



Sedimentary ancient DNA reveals past ecosystem and biodiversity changes on the Tibetan Plateau: Overview and prospects

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ABSTRACT

Alpine ecosystems on the Tibetan Plateau are being threatened by ongoing climate warming and intensified human activities. Ecological time-series obtained from sedimentary ancient DNA (sedaDNA) are essential for understanding past ecosystem and biodiversity dynamics on the Tibetan Plateau and their responses to climate change at a high taxonomic resolution. Hitherto only few but promising studies have been published on this topic. The potential and limitations of using sedaDNA on the Tibetan Plateau are not fully understood. Here, we (i) provide updated knowledge of and a brief introduction to the suitable archives, region-specific taphonomy, state-of-the-art methodologies, and research questions of sedaDNA on the Tibetan Plateau; (ii) review published and ongoing sedaDNA studies from the Tibetan Plateau; and (iii) give some recommendations for future sedaDNA study designs. Based on the current knowledge of taphonomy, we infer that deep glacial lakes with freshwater and high clay sediment input, such as those from the southern and southeastern Tibetan Plateau, may have a high potential for sedaDNA studies. Metabarcoding (for microorganisms and plants), metagenomics (for ecosystems), and hybridization capture (for prehistoric humans) are three primary sedaDNA approaches which have been successfully applied on the Tibetan Plateau, but their power is still limited by several technical issues, such as PCR bias and incompleteness of taxonomic reference databases. Setting up high-quality and open-access regional taxonomic reference databases for the Tibetan Plateau should be given priority in the future. To conclude, the archival, taphonomic, and methodological conditions of the Tibetan Plateau are favorable for performing sedaDNA studies. More research should be encouraged to address questions

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about long-term ecological dynamics at ecosystem scale and to bring the paleoecology of the Tibetan Plateau into a new era.

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1. Introduction

The Tibetan Plateau and its surrounding mountains (Fig. 1), with an average elevation exceeding 4000 m above sea level, represent a highly complex system where climatic, ecological, and socio-cultural dynamics interact strongly (Qiu, 2008; Royden et al., 2008; Zheng et al., 2000). The uplift of the plateau markedly changed the climate system, topography, species diversification, and biodiversity of eastern Asia, creating a unique geographical unit and alpine ecosystem in the world (Chen et al., 2021; Favre et al., 2015; Li et al., 2021). Ongoing climate warming is expected to cause (especially endemic) species loss on the Tibetan Plateau and poses a growing risk to its alpine ecosystems (Hopping et al., 2018; Klein et al., 2004; Lu et al., 2020; Piao et al., 2019). In conjunction with temperature changes, increasing precipitation, glacier melt, and permafrost thaw will shift hydrological cycles and terrestrial vegetation patterns (Zhong et al., 2019), thereby increasing the input of nutrients and dissolved organic matter into alpine lakes, which can alter their carbon cycling pathways, and strongly threaten aquatic biodiversity and the metabolic balance of lake ecosystems (Brothers et al., 2014; Woolway et al., 2020). In addition, intensified human activities (e.g., population explosion, land-cover/land-use changes, grazing activities) on the Tibetan Plateau have also exerted a growing pressure on alpine ecosystems over the last decades (Chen et al., 2013; Li et al., 2018; Liu et al., 2021a; Miede et al., 2014), but we do not yet know much about the interplay of humans and long-term ecological processes. Therefore,

it is important to understand how prehistoric humans migrated and adapted to the cold and hypoxic environment of the Tibetan Plateau and how they relate to past ecosystem changes, which has become an increasingly interdisciplinary research hotspot (Zhang et al., 2016).

Understanding the interactions between climate, the biosphere, and humans and the respective time scales of the interactions are prerequisites to securing alpine ecosystem functions and services (Pecl et al., 2017). However, observational studies reporting ecological turnover in response to climate and land-use changes, for example through satellite remote sensing or field survey data, remain scarce, which may originate from the time-lagged responses of biological variables to climate change (Alexander et al., 2018; Herzs Schuh et al., 2016; Svenning and Sandel, 2013; Williams et al., 2021). This highlights the necessity to set up and analyze long ecological time-series as can be obtained from natural environmental archives, such as lake sediments and permafrost deposits (Willis and Birks, 2006).

Traditional analyses of past biota are, to some extent, limited by biological remains or non-genetic molecules stored in the stratigraphic layers or sediments. Many fossil proxy records such as pollen, macrofossils, diatoms, ostracods, chironomids, and pigments have been widely used to track past community changes (Shen et al., 2020), but these morphology-based proxies are generally not identifiable to a high taxonomic level (e.g., species level) and exclude those taxa that do not leave well-preserved and recognizable fossil remains in the sediment (e.g., fish, mammals,

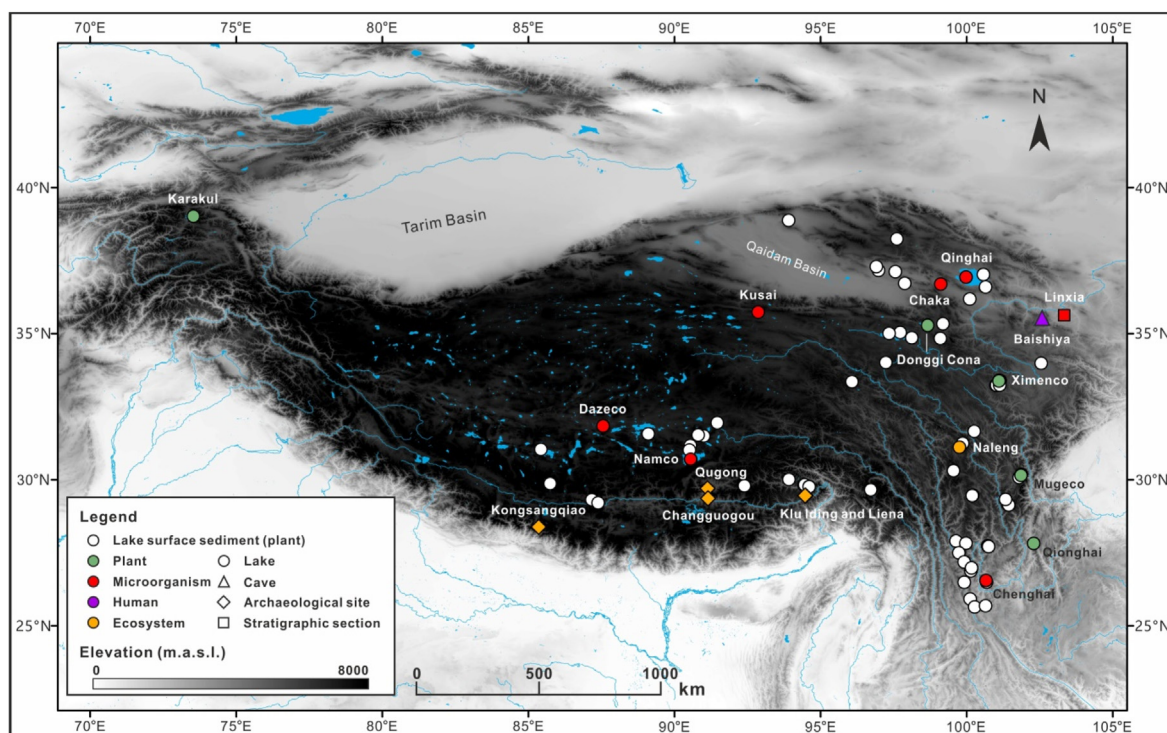


Fig. 1. Spatial distribution of lake surface sediments (Jia et al., 2022) and sedimentary ancient DNA records from the Tibetan Plateau mentioned in this paper.

bacteria). Pollen taxa such as *Potentilla*, *Rumex*, Poaceae, Fabaceae, Apiaceae, and Chenopodiaceae have been used, for example, as indicators of human activity on the Tibetan Plateau during the late Holocene (Li et al., 2019; Ma et al., 2020; Wischniewski et al., 2011, 2014). However, the low taxonomic resolution of the fossil pollen makes it difficult to disentangle human impacts from those of climate change, thus causing high ambiguity in data interpretation. This underlines the importance of having high-resolution proxies in paleoecological research.

Over the last two decades, the rapid development of sedimentary ancient DNA (sedaDNA) technologies has provided a new opportunity to unveil past ecosystem and biodiversity changes (Pedersen et al., 2015; Thomsen and Willerslev, 2015; Capo et al., 2021). SedaDNA refers to ancient environmental DNA that can be directly extracted from ancient sediment samples, without first isolating any target organisms, which is typically more degraded and fragmented than that from modern samples (Hofreiter et al., 2001; Taberlet et al., 2012a; Willerslev and Cooper, 2005). This approach already shows great potential for reconstructing past ecosystem changes across a full range of taxa such as plants, animals, fungi, and microorganisms at a high taxonomic resolution (Deiner et al., 2017; Pedersen et al., 2016; Wang et al., 2021; Willerslev et al., 2003, 2014). This field is witnessing exponential growth since the commercial launch of high-throughput sequencing technologies in 2005 (Taberlet et al., 2018). However, as an emerging proxy, the application of sedaDNA is currently limited in paleoecological studies on the Tibetan Plateau. Most studies have focused only on microorganisms (e.g., Dong et al., 2010; Lake Kusai, Hou et al., 2014; Lake Chaka, Jiang et al., 2007; Lake Dazeco, Liu et al., 2021c; Linxia Basin, Xu et al., 2022; Lake Qinghai, Yang et al., 2015, Fig. 1), while largely ignoring other biological groups.

In this paper, we provide updated knowledge of and a brief introduction to the suitable archives, region-specific taphonomy, as well as state-of-the-art methodologies of sedaDNA studies on the Tibetan Plateau. Furthermore, we present an overview of published and ongoing sedaDNA studies to date. Finally, we provide some outlook on future applications and developments of sedaDNA on the Tibetan Plateau.

2. Archives and taphonomy of sedaDNA

Ancient environmental DNA can potentially be recovered from a variety of archives on the Tibetan Plateau including lake sediments, permafrost deposits, ice cores, peats, cave sediments, and soils. Of these, lake sediments are very promising as they accumulate continuously and contain both autochthonous (produced within the lake) and allochthonous (originated from the catchment) organic matter. Moreover, lakes with sufficient depth to exhibit thermal stratification usually create a low-temperature (about 4 °C), low-oxygen, and low-level ultraviolet (UV) radiation environment at the water-sediment interface, which limits bio-turbation, sediment reworking, and microbial activity and thus facilitates DNA preservation (Giguët-Covex et al., 2019; Pansu et al., 2015; Parducci et al., 2017, 2019b). Leaching of DNA through stratigraphic layers by water has also been observed in some other archives such as cave sediments and soils (Andersen et al., 2011; Haile et al., 2007), but this phenomenon has not yet been reported from lake sediments, because they are frequently saturated with water (Anderson-Carpenter et al., 2011; Giguët-Covex et al., 2014; Sjögren et al., 2017). With 1424 lakes greater than 1 km² and a total lake area of $5 \times 10^4 \pm 791.4$ km² (Liu et al., 2021b), accounting for 57.2% of China's lake area (Zhang et al., 2020b), the Tibetan Plateau has the largest group of high-elevation lakes in the world (Wang and Dou, 1998). Thus, lake sediments seem to be promising and

abundant archives to unlock sedaDNA's secrets on the Tibetan Plateau.

We still have a poor understanding of the taphonomy of lake sedaDNA including all processes that influence its production, incorporation, transportation, deposition, and preservation (Birks and Birks, 2016; Capo et al., 2021; Giguët-Covex et al., 2019; Jia et al., 2022, Fig. 2). Environmental DNA in natural archives includes intracellular DNA, which belongs to organisms or cells, and extracellular DNA, which is released into the surrounding environment by the degradation of biological tissues and the lysis of cells (Pawlowski et al., 2020; Taberlet et al., 2018). Because the majority of cells are lysed rapidly in the environment (Nielsen et al., 2007), extracellular DNA is considered the main component of total sedaDNA (Dell'Anno and Danovaro, 2005; Parducci et al., 2017; Parducci et al., 2019b).

Environmental DNA production is a function of biotic (e.g., biomass, species type, life stage, diet, microbial activity) and abiotic (e.g., temperature, pH, stress) factors (Barnes and Turner, 2016; Rourke et al., 2022; Stewart, 2019). For example, Maruyama et al. (2014) report that juvenile bluegill sunfish from Lake Biwa release DNA at a higher rate than adults. A recent study on freshwater fish shows that temperature has a positive effect on DNA production, and the quantity of fish DNA at 16 °C is significantly higher than at 8 °C and 24 °C (probably related to high DNA degradation rate at 24 °C; Caza-Allard et al., 2021). After having been released into the environment, extracellular DNA molecules can be (i) enzymatically hydrolyzed by bacterial and fungal DNases, (ii) genetically transformed by microorganisms, and (iii) incorporated into soils or sediments by adsorbing to inorganic or organic surface reactive particles such as clay minerals and humic substances (Pedersen et al., 2015, Fig. 2). In particular, the adsorption process can inactivate DNases and reduce natural transformation frequency (Demanèche et al., 2001; Khanna and Stotzky, 1992; Nagler et al., 2018; Romanowski et al., 1991; Stotzky, 2000), which appears to be the primary mechanism for the long-term preservation of extracellular DNA in the environment (Blum et al., 1997; Kanbar et al., 2020; Ogram et al., 1988; Pietramellara et al., 2009). The adsorption capacity of DNA on soil and sediment particles has been experimentally studied as a function of several environmental variables, such as the pH and ionic strength of the medium, the mineralogy and grain size of the sorbent, and DNA fragment length (Cai et al., 2006; Freeman et al., 2020; Greaves and Wilson, 1969; Harrison et al., 2019; Kanbar et al., 2020; Levy-Booth et al., 2007; Lorenz and Wackernagel, 1987; Ogram et al., 1988; Pedreira-Segade et al., 2018; Romanowski et al., 1991; Yu et al., 2013).

Subsequently, incorporated or 'free' DNA molecules are physically transported from the catchment areas into the lake through groundwater or air (Fig. 2), and the rate and efficiency of this process is co-influenced by several environmental variables, such as topographic relief, erosion rates, hydrological connectivity, transport distances, and species distributions (Giguët-Covex et al., 2019). For example, the fast decay of fish DNA with increasing distance downstream is recently reported in two Amazonian rivers (Cantera et al., 2021), suggesting that DNA may degrade very quickly in the water and cannot be transported over long distances by natural rivers (Harrison et al., 2019). Nevertheless, a high similarity of fish species is found within 50–100 km downstream of the rivers (Cantera et al., 2021), implying that upstream DNA is likely to be transported into lakes with short runoff-transport distances in the catchment. In addition, DNA from taxa growing along the rivers and lakeshores may have a good chance of being transported into the lake, as exemplified by the overrepresentation of *Salix* and *Populus* DNA in lake surface sediments from the Tibetan Plateau and arid northwestern China (Jia, 2020; Li et al., 2021). With diverse landscapes and well-developed hydrographical networks in the

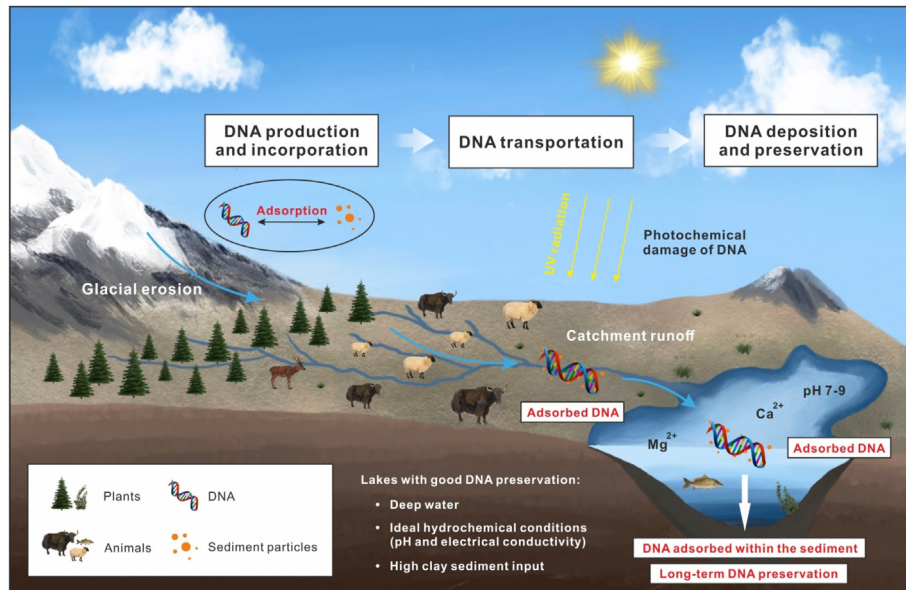


Fig. 2. Conceptual model of the taphonomic processes of extracellular DNA from lake sediments. After having been released into the environment, extracellular DNA can be incorporated into soil or sediment particles by adsorbing to, for example, clay minerals and humic substances, which is the primary mechanism responsible for long-term DNA preservation. Adsorbed DNA can subsequently be transported into the lake through catchment runoff. Most large lakes on the Tibetan Plateau are expected to have high upstream DNA inputs because of diverse landscapes and well-developed hydrographical networks in their catchments. However, high-level ultraviolet (UV) radiation on the Tibetan Plateau might photochemically damage DNA over long transport distances. During the deposition stage, terrestrial adsorbed DNA is expected to be well preserved in deep lakes with neutral to slightly alkaline water pH (7–9), intermediate water conductivities (100–500 $\mu\text{S cm}^{-1}$; suitable cation concentrations), and high clay sediment input. On the other hand, DNA from aquatic organisms (e.g., fish and macrophytes) might still be preserved in many Tibetan lakes characterized by high water pH (≥ 9) and conductivity ($> 1000 \mu\text{S cm}^{-1}$). After sediment particles are finally deposited and buried in the lake bottom, DNA is adsorbed within the sediment and stored in a cold and anoxic environment that limits bioturbation, sediment reworking, and microbial activity, which favors long-term DNA preservation. To conclude, we infer that some deep glacial lakes with freshwater and high clay sediment input, such as those from the southern and southeastern Tibetan Plateau, may have a high potential for sedimentary ancient DNA studies in the future.

catchments (Liu et al., 2022), most large lakes on the Tibetan Plateau are expected to have more upstream inputs compared with small lakes (or ponds) in the Arctic region that only receive inflow from limited streams (e.g., Alsos et al., 2018; Niemeyer et al., 2017). This means that their sedaDNA records not only give a local signal, but also have the potential to provide comprehensive information on species assemblages at the catchment scale (Clarke et al., 2018; Giguet-Covex et al., 2019). However, it should be noted that high-level UV radiation on the Tibetan Plateau (Peng et al., 2015) might photochemically damage DNA molecules over long transport distances (Jia et al., 2022).

Chemical characteristics of the lake water may be the main factors influencing the preservation of sedimentary DNA molecules before they reach the water-sediment interface (Fig. 2). Based on the metabarcoding data of 219 lakes from China (mainly from the Tibetan Plateau) and Siberia, Jia et al. (2022) report that plant DNA from surface sediments is well preserved in lakes with intermediate water conductivities (100–500 $\mu\text{S cm}^{-1}$) and neutral to slightly alkaline water pH (7–9). Air temperature is not a key factor in limiting plant DNA preservation. The underlying reason behind this is that such hydrochemical settings are suitable for the adsorption of extracellular DNA molecules to sediment particles. At pH < 5, the phosphate moieties of DNA have a net positive charge, which is opposite to the surface charge of most clay minerals and humic substances and therefore favors DNA adsorption (Greaves and Wilson, 1969), but acidic conditions catalyze hydrolytic processes and accelerate DNA degradation (Giguet-Covex et al., 2019; Lindahl, 1993; Lindahl and Nyberg, 1972; Seymour et al., 2018). Above pH 5, DNA has a net negative charge, and thus requires more cations to form bridges between the phosphate moieties of DNA and particle surfaces to enhance adsorption capacity. However, the efficiency of adsorption will be highly reduced at pH ≥ 9 , where DNA can be

desorbed almost completely from the negatively charged surface of clay minerals (Cai et al., 2006; Greaves and Wilson, 1969; Khanna and Stotzky, 1992; Levy-Booth et al., 2007; Ogram et al., 1988; Romanowski et al., 1991) even if their surface cations are saturated (Sheng et al., 2019). For example, plant DNA is poorly preserved in some lakes located in the central Tibetan Plateau with water pH > 9.5 (e.g., Lake Pengco, Lake Bamuco, Lake Shengco, and Lake Angrenjinco; Jia et al., 2022), which has also been evidenced by diatom DNA records from the same lakes (X. Mao, unpublished data). In this case, desorbed DNA will be free within the water column and vulnerable to DNase attack. Aside from water chemistry, the grain size and mineralogy of sediment particles also ought to be considered for DNA adsorption. For example, the capacity of sand to adsorb DNA is at least three orders of magnitude less than that of clay because of its smaller surface area (Levy-Booth et al., 2007; Romanowski et al., 1991). Clay minerals are the main minerals to adsorb DNA, but the capacity of different clay minerals (e.g., montmorillonite, illite, kaolinite, chlorite, and mica) to bind DNA is variable (Kanbar et al., 2020; Pietramellara et al., 2001; Poly et al., 2000).

After sediment particles are finally deposited and buried in the lake bottom, DNA molecules become theoretically stored in a cold and anoxic environment that limits bioturbation, sediment reworking, and microbial activity and facilitates long-term DNA preservation. However, many previous studies have reported strong ancient DNA degradation over burial time (Anderson-Carpenter et al., 2011; Crump et al., 2021; Dommain et al., 2020; Hofreiter et al., 2001, 2014; Pedersen et al., 2016), and this has also been observed in some Tibetan lakes in recent years (W. Jia, unpublished data). Although different perspectives have been proposed (Giguet-Covex et al., 2019; Parducci et al., 2019a), most lake sedaDNA studies so far have only been able to reach the Late

Pleistocene (<30 ka BP) and Holocene (Capo et al., 2021; Parducci et al., 2017; Rawlence et al., 2014), and only a few can reach >100 ka BP (Crump et al., 2021; Ekram et al., 2021). Thus, more systematic studies are still needed to understand the preservation mechanism of DNA molecules in ancient sediments and to try to extend the time window of this technique in the future.

It should be noted that there is potential taxonomic variability in sedaDNA preservation mechanisms (Boere et al., 2011). For example, some microorganisms can be dormant and able to remain viable in sediments for hundreds to thousands of years under suitable environmental conditions, thus posing a challenge in distinguishing between extracellular (ancient) and intracellular (modern) DNA signals (Capo et al., 2022). For aquatic organisms, their DNA might have a better chance of being preserved than those of terrestrial organisms. Without the runoff-transport process, their biological tissues and cells may be deposited at the lake bottom and buried by sediment particles, thus releasing their DNA directly into the sediment (Parducci et al., 2019b). For example, Heinecke et al. (2017) successfully extracted PCR-amplifiable aquatic plant DNA from a long sediment core of Lake Karakul (Tajikistan) with modern water pH 9.2 and conductivity $10,300 \mu\text{S cm}^{-1}$, while terrestrial plant taxa were rarely amplified. A recent study suggests that diatom DNA can be well preserved in a sediment core of Lake Namco, where modern water pH is 9.1 and conductivity is $1447 \mu\text{S cm}^{-1}$ (Anslan et al., 2022). This sheds new light on sedaDNA studies of aquatic taxa on the central and northern Tibetan Plateau, where many lakes are characterized by high water salinity and pH (Wang and Dou, 1998; Zheng, 1997), but more studies are still needed to assess the preservation of aquatic DNA in the future.

Based on the current knowledge of taphonomy and our research experiences, we conclude that sedaDNA studies could preferentially select deep lakes characterized by ideal hydrochemical conditions (intermediate electrical conductivities and neutral to slightly alkaline pH), clay mineral-rich and fine sediments, and medium-sized catchments with well-developed hydrographical networks, high erosion rates, and diverse ecosystems. From this perspective, some deep glacial lakes with freshwater (Jia et al., 2022; Liu et al., 2022) and high clay sediment input (Wang et al., 2012), such as those from the southern and southeastern Tibetan Plateau, may have a high potential for sedaDNA studies in the future. Aside from lakes, systematic taphonomic studies of sedaDNA in other archives such as cave sediments (e.g., Haouchar et al., 2014; Massilani et al., 2022) and soils (e.g., Ariza et al., 2022; Edwards et al., 2018) from the Tibetan Plateau are also highly encouraged in the future.

3. Methodological opportunities and limitations of sedaDNA approaches

Three main sedaDNA approaches are currently being used: (i) metabarcoding of certain taxonomic groups based on PCR amplification of a gene fragment (also described as 'metabarcoding' or 'marker') followed by high-throughput sequencing (Deiner et al., 2017; Taberlet et al., 2012b; Zinger et al., 2019), (ii) metagenomics of entire taxonomic assemblages based on untargeted shotgun sequencing (Coissac et al., 2016; Pedersen et al., 2016; Wang et al., 2021), and (iii) hybridization capture to enrich the DNA fragments from species of interest prior to shotgun sequencing (Murchie et al., 2020; Schulte et al., 2021; Slon et al., 2017).

With the development of next-generation sequencing technologies (Glenn, 2011; Metzker, 2010; Shendure and Ji, 2008), metabarcoding has shown a great potential for reconstructing long-term changes in different biological groups. Several sets of universal PCR primers suitable for sedaDNA studies have been designed for plants

(Taberlet et al., 2007), mammals (Giguët-Covex et al., 2014), fish (Miya et al., 2015), fungi (Seeber et al., 2022), diatoms (Stoof-Leichsenring et al., 2012), ostracods (Echeverría-Galindo et al., 2021), and bacteria (Belle and Parent, 2019). Metabarcodes amplified by these primers offer the opportunity to identify the target groups at high taxonomic levels (e.g., species level), and the bias in taxonomic resolution between different taxa can be further complemented by specific primers. For example, based on the *trnL* P6 loop amplified by the 'g' and 'h' primers (Taberlet et al., 2007), about half of terrestrial and aquatic plant taxa derived from surface sedimentary DNA from more than 250 lakes in China (mainly from the Tibetan Plateau) and Siberia can be identified to species level at 100% identity, but the taxonomic resolution of Cyperaceae, Poaceae, and Asteraceae, three dominant families on the Tibetan Plateau (Hou, 2001; Miehe et al., 2019), is quite low (Li et al., 2021; Stoof-Leichsenring et al., 2020, 2022). Fortunately, specific primers have already been designed for these three plant families (Baamrane et al., 2012; Willerslev et al., 2014), allowing further tracking of their intraspecific variations. It should be kept in mind that some biases produced in laboratory experiments, sequencing, and bioinformatics may add uncertainty to the data interpretation (Alberdi et al., 2018; Deiner et al., 2017; Mathieu et al., 2020; Zinger et al., 2019). Cyperaceae, Poaceae, and Amaranthaceae (Chenopodiaceae), for example, are consistently underestimated in the surface dataset of the Tibetan Plateau (Jia, 2020; Li et al., 2021), as well as in other Arctic studies (Alsos et al., 2015, 2018), which is probably due to imbalances in PCR amplification efficiency. Nevertheless, due to lower sequencing cost per sample (compared to metagenomics and hybridization capture) and the availability of PCR primers for many ecologically important taxonomic groups, metabarcoding is currently still the most widely used approach in sedaDNA studies.

Since it is time- and resource-consuming to analyze each group of organisms by the metabarcoding approach with different PCR primers, metagenomics seems to be a complementary choice to reveal the composition and diversity of entire biota at ecosystem scale. Unlike PCR-based approaches, metagenomics theoretically allows the researcher to target and sequence all DNA fragments from environmental samples without taxonomic biases and achieve broad taxonomic resolutions and a better estimation of the relative abundance of taxa (Capo et al., 2021; Parducci et al., 2019a). Because all information from prokaryotes to eukaryotes is retained, this approach can further our understanding of interspecific interactions (e.g., prokaryotic-eukaryotic and host-parasite interactions), the functional characteristics of their genomes, and phylogenomics over geological time (Malé et al., 2014; Pedersen et al., 2016; Wang et al., 2021). Notably, it is possible to detect cytosine (C) to thymine (T) substitution at the ends of DNA fragments, which is caused by the deamination of cytosine bases and has become a common way to distinguish ancient DNA from potential contamination (Briggs et al., 2007; Jónsson et al., 2013; Willerslev and Cooper, 2005). To prevent the signal of focal species being diluted or suppressed by other communities such as bacteria in the samples, it is possible to enrich their DNA via hybridization capture with specific baits or probes before shotgun sequencing (Murchie et al., 2020; Schulte et al., 2021; Slon et al., 2017). Due to the difficulty to sequence long DNA fragments and relatively high sequencing cost, both methods are so far little used for sedaDNA studies (Capo et al., 2021).

For accurate taxonomic identification, a high-quality reference database is crucial (Taberlet et al., 2012b). Two taxonomic reference databases, for example, have been widely used in previous plant DNA metabarcoding studies on the Tibetan Plateau, which are the Arctic-Boreal vascular plant and bryophyte database (Soininen et al., 2015; Sønstebo et al., 2010; Willerslev et al., 2014) and the European Molecular Biology Laboratory (EMBL) nucleotide

database (Kanz et al., 2005). However, such databases have been recognized as being incomplete for the Tibetan Plateau, where a large number of endemic plant species are distributed (Jia, 2020; Li et al., 2021; Liu et al., 2021d), especially on the southeastern Tibetan Plateau (Sun et al., 2017). For most metagenomic studies, the majority of reads (>90%) cannot be assigned to any taxonomic names, mainly because of the lack of reference genomes in the databases (Parducci et al., 2019a; Pedersen et al., 2016; Schulte et al., 2021; Wang et al., 2021). Moreover, there is a strong bias towards mammals, agricultural and medicinal plants, and pathogenic microorganisms in available genomic databases (Capo et al., 2021), but promisingly, this will be changed by increasing genomic reference sequences of different biological groups from different regions. For example, the PhyloNorway plant genome database, constructed by sequencing 1541 Arctic and boreal plant specimens collected from herbaria, was recently introduced by Wang et al. (2021), which is important to promote metagenomic studies on ancient plant communities in the Arctic and sub-Arctic regions. Therefore, the improvement of the public nucleotide databases and, at the same time, the customization of a regional taxonomic reference database for the Tibetan Plateau should be given priority in the future.

4. Promising examples of sedaDNA research from the Tibetan Plateau

Although sedaDNA has received increasing attention over the past two decades, few studies have focused on the Tibetan Plateau. To date (searched on Web of Science and Google Scholar on July 20, 2022, keywords 'ancient DNA', 'sedimentary DNA', and 'Tibetan Plateau'), there are around fifteen sedaDNA records from eight lakes, five archaeological sites, one stratigraphic section, and one cave available from the Tibetan Plateau, covering a broad range of organisms from terrestrial (terrestrial plants, wild animals, and prehistoric humans) to aquatic (microalgae, bacteria, archaea, macrophytes) environments (Fig. 1). In this section, we introduce some exemplary studies and highlight the power of sedaDNA to advance the understanding of long-term ecological and environmental dynamics on the Tibetan Plateau.

4.1. Climate and environmental changes inferred from microbial DNA metabarcoding

Microbiologists are the pioneers in sedaDNA research on the Tibetan Plateau and microbial DNA metabarcoding has been successfully applied to reflect past climate and environmental conditions. Hou et al. (2014), Li et al. (2016), and Yang et al. (2016) investigated the temporal dynamics of microalgae using ancient DNA in the sediment cores from Lake Kusai (via quantitative PCR, denaturing gradient gel electrophoresis, and 23S rDNA) and Lake Qinghai (via 23S rDNA and 18S rDNA) over the last 3100 years and 18,500 years, respectively. They found that microalgal communities in Lake Kusai are mainly composed of *Synechococcus* (Cyanophyceae), the haptophyte *Isochrysis*, and a small amount of *Gloeotilopsis sterilis*, a species of chlorophytes of the family Ulotrichaceae. The abundance of *Synechococcus* is positively correlated with changes in precipitation (Shao et al., 2005), Asian monsoon strength (Wang et al., 2005), and solar radiation (Solanki et al., 2004; corresponding to warm-humid climate stages), whereas the abundance of *Isochrysis* is negatively correlated with these events (corresponding to cold-dry climate stages). Due to the potential competition between *Synechococcus* and *Isochrysis*, their ratios are expected to be a proxy for paleoclimate reconstructions. In contrast, microalgal communities in Lake Qinghai are much more diverse and complex than those in Lake Kusai, which are dominated by twelve genera within eight major classes. Trebouxiophyceae (*Chlorella*) and

Eustigmatophyceae (*Nannochloropsis*) show higher abundance than others during the cold periods, while Phaeophyceae (*Ectocarpus* and *Colpomenia*), Xanthophyceae (*Vaucheria*), Chlorophyceae (*Chlamydomonas*, *Dunaliella*, and *Spermatozopsis*), Bacillariophyceae (*Fistulifera*), Cyanophyceae (*Synechococcus*), and Dinoflagellate (*Woloszynskiahialophila* and *Scrippsiellahangoei*) are more abundant during the warm periods. Based on the differences in microalgal adaptation to climate change, they propose a phytoplankton index to indicate paleo-temperature changes in the Lake Qinghai region over the last 18,500 years, which is highly consistent with the variations of Asian summer monsoon index, Westerlies climate index (An et al., 2012), and redness index (Ji et al., 2009) derived from the same lake, and with solar radiation (Berger and Loutre, 1991). In addition, lower abundance of Dinoflagellate can indirectly reflect lower nutrition levels and higher salinity of the lake, and vice versa.

Aside from microalgae, ammonia-oxidizing archaea (AOA) in the sediment core of Lake Qinghai have also been studied by Yang et al. (2015). The archaeal *amoA* genes (encoding the alpha subunit of ammonia monooxygenases) were amplified by the specific primer set of Arch-amoAF and Arch-amoAR (Francis et al., 2005). The results indicate that the abundance variation of *Nitrososphaera*, the dominant AOA genus, is negatively correlated with total organic carbon content, which is an indicator of monsoonal precipitation through its linkage to nutrient levels and primary productivity of the lake (An et al., 2012). This finding suggests that a large amount of allochthonous nutrients are transported into the lake via high rainfall during the warm periods, resulting in high trophic conditions and increased primary productivity of the lake, which inhibits the AOA growth because of their oligotrophic physiology (Herrmann et al., 2009). Meanwhile, lake salinity increases due to high evaporation, which is unfavorable for the AOA growth because they prefer low-salinity environment (Mosier and Francis, 2008). On the contrary, the growth of AOA will be facilitated by low nutrient input and low salinity of the lake during the cold periods. To conclude, these studies suggest that microalgal and archaeal DNA from lake sediments can be an additional and promising proxy for paleoclimate and paleoenvironmental reconstructions on the Tibetan Plateau.

Looking at ancient cyanobacterial communities, Yan et al. (2019) and Yan et al. (2020) applied the metabarcoding approach to analyze cyanobacterial DNA from a sediment core of Lake Chenghai over the last 100 years and 1000 years, respectively. The 16S rDNA V4 region was amplified by the specific primers 515F/806R (Caporaso et al., 2011). They report that the dominant genera are *Aphanizomenon* (24.2%), followed by *Synechococcus* (15.2%) and *Sphaerospermopsis* (7.1%), and the bloom of these taxa after 1980 CE is directly linked to high nutrient levels associated with anthropogenic disturbances and lake level declines. Furthermore, cyanobacterial abundance and microbial diversity show a significant increase during the Medieval Warm Period (930–1350 CE) and the Current Warm Period (1900 CE–present), but a decline during the Little Ice Age (1400–1850 CE). Accordingly, they predict that cyanobacterial biomass in Lake Chenghai and other subtropical lakes may continue to increase with the future impact of global warming and intensified human activities, which will threaten lake water quality and ecosystem health.

Diatom DNA in freshwater sediments is commonly used as an indicator for environmental reconstructions (Dulias et al., 2017; Epp et al., 2015), but little is known about how well diatom signals from sedaDNA correlate with fossil records in brackish and saline lakes on the Tibetan Plateau. A recent study explored this question by analyzing sedaDNA and fossil diatom records across a sediment core covering the last 900 years from Lake Namco (Anslan et al., 2022). Following a prior metabarcoding study in the same lake

(Kang et al., 2021), they applied the modified primers Diat_rbcL_709F and Diat_rbcL_808Rd to amplify the *rbcL* gene fragments to potentially capture a wider range of diatoms that may be endemic for the region. The results show a concordant pattern of diatom richness and community structures from sedaDNA and fossil data. Some differences are also reported, which are caused by the absence of reference diatom sequences, but may also be the case for dissolved diatom valves in brackish and alkaline pH water. Nevertheless, both proxies reveal an identical breakpoint in diatom community shifts in 1950 CE, which is likely due to the impact of current climate warming on the Tibetan Plateau. This suggests that the environmentally driven signals reflected by diatom communities can be successfully recovered by sedaDNA. Moreover, higher diatom diversity can be recovered by the metabarcoding approach compared to fossil records, indicating the higher sensitivity of sedaDNA analysis.

4.2. Plant DNA metabarcoding reveals past vegetation and plant diversity

A recent study of Lake Naleng, a glacially-formed alpine lake in the Hengduan Mountains, used ancient plant DNA records (the *trnL* P6 loop amplified by the *g-h* primers; Taberlet et al., 2007) to reconstruct the trajectory of terrestrial plant richness since the last deglaciation (Liu et al., 2021d). The results show that the highest terrestrial plant richness occurs between 14 and 10 ka BP when the ice-free area was dominated by alpine meadow, and the lowest terrestrial plant richness occurs between 10 and 3.6 ka BP when forests invaded (Fig. 3). Furthermore, Spearman rank-correlation tests indicate that changes in terrestrial plant richness are directly triggered by the alpine habitat area above the treeline across time. These results suggest that plant diversity in high-mountain regions will not be expected to increase with warm-induced forest advance. Ongoing climate warming on the Tibetan Plateau will induce a pronounced loss of terrestrial plant richness in the lake catchments during 2050–2300 CE, which is simulated by the Representative Concentration Pathway 4.5 emissions scenario based on the past relationship between alpine habitat area and terrestrial plant richness.

Macrophytes play a key role in the structure and function of aquatic ecosystems and are valuable ecological indicators for lake health and condition (Chambers et al., 2008; Sayer et al., 2010), but

their composition and diversity in response to different environmental variables are not fully understood. A case study applied the metabarcoding approach (the *trnL* P6 loop amplified by the *g-h* primers; Taberlet et al., 2007) to surface sediments from 50 lakes on the Tibetan Plateau and showed that modern macrophyte richness and composition are highly influenced by mean July temperature and lake water conductivity. Highest richness is found when mean July temperatures are above 12 °C and lake water conductivities are 40–400 $\mu\text{S cm}^{-1}$, while extremely low richness is detected in some saline lakes with water conductivities $>1000 \mu\text{S cm}^{-1}$. A pronounced change from emergent to submerged taxa combined with an increase in macrophyte richness in Lake Naleng is found at 14 ka BP. Based on the modern results described above, this important turnover could be explained by an increase in temperature and water conductivity due to glacial runoff and high evaporation during the deglaciation (Stoof-Leichsenring et al., 2022).

To understand the long-term dynamics of macrophytes and their potential drivers in the westernmost Tibetan Plateau, Heinecke et al. (2017) analyzed a sediment core from Lake Karakul (Tajikistan) with macrophyte remains and ancient plant DNA amplified by the *g-h* primers (Taberlet et al., 2007). They reconstructed the changes in the composition and productivity of submerged vegetation and investigated their relationship to environmental changes over the last 29 ka BP. The results show that *Stuckenia* (Potamogetonaceae), a dominant taxon distributed at low lake levels due to its high light demands for growth, occurs throughout the entire timespan, even in some phases where no plant macrofossils are detected. *Chara* (Characeae), a deeper-water taxon with no preserved plant macrofossils in the core, seems to spread when growing conditions are unsuitable for *Stuckenia*. This turnover suggests that lake level changes may be the main driver of macrophyte dynamics in Lake Karakul, as also evidenced by elemental and isotopic records of *Stuckenia* leaf remains from the sediment core.

From the above examples and our prior studies on many lake surface sediments (Jia et al., 2022; Li et al., 2021; Liu et al., 2021e; Stoof-Leichsenring et al., 2020, 2022), we conclude that future applications of plant DNA metabarcoding on the Tibetan Plateau seem promising. Worth mentioning is that more projects on terrestrial and aquatic plants from other lakes, such as Lake Ximenco, Lake Mugeco, Lake Qionghai, and Lake Donggi Cona (Fig. 1), are currently being carried out in the paleogenetic

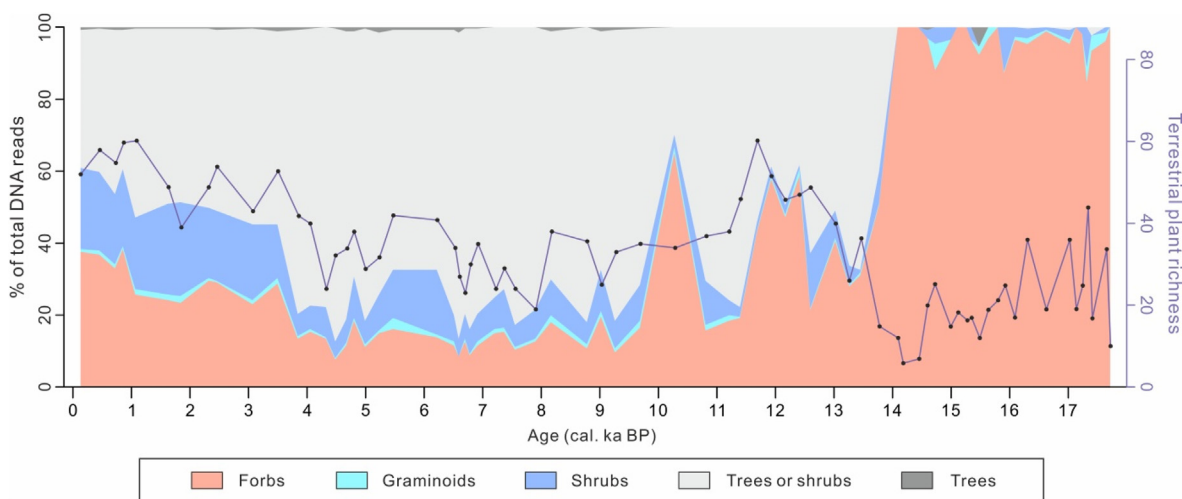


Fig. 3. Summary of the percentage abundance of terrestrial plant functional groups and terrestrial plant richness in the sedimentary ancient DNA records from Lake Naleng since the last deglaciation (Liu et al., 2021d).

laboratories at Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research (AWI), which will present a better understanding of vegetation dynamics on the Tibetan Plateau under current warming scenarios.

4.3. Metagenomic studies on alpine ecosystem changes and early agricultural developments

To assess the changes in whole biological groups since the last deglaciation on the southeastern Tibetan Plateau, the metagenomic approach has also been applied to DNA extracts from Lake Naleng (Liu et al., 2021d; see the first paragraph of Section 4.2). The DNA libraries were prepared following the established protocol of single-stranded DNA library preparation (Gansauge et al., 2017). The results show that the classified taxa are mainly from three domains, that is, bacteria, archaea, and eukaryotes, consistent with other metagenomic studies from Arctic lakes (Parducci et al., 2019a). Likewise, extremely low read abundances of eukaryotes are found in this study, which is dominated by terrestrial taxa. Although few reads are mapped to plants, the metagenomic approach retrieves more taxa and shows higher taxonomic resolution than the metabarcoding approach, thereby allowing a comprehensive interpretation of changes in terrestrial plant communities and associated organisms (e.g., mammals) in response to past climate change. The findings also show the response of aquatic communities to glacial-interglacial climate change. Overall, these results reveal non-random processes of community assembly associated with the interaction of abiotic and biotic factors in terrestrial and aquatic ecosystems. Such paleo-assemblage processes provide a window into the possible community organization of the southeastern Tibetan Plateau under future climate warming (S. Liu, unpublished data).

The metagenomic approach has also been applied in archaeological studies. To assess the history of alpine farming and animal husbandry in the Yarlung Zangbo Basin, Yang (2019) obtained metagenomic DNA data from 3000-year-old sediments from four archaeological sites including Changguogou, Klu lding, Liena, and Qugong. The DNA libraries were prepared following the protocol of double-stranded DNA library preparation (Meyer and Kircher, 2010). Their preliminary results show that foxtail millet (*Setaria italica*) and barley (*Hordeum vulgare*) are the major food sources for the early agricultural populations, while fishing and hunting are also important activities. Species detected by sedaDNA are in accordance with the results of traditional archaeobotanical and zooarchaeological studies. In another study from the Kongsangqiao archaeological site (Q. Sun and Z. Gu, unpublished data), twelve reads are assigned to *Oryza sativa*. Of these, five reads are annotated as *Oryza sativa Indica*, which can be distinguished from *Oryza sativa Japonica*. These findings are also consistent with archaeobotanical evidence, which might be the earliest record of indica rice in China, but further studies are still needed in the future. To conclude, ancient DNA can be well preserved in archaeological sediments and could be used as a proxy to reconstruct the early agricultural history of the southern Tibetan Plateau.

4.4. Tracing the history of prehistoric humans by hybridization capture

Denisovans, an extinct sister group of Neanderthals, are thought to be closely related to prehistoric humans on the Tibetan Plateau, and they may have genetic contributions to the adaptation of modern Tibetans to high-elevation environments (Huerta-Sanchez et al., 2014). In a study by Chen et al. (2019), half of a hominin mandible (160 ka BP) found in the Baishiya Karst Cave on the northeastern Tibetan Plateau has been assigned to Denisovans.

However, this identification is ambiguous because it is only based on a single amino acid position. To further explore the hominin occupation history of the Baishiya Karst Cave, Zhang et al. (2020a) extracted ancient DNA from cave sediments over the last 200 ka. After library preparation (Gansauge et al., 2017), mammalian and human mitochondrial DNA (mtDNA) was enriched by hybridization capture with 242 mammalian mtDNA probe sets (Slon et al., 2017) and a human mtDNA probe set (Fu et al., 2013), respectively. The results show that between 71% and 100% of the ancient mtDNA fragments that covered informative positions can be matched to the Denisovan reference genome. In the phylogenetic trees, the mtDNA sequences are clustered to a clade with the mtDNA sequences of two published Denisovan individuals (Denisova 3 and 4). These results indicate that Denisovans occupied the Baishiya Karst Cave at 100 ka and 60 ka BP, implying a long occupation of the Tibetan Plateau by prehistoric humans who may have carried a genetic haplotype that allows adaptation to the cold and hypoxic environment (Huerta-Sanchez et al., 2014).

5. Outlook of sedaDNA research on the Tibetan Plateau

Based on past and present publications and our research experience, we aim to provide some recommendations and guidelines for future sedaDNA works on the Tibetan Plateau in this section.

- (i) More systematic studies of taphonomic processes (i.e., DNA production, incorporation, transportation, deposition, preservation) must be carried out before sedaDNA reaches its full potential to reconstruct past biota. The lack of taphonomic investment is one of the main reasons why sedaDNA results have long been questioned by part of the paleoecology community. For example, as two established proxies for vegetation reconstruction, the modern taphonomic processes of pollen and plant macrofossil have been well studied over the last 50 years (Birks and Birks, 2016; Xu et al., 2016), but similar studies on sedaDNA remain scarce, resulting in controversies over the reliability of ancient plant DNA signals in the last decade (Alsos et al., 2020; Birks et al., 2012; Birks and Birks, 2016; Parducci et al., 2012a, 2012b). To do this, more modern-process studies on surface sediments based on detailed field surveys and environmental data are needed on the Tibetan Plateau in the near future, which would provide the knowledge basis for study site selection and the calibration and interpretation of ancient DNA records. Furthermore, the establishment of a standard method or protocol for assessing sedaDNA data quality (or DNA preservation conditions) before downstream data analysis is highly recommended (e.g., Jia et al., 2022).
- (ii) Trying to overcome methodological limitations and improving taxonomic reference databases. There is still much room for technological development, indicating the necessity to increase discussion and collaboration between molecular biologists and paleoecologists. For example, DNA extraction protocols can be further optimized to yield more DNA from different types of sediments (Murchie et al., 2020). For the metabarcoding approach, other metabarcodes could also be designed and tested to reveal intraspecific genetic differences. In the next step, three specific primers, targeting the ITS (internal transcribed spacer) region (Baamrane et al., 2012; Willerslev et al., 2014), are recommended to be tested on the Tibetan Plateau to determine to what extent they could improve the taxonomic resolution of Cyperaceae, Poaceae, and Asteraceae. In addition, high-quality and open-access taxonomic reference databases are urgently needed. Unlike the Arctic and boreal regions, few databases have yet

been established or shared for the Tibetan Plateau. Currently, the iFlora, a vascular plant DNA database including the barcode sequences of 39,971 species representing 300 families and 3434 genera in China, has been developed with the efforts of many Chinese universities and scientific institutions, especially the Kunming Institute of Botany, Chinese Academy of Sciences (Li et al., 2012), thus the establishment of a regional plant DNA reference database for the Tibetan Plateau in the foreseeable future is very promising. To do this, differences in vegetation patterns between different regions of the Tibetan Plateau need to be considered. For example, the southeastern Tibetan Plateau, also called 'the Hengduan Mountains', is one of the world's biodiversity hotspots (Myers et al., 2000), which is characterized by a series of north-south-oriented mountain ranges separated by deep river valleys (Li et al., 2015), resulting in well-developed elevational zonation of local vegetation covering temperate to alpine flora (Zhang et al., 2009). Thus, plants from the surrounding areas of the Tibetan Plateau, especially those from lower elevations, should also be included in the construction of taxonomic reference databases. Other good news is that the Earth BioGenome Project has also been launched, aiming to sequence, catalog, and characterize the genomes of all Earth's eukaryotes (Lewin et al., 2018), which will highly promote future applications of metagenomics in sedaDNA studies.

- (iii) Encouraging and conducting more sedaDNA studies on the Tibetan Plateau to provide a more comprehensive view of alpine ecosystem and biodiversity changes during the Late Quaternary. Tracking the long-term dynamics of a full range of taxa at a high taxonomic resolution across different elevational gradients on the Tibetan Plateau and their responses to past climate and environmental changes is important for accurately assessing the potential impacts of current climate warming on alpine ecosystem structure and function, which is becoming possible with the emergence and fast development of sedaDNA technologies. More information relating to biodiversity monitoring and conservation, including biological distributions, migrations, invasions, and extinctions, especially the history of endemic and endangered species (e.g., *Saussurea* spp., *Pantholops hodgsonii*, and *Panthera uncia*; Chen and Yuan, 2015; State Forestry Administration of China, 1998; Wang et al., 2009; Xiao et al., 2019), and species of high economic and medicinal value (e.g., *Curcuma* spp. and *Rheum* spp.; Liang et al., 2022; Zhang et al., 2022), can also be obtained from sedaDNA studies, which could provide valuable and quantitative advice to policymakers for restoration and management of alpine ecosystems on the Tibetan Plateau. Prehistoric human activities on the Tibetan Plateau, such as occupation, migration, crop spread, livestock domestication, and high-elevation adaptation, are current research hotspots in environmental archaeology, but most of previous studies are based on archaeological remains (e.g., Chen et al., 2015; Chen et al., 2022; Cheng et al., 2021; Meyer et al., 2017; Zhang et al., 2016). We believe that sedaDNA can provide more straightforward evidence for these important research questions (e.g., Curtin et al., 2021; Giguët-Covex et al., 2014; Jacobs et al., 2019; Zhang et al., 2020a). For example, the *Endothelial Pas1* (*EPAS1*) gene found in modern Tibetan populations is assumed to be related to their adaptation to hypoxic environments (Huerta-Sanchez et al., 2014), which can possibly be traced through ancient DNA in cave or archaeological sediments. This might answer when such genes started to appear and spread in plateau populations and how these populations are genetically related to

modern Tibetans (Zhang et al., 2018). In addition, hybridization capture could be a next attempt to target ancient crop (e.g., *Oryza*, *Triticum*, *Zea*) and livestock (e.g., *Bos*, *Ovis*, *Sus*) DNA from sediments (Gasc et al., 2016), which may uncover the early history of agricultural developments on the Tibetan Plateau.

The current number of sedaDNA records from the Tibetan Plateau and China is far lower than those from Europe, North America, and the Arctic region (Jia, 2020; Pedersen et al., 2015), suggesting that more attention should be given to this important region in the future. With the improvement of experimental techniques and taxonomic reference databases, we believe more sedaDNA records will be published in the upcoming years, which could address questions about long-term ecological dynamics at ecosystem scale and bring the paleoecology of the Tibetan Plateau into a new era.

Author contributions

All authors have made substantial contributions to the manuscript. Ulrike Herzschuh and Weihan Jia designed the study. Weihan Jia led the writing of manuscript and compiled the contributions to Sections 4 and 5 from co-authors. All co-authors reviewed and edited the final manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The major part of the data employed in this review has been published, either in master's or doctoral theses or widely available journals.

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