological
1028 and archaeological data. *Journal of Quaternary Science* **24**: 317–321.

1029 Madeja J, Wacnik A, Wypasek E, Chandran A, Stankiewicz E. 2010. Integrated palynological and
1030 molecular analyses of late Holocene deposits from Lake Miłkowskie (NE Poland): Verification
1031 of local human impact on environment. *Quaternary International* **220**: 147–152.

1032 Madeja J. 2015. A new tool to trace past human presence from lake sediments: the human-
1033 specific molecular marker Bacteroides strain HF 183. *Journal of Quaternary Science* **30**: 349–
1034 354.

1035 Magyari EK, Major A, Balint M, Nedli J, Braun M, Racz I, Parducci L. 2011. Population dynamics
1036 and genetic changes of Picea abies in the South Carpathians revealed bypollen and ancient
1037 DNA analyses. *BMC Evolutionary Biology* **11**: 66.

1038 Marková S, Černý M, Rees D, Stuchlík E. 2006. Are they still viable? Physical conditions and
1039 abundance of *Daphnia pulicaria* resting eggs in sediment cores from lakes in the Tatra
1040 Mountains. *Biologia* **61**: 135–146.

1041 Mascher M, Schuenemann VJ, Davidovich U, Marom N, Himmelbach A, Hübner S, Korol A,
1042 David M, Reiter E, Riehl S, *et al.* 2016. Genomic analysis of 6,000-year-old cultivated grain
1043 illuminates the domestication history of barley. Nature Genetics 48: 1089–1093.

1044 Matisoo-Smith E, Roberts K, Welikala N, Tannock G, Chester P, Feek D, Flenley J. 2008.
1045 Recovery of DNA and pollen from New Zealand lake sediments. *Quaternary International* **184**:
1046 139–149.

1047 Matsuki Y, Isagi Y, Suyama Y. 2007. The determination of multiple microsatellite genotypes
1048 and DNA sequences from a single pollen grain. *Molecular Ecology Notes* **7**: 194–198.

1049 Matsuki Y, Tateno R, Shibata M, Isagi Y. 2008. Pollination efficiencies of flower-visiting insects
1050 as determined by direct genetic analysis of pollen origin. *American Journal of Botany* **95**: 925–
1051 930.

1052 Menzel P, Ng KL, Krogh A. 2016. Fast and sensitive taxonomic classification for metagenomics
1053 with Kaiju. Nature Communications 7: 11257.

1054 Mergeay J, Vanoverbeke J, Verschuren D, De Meester L. 2007. Extinction, recolonization, and
1055 dispersal through time in a planktonic crustacean. *Ecology* **88**: 3032–3043.

1056 Mitchell A, Bucchini F, Cochrane G, Denise H, Hoopen Ten P, Fraser M, Pesseat S, Potter S,
1057 Scheremetjew M, Sterk P, et al. 2016. EBI metagenomics in 2016--an expanding and evolving
1058 resource for the analysis and archiving of metagenomic data. *Nucleic Acids Research* **44**:
1059 D595–603.

1060 Murray DC, Coghlan ML & Bunce M, 2015. From Benchtop to Desktop: Important
1061 Considerations when Designing Amplicon Sequencing Workflows. *PLoS ONE*, **10**, p.e0124671.

1062 Nagata N, Saito C, Sakai A, Kuroiwa H, Kuroiwa T. 1999. The selective increase or decrease of
1063 organellar DNA in generative cells just after pollen mitosis one controls cytoplasmic
1064 inheritance. *Planta* 209: 53–65.

1065 Nakazawa F, Uetake J, Suyama Y, Kaneko R, Takeuchi N, Fujita K, Motoyama H, Imura S, Kanda H.
1066 2013. DNA analysis for section identification of individual Pinus pollen grains from Belukha glacier,
1067 Altai Mountains, Russia. *Environmental Research Letters* 8: 014032.

1068 Nesje A. 1992. A Piston Corer for Lacustrine and Marine Sediments. *Arctic and Alpine Research*
1069 24: 257.

1070 Orlando L, Gilbert MTP, Willerslev E. 2015. Reconstructing ancient genomes and epigenomes.
1071 *Nature Reviews Genetics* 16: 395–408.

1072 Orlando L, Ginolhac A, Zhang G, Froese D, Albrechtsen A, Stiller M, Schubert M, Cappellini E,
1073 Petersen B, Moltke I, *et al.* 2014. Recalibrating Equus evolution using the genomesequence of
1074 an early Middle Pleistocene horse. *Nature* 498: 74–78.

1075 Ounit R, Wanamaker S, Close TJ, Lonardi S. 2015. CLARK: fast and accurate classification of
1076 metagenomic and genomic sequences using discriminative k-mers. BMC *Genomics* 16: 236.

1077 Paffetti D, Vettori C, Caramelli D, Vernesi C, Lari M, Paganelli A, Paule L, Giannini R. 2007.
1078 Unexpected presence of Fagus orientalis complex in Italy as inferred from 45,000-year-old
1079 DNA pollen samples from Venice lagoon. *BMC Evolutionary Biology* 7: S6.

1080 Pal S, Gregory-Eaves I, Pick FR. 2015. Temporal trends in cyanobacteria revealed through DNA
1081 and pigment analyses of temperate lake sediment cores. Journal of *Paleolimnology* 54: 87–
1082 101.

1083 Pansu J, Giguet-Covex C, Ficetola GF, Gielly L, Boyer F, Zinger L, Arnaud F, Poulenard J, Taberlet
1084 P, Choler P. 2015. Reconstructing long-term human impacts on plant communities: an
1085 ecological approach based on lake sediment DNA. *Molecular Ecology* 24: 1485–1498.

1086 Parducci L, Suyama Y. 2011. Single-Pollen Genotyping of Holocene Lake Sediments.Isagi Y,
1087 Suyama Y eds. Ecological Research Monographs. Single-Pollen Genotyping. Tokyo: Springer
1088 Japan, 101–109.

1089 Parducci L, Jørgensen T, Tollefsrud MM, Elverland E, Alm T, Fontana SL, Bennett KD, Haile J,
1090 Matetovici I, Suyama Y, *et al.* 2012. Glacial survival of boreal trees in northern Scandinavia.
1091 *Science* 335: 1083–1086.

1092 Parducci L, Matetovici I, Fontana SL, Bennett KD, Suyama Y, Haile J, Kjær KH, Larsen NK, Drouzas
1093 AD, Willerslev E. 2013. Molecular- and pollen-based vegetation analysis in lake sediments
1094 from central Scandinavia. *Molecular Ecology* 22: 3511–3524.

1095 Parducci L, Suyama Y, Lascoux M, Bennett KD. 2005. Ancient DNA from pollen: a genetic record
1096 of population history in Scots pine. *Molecular Ecology* 14: 2873–2882.

1097 Parducci L, Väliranta M, Salonen JS, Ronkainen T, Matetovici I, Fontana SL, Eskola T, Sarala P,
1098 Suyama Y. 2015. Proxy comparison in ancient peat sediments: pollen, macrofossil and plant
1099 DNA. *Philosophical Transactions of the Royal Society B: Biological Sciences* 370: 20130382.

1100 Poulain AJ, Aris-Brosou SEP, Blais JM, Brazeau M, Keller WB, Paterson AM. 2015. Microbial DNA
1101 records historical delivery of anthropogenic mercury. *The ISME Journal* 9: 2541–2550.

Paus A, Boessenkool S, Brochmann C, Epp LS, Fabel D, Haflidason H, Linge H. 2015. Lake Store Finnsjøen – a key for understanding Lateglacial/early Holocene vegetation and ice sheet dynamics in the central Scandes Mountains. *Quaternary Science Reviews* 121: 36–51.

Pääbo S. 1984. Molecular cloning of Ancient Egyptian mummy DNA. *Nature* 314: 644–645.

Pedersen MW, Ginolhac A, Orlando L, Olsen J, Andersen K, Holm J, Funder S, Willerslev E, Kjær KH. 2013. A comparative study of ancient environmental DNA to pollen and macrofossils from lake sediments reveals taxonomic overlap and additional plant taxa. *Quaternary Science Review* 75: 161–168.

Pedersen MW, Overballe-Petersen S, Ermini L, Sarkissian CD, Haile J, Hellstrom M, Spens J, Thomsen PF, Bohmann K, Cappellini E, *et al.* 2015. Ancient and modern environmental DNA. *Ph*ilosophical Transactions of the Royal Society B: Biological Sciences 370: 20130383.

Pedersen MW, Ruter A, Schweger C, Friebe H, Staff RA, Kjeldsen KK, Mendoza MLZ, Beaudoin AB, Zutter C, Larsen NK, et al. 2016. Postglacial viability and colonization in North America's ice-free corridor. *Nature* 537: 45–49.

Petersen G, Johansen B, Sedeberg O. 1996. PCR and sequencing form a sing pollen grain. Plant Molecular Biology 31: 189–191.

Pietramellara G, Ascher J, Borgogni F, Ceccherini MT, Guerri G, Nannipieri P. 2009. Extracellular DNA in soil and sediment: fate and ecological relevance. Biology and Fertility of Soils 45: 219–235.

Pollard HG, Colbourne JK, Keller W. 2003. Reconstruction of centuries-old Daphnia communities in a lake recovering from acidification and metal contamination. *AMBIO: A Journal of the Human Environment* 32: 214–218.

Pornon A, Escaravage N, Burrus M, Holota H, Khimoun A, Mariette J, Pellizzari C, Iribar A, Etienne R, Taberlet P, *et al.* 2016. Using metabarcoding to reveal and quantify plant-pollinator interactions. *Scientific Reports* 6: 27282.

Porter TM, Golding GB, King C, Froese D, Zazula G, Poinar HN. 2013. Amplicon pyrosequencing late Pleistocene permafrost: the removal of putative contaminant sequences and small-scale reproducibility. *Molecular Ecology Resources* 13: 798–810.

Poté J, Ackermann R, Wildi W. 2009. Plant leaf mass loss and DNA release in freshwater sediments. *Ecotoxicology and Environmental Safety* 72: 1378–1383.

Poté J, Rosselli W, Wigger A, Wildi W. 2007. Release and leaching of plant DNA in unsaturated soil column. *Ecotoxicology and Environmental Safety* 68: 293–298.

Randlett M-È, Coolen M, Stockhecke M, Pickarski N, Litt T, Balkema C, Kwiecien O, Tomonaga Y, Wehrli B, Schubert CJ. 2014. Alkenone distribution in Lake Van sediment over the last 270 ka: influence of temperature and haptophyte species composition. *Quaternary Science Reviews* 104: 53–62.

Ravasi DF, Peduzzi S, Guidi V, Peduzzi R, Wirth SB, Gilli A, Tonolla M. 2012. Development of a real-time PCR method for the detection of fossil 16S rDNA fragments of phototrophic sulfur bacteria in the sediments of Lake Cadagno. *Geobiology* 10: 196–204.

Rawlence NJ, Lowe DJ, Wood JR, Young JM, Churchman GJ, Huang Y-T, Cooper A. 2014. Using palaeoenvironmental DNA to reconstruct past environments: progress and prospects. *Journal of Quaternary Science* **29**: 610–626.

Renberg I, Hansson H. 2008. The HTH sediment corer. *Journal of Paleolimnology* **40**: 655–659.

Reid VA, Carvalho GR, George DG, Griffiths HI. 2002. A technique for the molecular genetic analysis of Daphnia resting eggs from sub-recent lake sediments. *Journal of Paleolimnology* **27**: 481–486.

Roberts AV. 2007. The use of bead beating to prepare suspensions of nuclei for flow cytometry from fresh leaves, herbarium leaves, petals and pollen. *Cytometry* Part A **71**: 1039–1044.

Savichtcheva O, Debroas D, Kurmayer R, Villar C, Jenny JP, Arnaud F, Perga ME, Domaizon I. 2011. Quantitative PCR Enumeration of Total/Toxic Planktothrix rubescens and Total Cyanobacteria in Preserved DNA Isolated from Lake Sediments. *Applied and Environmental Microbiology* **77**: 8744–8753.

Sawyer S, Krause J, Guschanski K, Savolainen V, Pääbo S. 2012. Temporal Patterns of Nucleotide Misincorporations and DNA Fragmentation in Ancient DNA. PLOS ONE 7: e34131.

Schnell I B, Bohmann K, Gilbert M T P. 2015. Tag jumps illuminated - reducing sequence-to-sample misidentifications in metabarcoding studies. *Molecular Ecology Resources* **15**: 1289–1303.

Schubert M, Ginolhac A, Lindgreen S, Thompson JF, Al-Rasheid KAS, Willerslev E, Krogh A, Orlando L. 2012. Improving ancient DNA read mapping against modern reference genomes. *BMC Genomics* **13**: 178.

Segata N, Waldron L, Ballarini A, Narasimhan V, Jousson O, Huttenhower C. 2012. Metagenomic microbial community profiling using unique clade-specific marker genes. *Nature Methods* **9**: 811–814.

Shapiro B, Hofreiter M. 2012. Ancient DNA: methods and protocols (B Shapiro and M Hofreiter, Eds.). New York Dordrecht Heidelberg London: Springer.

Sharon I, Banfield JF. 2013. Microbiology. Genomes from metagenomics. *Science* **342**: 1057–1058.

Sjögren P, Edwards ME, Gielly L, Langdom CT, Croudace IW, Merkel MKF, Fonville T, Alsos IG. 2016. Lake sedimentary DNA accurately records 20th century introductions of exotic conifers in Scotland. *New Phytologist*: doi: 10.1111/nph.14199

Sobek S, Durisch-Kaiser E, Zurbrügg R. 2009. Organic carbon burial efficiency in lake sediments controlled by oxygen exposure time and sediment source. *Limnology and Oceanography* **54**: 2243–2254.

Soininen EM, Valentini A, Coissac E, Miquel C, Gielly L, Brochmann C, Brysting AK, Sønstebø JH, Ims RA, Yoccoz NG, *et al.* 2009. Analysing diet of small herbivores: the efficiency of DNA barcoding coupled with high-throughput pyrosequencing for deciphering the composition of complex plant mixtures. *Frontiers in Zoology* **6**: 16.

1181 Southworth D. 1974. Solubility of pollen exines. American Journal of Botany 61: 36–44.

1182 Stager JC, Sporn LA, Johnson M, Regalado S. 2015. Of Paleo-Genes and Perch: What if an
1183 'Alien' Is Actually a Native? *PLOS ONE* **10** (3): e0119071

1184 Stewart JR, Lister AM, Barnes I, Dalén L. 2010. Refugia revisited: individualistic responses of
1185 species in space and time. *Proceedings of the Royal Society B*: *Biological Sciences* **277**: 661–
1186 671.

1187 Stoof-Leichsenring KR, Epp LS, Trauth MH, Tiedelman R. 2012. Hidden diversity in diatoms of
1188 Kenyan Lake Naivasha: a genetic approach detects temporal variation. *Molecular Ecology* **21**:
1189 1918–1930.

1190 Stoof-Leichsenring KR, Herzschuh U, Pestryakova LA, Klemm J, Epp LS, Tiedelman R. 2015.
1191 Genetic data from algae sedimentary DNA reflect the influence of environment over
1192 geography. *Scientific Reports* **5**: 12924.

1193 Stull GW, Moore MJ, Mandala VS, Douglas NA, Kates H-R, Qi X, Brockington SF, Soltis PS, Soltis
1194 DE, Gitzendanner MA. 2013. A targeted enrichment strategy for massively parallel sequencing
1195 of angiosperm plastid genomes. Applications in Plant Sciences **1**: 1200497.

1196 Sunagawa S, Mende DR, Zeller G, Izquierdo-Carrasco F, Berger SA, Kultima JR, Coelho LP,
1197 Arumugam M, Tap J, Nielsen HB, *et al.* 2013. Metagenomic species profiling using universal
1198 phylogenetic marker genes. *Nature Methods* **10**: 1196–1199.

1199 Suyama Y, Matsuki Y. 2015. MIG-seq: an effective PCR-based method for genome-wide single-
1200 nucleotide polymorphism genotyping using the next-generation sequencing platform.
1201 *Scientific Reports* **5**: 16963.

1202 Suyama Y, Kawamuro K, Kinoshita I, Yoshimura K, Tsumura Y, Takahara H. 1996. DNA sequence
1203 from a fossil pollen of *Abies* spp. from Pleistocene peat. *Genes & Genetic Systems* **71**: 145–
1204 149.

1205 Sønstebø JH, Gielly L, Brysting AK, Elven R, Edwards M, Haile J, Willerslev E, Coissac E, Rioux D,
1206 Sannier J, *et al.* 2010. Using next-generation sequencing for molecular reconstruction of past
1207 Arctic vegetation and climate. *Molecular Ecology Resources* **10**: 1009–1018.

1208 Taberlet P, Coissac E, Hajibabaei M, Rieseberg LH. 2012a. Environmental DNA. *Molecular*
1209 *Ecology* **21**: 1789–1793.

1210 Taberlet P, Coissac E, Pompanon F, Brochmann C, Willerslev E. 2012b. Towards next-
1211 generation biodiversity assessment using DNA metabarcoding. *Molecular Ecology* **21**: 2045–
1212 2050.

1213 Taberlet P, Coissac E, Pompanon F, Gielly L, Miquel C, Valentini A, Vermat T, Corthier G,
1214 Brochmann C, Willerslev E. 2007. Power and limitations of the chloroplast *trn*L (UAA) intron
1215 for plant DNA barcoding. *Nucleic Acids Research* **35**: e14.

1216 Thomsen PF, Willerslev E. 2015. Environmental DNA–an emerging tool in conservation for
1217 monitoring past and present biodiversity. *Biological Conservation* **183**: 4–18.

1218 Valentini A, Pompanon F, Taberlet P. 2009. DNA barcoding for ecologists. *Theoretical and*
1219 *Applied Genetics* **24**: 110–117.

Vos M, Wolf AB, Jennings SJ, Kowalchuk GA. 2013. Micro-scale determinants of bacterial diversity in soil. *FEMS Microbiology Reviews* **37**: 936–954.

Vuillemin A, Ariztegui D, Leavitt PR, Bunting L, the PASADO Science Team. 2016. Recording of climate and diagenesis through sedimentary DNA and fossil pigments at Laguna Potrok Aike, Argentina. *Biogeosciences* **13**: 2475–2492.

Wang Y, Navin NE. 2015. Advances and applications of single-cell sequencing technologies. *Molecular Cell* **58**: 598–609.

Warinner C, Speller C, Collins MJ. 2015. A new era in palaeomicrobiology: prospects for ancient dental calculus as a long-term record of the human oral microbiome. P*hilosophical Transactions of the Royal Society B: Biological Sciences* **370**: 20130376.

Weiß CL, Dannemann M, Prufer K, Burbano HA, Pickrell JK. 2015. Contesting the presence of wheat in the British Isles 8,000 years ago by assessing ancient DNA authenticity from low-coverage data. *eLife* **4**: e10005.

Wilke A, Bischof J, Harrison T, Brettin T, D'Souza M, Gerlach W, Matthews H, Paczian T, Wilkening J, Glass EM, *et al.* 2015. A RESTful API for accessing microbial community data for MG-RAST. *PLoS Computational Biology* **11**: e1004008.

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

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

Willerslev E, Hansen AJ, Binladen J, Brand TB, Gilbert MTP, Shapiro B, Bunce M, Wiuf C, Gilichinsky DA, Cooper A. 2003. Diverse plant and animal genetic records from Holocene and Pleistocene sediments. Science **300**: 791–795.

Willerslev E, Hansen AJ, Christensen B, Steffensen JP, Arctander P. 1999. Diversity of Holocene life forms in fossil glacier ice. *Proceedings of the National Academy of Sciences US*A 96: 8017–8021.

Wetzel, R. G. 2001. Limnology. Lake and River Ecosystems, 3rd ed., Academic press, San Diego.

Wood DE, Salzberg SL. 2014. Kraken: ultrafast metagenomic sequence classification using exact alignments. *Genome Biology* **15: R46.**

Wooller MJ, Gaglioti B, Fulton TL, Lopez A, Shapiro B. 2015. Post-glacial dispersal patterns of Northern pike inferred from an 8800 year old pike (*Esox* cf. *lucius*) skull from interior Alaska. *Quaternary Science Reviews* **120**: 118–125.

Wood JR, Wilmshurst JM. 2016. A protocol for subsampling Late Quaternary coprolites for multi-proxy analysis. *Quaternary Science Reviews* **138**: 1–5.

Wright HE, Mann DH, Glaser PH. 1984. Piston Corers for Peat and Lake Sediments. *Ecology* **65**: 657–659.

Xiong J, Liu Y, Lin X, Zhang H, Zeng J, Hou J, Yang Y, Yao T, Knight R, Chu H. 2012. Geographic distance and pH drive bacterial distribution in alkaline lake sediments across Tibetan Plateau. *Environmental Microbiology* **14**: 2457–2466.

Xu Z-H, Jiang X-D, Wang G-Z, He J-F, Cai M-H, Wu L-S, Jiang J-L, Chen X-L. 2011. DNA extraction, amplification and analysis of the 28S rRNA portion in sediment-buried copepod DNA in the Great Wall Bay and Xihu Lake, Antarctica. *Journal of Plankton Research* **33**: 917–925.

Yang J, Jiang H, Dong H, Hou W, Li G, Wu G. 2015. Sedimentary archaeal amoA gene abundance reflects historic nutrient level and salinity fluctuations in Qinghai Lake, Tibetan Plateau. *Scientific Reports* **5**: 1–9.

Yoccoz NG, Bråthen KA, Gielly L, Haile J, Edwards ME, Goslar T, Stedingk Von H, Brysting AK, Coissac E, Pompanon F, *et al.* 2012. DNA from soil mirrors plant taxonomic and growth form diversity. *Molecular Ecology* **21**: 3647–3655.

Yoccoz N. 2012. The future of environmental DNA in ecology. *Molecular Ecology* **21**:

2031–2038.

Zhang Q, Liu Y. 2003. Examination of the cytoplasmic DNA in male reproductive cells to determine the potential for cytoplasmic inheritance in 295 angiosperm species. *Plant and Cell Physiology* **44**: 1–11.

Ziesemer KA, Mann AE, Sankaranarayanan K, Schroeder H, Ozga AT, Brandt BW, Zaura E, Waters-Rist A, Hoogland M, Salazar-García DC, et al. 2015. Intrinsic challenges in ancient microbiome reconstruction using 16S rRNA gene amplification.

Supporting Information

Additional Information may be found in the online version of this article

Note S1

Additional information on bioinformatic tools for metabarcoding datasets relevant to this article.

Figure legends

Figure 1.

Number of publications retrieved from Web of Science data bank in August 2016, using 'ancient DNA' as search term, categorized according to the study organisms.

Figure 2.

Biotic palaeoenvironmental proxies in lake sediments. **a**, sequential environmental development for a temperate region, in which the lake sediments start accumulating as glacial ice retreats, incorporating glacially eroded debris and the sparse pioneering biota (1), which later is replaced by a tundra-steppe community (2), then the boreal forest establishes (3) before eventually being replaced by a temperate forest (4). **b**, by identifying organisms detectable by DNA, macro- and microfossils and accumulated and preserved in the lake sediments, **c**, it is possible to reconstruct the environments through time. It is important to notice that preservation or rate of degradation is strongly correlated with the age of the sediments and that the input concentration **d**, varies in different climatic environments from these three proxies. **e**, In addition the resulting DNA profile, as well as macro and microfossils is influenced by taphonomic processes such as differences in biomass production and the distance from source to deposit. This is why a combination of all these three proxies makes a more robust palaeoenvironmental reconstruction. kyr BP: thousand years before present.

Figure 3.

Spatial distribution of larger lakes (blue squares) in the world (source: Natural Earth). The map shows that lakes are widely distributed geographically and present in many different environments. Photos exemplify different types of lake environments, (1) Lake Comarum, South Greenland. (2) Fiskvatn, Troms, Norway. (3) Olive-backed lake, Kamchatka. (4) Lake Milluni, Bolivia. (5) Buyan, Bali. (6) Lake Waikaremoana, North Island, New Zealand.

Figure 4.

A full bodysuit, shoe cover, hairnet, facemask, hood, gloves, and sleeve guards are necessary during DNA extraction from a sediment core for aDNA analyses in an aDNA lab (a). During

subsampling in a clean lab, surface contamination from the sediment core is removed with sterilized razors (b), and non-contaminated material from within the intact cores is extracted for DNA extraction (c).

Figure 5.

Schematic illustration of the changes in the organellar DNA from the four types of generative cells present in pollen. When chloroplast (p) or mitochondrial (m) DNA is present in the mature generative pollen cells (m+ or p+), the DNA content per organelle increases after pollen mitosis one (PMI) and during developmental stages from uni-nucleate to bi- (or tri-) cellular pollen (stages 1-5)and it decreases when organellar DNA is absent (m- or p-). All the changes in organellar DNA in the generative cells, whether an increase or a decrease, occur just after PMI, and the mitochondrial DNA and plastid DNA contents are regulated independently in the vegetative cells. An increase in the organellar DNA occurs with biparental/paternal inheritance (B/P) of the corresponding organelle, while a decrease occurs with maternal inheritance (M) of the corresponding organelle. Modified from Nagata *et al*. (1999).

Figure 6.

Venn diagrams showing the proportion of plant taxa commonly detected by different proxies: metabarcoding and shotgun metabarcoding of *sed*aDNA (blue), vegetation survey (light green), pollen (yellow) and macrofossils (dark green). These proportions have so far varied among different studies and the lower panel shows what we can theoretically expect with shotgun metabarcoding when a full genome reference library is developed.

Figure 7.

Representation of the chain of processes involved in the transformation of plant information present in the three lake sediment assemblages types: pollen (P), macrofossils (M), ancient DNA (aDNA). Current understanding of the processes is indicated as good (+++), reasonable (++) or poor (+). The publications upon which the levels of understanding are based are shown in Table 1. Modified from Birks & Birks (2016) and based on Jackson's (2012) general conceptual model for the representation of floristic material in palaeoecological assemblages.

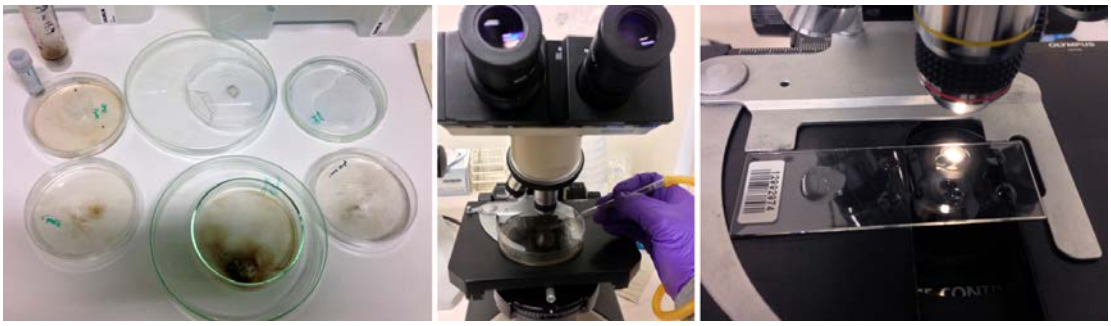
Box 1.

Isolating single pollen grains for direct PCR

1) Sieving sediment. Place a small amount (ca. 0.2-0.5 ml) of sediment onto a sterile filter with mesh size slightly larger than the target pollen grains. Add water and stir sediment with a small spatula. Wash sediment through the filter into a petri dish. Top up the petri dish with water.

2) Isolating pollen grains. Dilute sample so grains are adequately spaced in the petri dish, and scan at 100-200x magnification (depending on size of pollen). Once a target grain has been found, switch to a lower magnification, ensuring the grain can still be seen in the field of view. Place the tip of a glass pipette into the field of view, and slowly lower it down into the water beside the pollen grain. Capillary action will ensue. Capture the pollen grain as quickly as possible and remove pipette from the water. Transfer water from the pipette onto a microscope slide. Check the droplet at 200-400x magnification to ensure the correct pollen grain is present. For larger pollen grains and plant fragments, a 1-10 µl pipette can be used to isolate the specimen.

3) Dilution. Use a 1-10 µl pipette to add several droplets of water to the microscope slide. Capture the pollen in a glass pipette, avoiding as much debris as possible. Transfer the water from the pipette into a clean water droplet. Check to ensure the pollen grain is still present. Repeat this process until the pollen grain is isolated (i.e. no debris is transferred with the grain). For the final step, capture the grain in the glass pipette, and transfer to a PCR tube. The remainder of the final droplet can be transferred to another PCR tube as a PCR control for that pollen grain.



1377 Left to right: Petri dishes containing filtered sediment samples; using a glass pasteur pipette
1378 to isolate pollen grain from petri dish; microscope slide with water droplets for sequential
1379 dilution of pollen grain.
1380

1381 Table 1.

1382 Ancient DNA related publications from lake sediments sorted by year. Studies have been
1383 organized according to sample type, method used for DNA extraction [environmental DNA
1384 (eDNA) or DNA isolated from tissues] and research environment investigated.

1385

Author	Sample type	Method	Region	Environment
Coolen & Overmann (1998)	bacteria	eDNA	Mahoney Lake, British Columbia, Canada	temperate, low altitude
Limburg & Weider (2002)	daphnia	isolation	Belauer See Lake, Germany	temperate, low altitude
Reid *et al.* (2002)	daphnia	isolation	English Lake District, UK	temperate, low altitude
Pollard *et al.* (2003)	daphnia	isolation	Hannah Lake, Sudbury, Ontario, Canada	temperate, low altitude
Coolen *et al.* (2004)	diatoms	eDNA	Ace Lake, eastern Antarctica	antarctic, low altitude
Bissett *et al.* (2005)	copepods	isolation	Lake Terrasovoje, Antarctica	antarctic, low altitude
Parducci *et al.* (2005)	pollen	isolation	Holtjärnen, central Sweden	temperate, low altitude
D'Andrea *et al.* (2006)	algae	filtration> eDNA	Søndre Strømfjord, western Greenland	arctic, low altitude
Marková *et al.* (2006)	daphnia	isolation	Lakes on Tatra Mts, Carpathians, Hungary	temperate, high altitude
Jiang *et al.* (2007)	bacteria	eDNA	Lake Chaka, Tibetan plateau	temperate, high altitude
Mergeay *et al.* (2007)	daphnia	isolation	Lake Naivasha, Kenya	temperate, low altitude

Matisoo-Smith *et al.* (2008)	plants and metazoan	eDNA	Round Lake, New Zealand	temperate, low altitude
Coolen *et al.* (2008)	bacteria	eDNA	Ace Lake, Antarctica	antarctic low altitude
Madeja *et al.* (2009)	bacteria> human	eDNA	Great Mazurian Lake District, NE Poland	temperate, low altitude
Kojima *et al.* (2009)	bacteria	filtration> eDNA	Lake Mizugaki, Central Japan	temperate, low altitude
Epp *et al.* (2010)	rotifers	eDNA	Lake Sonachi, Rift Valley, Kenya	temperate, high altitude
Madeja *et al.* (2010)	bacteria> human	eDNA	Lake Miłkowskie, north-eastern Poland	temperate, low altitude
Anderson-Carpenter *et al*. (2011)	plants	isolation	Great Lakes, North America	temperate, low altitude
Savichtcheva *et al.* (2011)	bacteria	eDNA + isolataion	Lake Geneva, Bourget, Annecy, French Alps	temperate, low altitude
Xu *et al.* (2011)	bacteria	eDNA	Great Wall Bay, Xihu Lake, Antarctica	antarctic low altitude
Epp *et al.* (2011)	diatom	eDNA	Lake Naivasha, Kenya	tropical, high altitude
Magyari *et al.* (2011)	plants	isolation	Retezat Mountains, South Carpathians, Hungary	temperate, high altitude
Parducci *et al.* (2012)	plants	eDNA	Central Norway and Sweden	temperate, low altitude
Stoof-Leichsenring *et al.* (2102)	diatoms	eDNA	Kenya, Lake Naivasha	tropical, high altitude
Xiong *et al.* (2012)	bacteria	eDNA	Tibetan Plateau	temperate, high altitude
Ravasi *et al.* (2012)	bacteria	eDNA?	Lake Cadagno, Swiss Alps	temperate, high altitude

Pedersen *et al.* (2013)	plants	eDNA	Southern Greenland	arctic, low altitude
Parducci *et al.* (2013)	plants	eDNA	Central Norway and Sweden	temperate, low altitude
Domaizon *et al.* (2013)	bacteria	eDNA	Lake Bourget, French Alps	temperate, low altitude
Giguet-Covex *et al.* (2014)	plants and metazoan	eDNA	Lake Anterne, French Alps	temperate, high altitude
Boessenkool *et al.* (2013)	plants	eDNA	Mt Gahinga, eastern Africa	temperate, high altitude
Belle *et al.* (2014)	bacteria	eDNA	Lake Narlay, Jura Mountains, France	temperate, low altitude
Hou *et al.* (2014)	plankton	eDNA	Tibetan Plateau	temperate, high altitude
Randlett *et al.* (2014)	phytoplankton	eDNA	Lake Van, Turkey	temperate, high altitude
Madeja *et al.* (2015)	bacteria> human	eDNA	Great Mazurian Lake District, Poland	temperate, low altitude
Kyle *et al.* (2015)	bacteria	eDNA	Bjørkelangen, Gjersjøen, Hemnessjø lakes, Norway	temperate, low altitude
Pal *et al.* (2015)	bacteria	eDNA	Gatineau Park lakes, western Quebec	temperate, low altitude
Pansu *et al.* (2015)	plants	eDNA	Lake Anterne, French Alps	temperate, high altitude
Paus *et al.* (2015)	plants	eDNA	Dovre region, central Norway	temperate, low altitude
Stoof-Leichsenring *et al.* (2015)	algae	eDNA	North Siberia	arctic, low altitude
Epp *et al.* (2015)	algae vascular plants	eDNA	North Greenland	arctic, low altitude

Capo *et al.* (2015)	bacteria	eDNA	Lake Bourget, French Alps	temperate, low altitude
Yang *et al.* (2015)	bacteria	eDNA	Qinghai Lake, Tibetan Plateau	temperate, high altitude
Poulain *et al.* (2015)	bacteria	eDNA	Hudson Bay, Ontario, Canada,	arctic, low altitude
Wooller *et al.* (2015)	fish	isolation	Quartz Lake, Alaska	arctic, low altitude
Etienne *et al.* (2015)	bacteria> human	eDNA	Lorraine Plateau, north-eastern France	temperate, low altitude
Stager *et al.* (2015)	fish	eDNA	Lower Saint Regis Lake, Franklin County, NY, US	temperate, low altitude
Li *et al.* (2016)	phytoplankton	eDNA	Qinghai Lake, Tibetan Plateau	temperate, high altitude
Vuillemin *et al.* (2016)	bacteria >taphonomy	eDNA	Laguna Potrok Aike, Argentina	temperate, low altitude
Alsos Greve *et al.* (2016)	plants	eDNA	Lake Skartjørna, Svalbard	arctic, low altitude
Pedersen *et al.* (2016)	plants	eDNA	Peace River, Alberta and British Columbia, Canada	temperate, low altitude
Sjögren *et al.* (2016)	plants	eDNA	Spectacle Loch, Loch of the Lowes, Scotland, UK	temperate, low altitude

1386