TOPICAL REVIEW – TEMATICKÁ SYNTÉZA

# Digging deeper: Plant sedimentary ancient DNA in archaeobotanical studies

## Hlouběji do minulosti: Rostlinná sedimentární starobylá DNA v archeobotanickém výzkumu

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*The analysis of plant sedimentary ancient DNA (sedaDNA) is increasingly being applied in paleoecology and is now gaining relevance in archaeobotany and archaeology. This review outlines the practices involved in sedaDNA sampling, extraction, and analysis, emphasising contamination prevention and methodological considerations. Various genetic methods (metagenomics, metabarcoding, and target enrichment) are discussed concerning their strengths, limitations, and suitability for specific research questions. This review also examines the methodological advances, applications, and challenges associated with plant sedaDNA research, especially highlighting its potential to refine paleoenvironmental reconstructions when combined with other traditional archaeobotanical and palaeoecological methods. SedaDNA can allow researchers to detect various plant species, including those that may not have left morphologically determinable remains, thereby offering a more comprehensive understanding of past ecosystems. The approach provides crucial evidence for analysing long-term vegetation changes, climate impacts, and the role of humans in shaping biodiversity. Moreover, sedaDNA may also allow the detection of species used or encountered by humans at the site level. As sequencing technologies and bioinformatic tools continue to advance, sedaDNA will further enhance interdisciplinary research by connecting traditional methods.*

sedaDNA – environmental archaeology – palaeobotany – palaeoecology – vegetation

*Analýza rostlinné sedimentární starobylé DNA (sedaDNA) se v posledních letech stále častěji uplatňuje v paleoekologii a postupně získává význam i v archeobotanice a archeologii. Tato přehledová studie shrnuje postupy odběru vzorků, extrakce a analýzy sedaDNA se zvláštním důrazem na prevenci kontaminace a klíčové metodologické aspekty. Diskutujeme různé genetické přístupy – metagenomiku, metabarkódování (metabarcoding) a hybridizační obohacování cílových sekvencí (target enrichment) – a hodnotíme jejich přednosti, omezení i vhodnost pro různé typy výzkumných otázek. Text se dále věnuje metodologickému vývoji, možnostem využití i současným výzvám výzkumu rostlinné sedaDNA a zdůrazňuje její potenciál zpřesnit paleoenvironmentální rekonstrukce při kombinaci s tradičními archeobotanickými a paleoekologickými metodami. SedaDNA umožňuje identifikovat široké spektrum rostlinných druhů, včetně těch, které po sobě nezanechaly morfologicky určitelné zbytky, a přispívá tak k ucelenějšímu obrazu minulých ekosystémů. Tento přístup poskytuje zásadní informace pro studium dlouhodobých změn vegetace, vlivu klimatu i role člověka při utváření biodiverzity. SedaDNA může rovněž napomoci k odhalení druhů, které lidé na lokalitě využívali nebo s nimi přicházeli do kontaktu. S dalším rozvojem sekvenačních technologií a bioinformatických nástrojů bude sedaDNA stále více podporovat interdisciplinární výzkum propojením tradičních metod.*

sedaDNA – environmentální archeologie – paleobotanika – paleoekologie – vegetace

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## Introduction

Until recently, the study of past vegetation in sedimentary and archaeological contexts relied on macroscopic analyses (plant macroremains; *Pearsall 2015*) and microscopic approaches (palynology and phytolith analysis; *Piperno 2006*; *Birks-Berglund 2018*). But while these methods have provided valuable insights into past environmental changes and human-plant interactions for decades, they also have inherent limitations. Recent advances in DNA sequencing technologies have introduced a third avenue of investigation: molecular-level analysis (*Pedersen et al. 2015*). Early studies suggest that sedimentary ancient DNA (sedaDNA) has the potential to revolutionise the field. *Willerslev et al.* (*2003*) showed that ancient plant DNA can persist in permafrost and cave sediments, providing valuable information even where macrofossils are absent, thus offering unique insights into Pleistocene environments. *Sønstebø et al.* (*2010*) demonstrated the utility of the chloroplast P6 loop as a plant barcode for reconstructing Arctic flora from permafrost soils, highlighting its potentially higher taxonomic resolution compared to pollen records. However, ongoing discussions continue to explore the limitations and methodological challenges associated with this approach.

The extraction of DNA from ancient sediments allows researchers to gain insights into past biodiversity, community dynamics, and environmental conditions at archaeological sites (*Özdoğan et al. 2024*). Plant sedaDNA analysis detects DNA preserved directly in archaeological sediments. This DNA can originate from plants growing at or around the site, as well as from plants brought to the site and used or processed by people. Identifying this DNA can therefore provide evidence of which plants were present at a site and help reconstruct past vegetation and aspects of plant use by humans. SedaDNA studies have been conducted across a wide range of environments, including polar regions (*Kjær et al. 2022*), the tropics (*Bremond et al. 2017*), and the temperate zone (*Hudson et al. 2022*). With the use of various genetic methods in sedaDNA analysis, scientists can detect diverse assemblages of organisms from prokaryotes to eukaryotes, including plants. A focus on plants has allowed us to reconstruct past vegetation composition and dynamics at sites, and possibly a connection between past human societies and the surrounding environment. For example, *Ter Schure et al.* (*2022*) documented plant collection by hunter-gatherers, while *Van Vugt et al.* (*2022*) and *Zetter et al.* (*2021*) illustrated how deforestation and grazing shaped the development of mountain vegetation. This approach provides information about the diversity of plants that once inhabited an area, even those that may not have left more substantial remains.

Natural and anthropogenic archives such as peat deposits, lake sediments, and archaeological layers serve as repositories of palaeoenvironmental information. By analysing various proxies contained within these sediments, we can gain indirect insight into past environmental conditions, vegetation dynamics, and human-environment interactions. These reconstructions often rely heavily on traditional methods using plant macro- and microremains such as charcoal, seeds and fruits, pollen, and phytoliths (*Pearsall 2015*). These methods may recover only a limited portion of past botanical assemblages, as many plants used by prehistoric humans, especially soft plant parts, are rarely preserved in sediments (*Behre 2008*). SedaDNA analysis can transform archaeobotany by bridging plant, animal, and microbial proxies to reveal human-environment interactions in unprecedented high-resolution detail (*Zampirolo et al. 2024*).

In this review, we discuss if, when and how the sedaDNA approach can be implemented and show that plant sedaDNA does not merely complement traditional botanical analyses but fundamentally alters how we reconstruct past ecosystems and human impact. By integrating sedaDNA with macrofossil, pollen, and anthracological records, and others, we can refine our understanding of plant domestication, vegetation impact, and environmental change over time. An awareness of conditions suitable for the preservation of sedaDNA molecules and methods of field sampling should facilitate this cooperation. This review critically examines not only the strengths but also the limitations of plant sedaDNA in archaeobotany and geoarchaeology, particularly concerning the preservation of genetic records. Finally, based on the research worldwide, we explore the potential of plant sedaDNA and summarise suggestions for its complementary application in current archaeobotanical methodologies.

## The role of sedimentary and environmental conditions in sedaDNA preservation

Despite the clear potential of sedaDNA, the approach itself faces unresolved issues. The most important research questions that still need to be answered are the taphonomy and preservation of DNA in different depositional environments. Following the death of an organism, the DNA molecule can undergo up to three different processes in a sediment: 1) the degradation of the molecule by enzymes produced by microorganisms; 2) the incorporation of parts of the molecule by other organisms (bacteria, bdelloids); and 3) the binding of DNA into the sediments (*Pedersen et al. 2015*; *Jia et al. 2022*). Sediments (colloids and minerals) have been proven to be indispensable in DNA preservation (*Cai et al. 2006*), because individual sediment particles bind DNA and thus protect it to some extent from degradation processes, slowing down the decay of the molecule. Furthermore, according to *Giguet-Covex et al.* (*2019*), high salt concentration and high pH also prevent microbial DNA degradation.

It has been reported in various studies that good DNA preservation in sediments is the result of a combination of factors that are necessary to slow degradation, including a cool temperature, darkness, and an anoxic environment (*Yoca et al. 2021*). UV light exposure can damage DNA molecules, whereas cold and anoxic environments inhibit the activity of microorganisms that would otherwise damage the molecules with their enzymes. Therefore, ideal environments for DNA preservation are caves, which offer a combination of these factors and act as cooling boxes throughout the year. Another ideal environment is permafrost. This is exemplified by a groundbreaking study from Greenland (*Kjær et al. 2022*), which recovered the oldest known preserved ancient DNA, dating back approximately two million years. In addition to these terrestrial sources, sedaDNA has been successfully retrieved from marine (*Armbrecht 2020*) and lacustrine sediments (*Giguet-Covex et al. 2019*). For example, sedaDNA has been preserved very well in Scandinavian lake sediments dating to the Last Glacial Maximum (~20,000 BP; *Alsos et al. 2020*) and in Alpine sediments from the early Holocene (*Chen et al. 2016*; *Zetter et al. 2021*; *Garcés-Pastor et al. 2022*).

And yet, sedaDNA studies have surprisingly also succeeded in warmer climates, with examples including lake sediments in Indonesia preserving aDNA up to one million years (*Ekram et al. 2021*), Ethiopian lake sediments spanning the last 4,000 years (*Epp et al.*

*2019a*), and cave sediments from Australia dating back 20,000 years (*Haouchar et al. 2014*). Similarly, in Europe's warmer regions, sites such as Dordogne, France (Upper Palaeolithic; *Braadbaart et al. 2020*), and El Salt, Spain (Middle Palaeolithic; *Rampelli et al. 2021*), demonstrate sedaDNA's persistence under warm conditions. In such contexts, sediment composition (e.g. clays and carbonates), fast burial, and relatively stable microclimatic conditions appear to reduce the harmful influence of higher temperatures. According to *Freeman et al.* (*2023*) and *Brown et al.* (*2025*), minerals such as smectite clays and calcite significantly enhance DNA preservation through adsorption and nanoconfinement mechanisms, effectively protecting it from degradation.

Although mineral composition and microenvironmental stability can enhance DNA preservation, significant variation may still occur between horizons within a single sediment profile due to small-scale differences in conditions. In the study by *Ter Schure et al.* (*2022*), aDNA concentrations differ markedly, with the highest values found in the lower (older) horizons. The authors suggest that these differences may be linked to freeze-thaw processes (observed through micromorphology) that negatively affected aDNA preservation in the younger horizons, leading to lower concentrations. In addition, sediment composition and organic matter content may also play an important role: the older horizons with higher aDNA concentrations contain more clay and organic matter compared to the horizons with lower concentrations. However, it is widely held that the processes of binding and preservation of DNA in different sediments (minerals and organic matter) have not yet been fully resolved (*Parducci et al. 2019*; *Braadbaart et al. 2020*; *Kanbar et al. 2020*; *Freeman et al. 2023*; *Hudson et al. 2023*). This issue is particularly relevant in archaeological contexts, where sediments are often heterogeneous mixtures of mineral particles, pottery fragments, charcoal, faunal and plant remains, and other organic matter. Unlike relatively homogeneous sedimentary archives such as lake sediments or peat deposits, archaeological deposits may exhibit substantial variability even within a single stratigraphic unit (see *Fig. 1*). Consequently, identifying the specific sediment fraction responsible for aDNA adsorption and preservation can be challenging, and interpretations are frequently based on the dominant sedimentary characteristics of the sampled horizon (e.g. *Gelabert et al. 2021*).

## Key considerations before undertaking sedaDNA analysis

The mobility of aDNA within the sediment is strongly influenced by the depositional environment, with lacustrine and terrestrial sediments exhibiting distinct physical and geochemical conditions that affect DNA stability. In lacustrine environments, sediments remain largely undisturbed, become compacted over time, and experience little to no vertical leaching of materials following deposition (*Capo et al. 2021*). *Giguet-Covex et al.* (*2019*) demonstrated that the presence of plant sedaDNA in lake sediments can preserve stratigraphic fidelity over time, even spanning thousands of years. This phenomenon is attributed to the sedimentary conditions, such as fine particle size and water saturation, which impede vertical DNA movement. Changes in plant communities through time are in agreement with other records, and the presence of taxa is time-restricted (*Parducci et al. 2019*; *Crump et al. 2021*).

By contrast, terrestrial sediments—often of particular interest to archaeologists—present a more complex situation. As *Massilani et al.* (*2022*) have demonstrated, movement

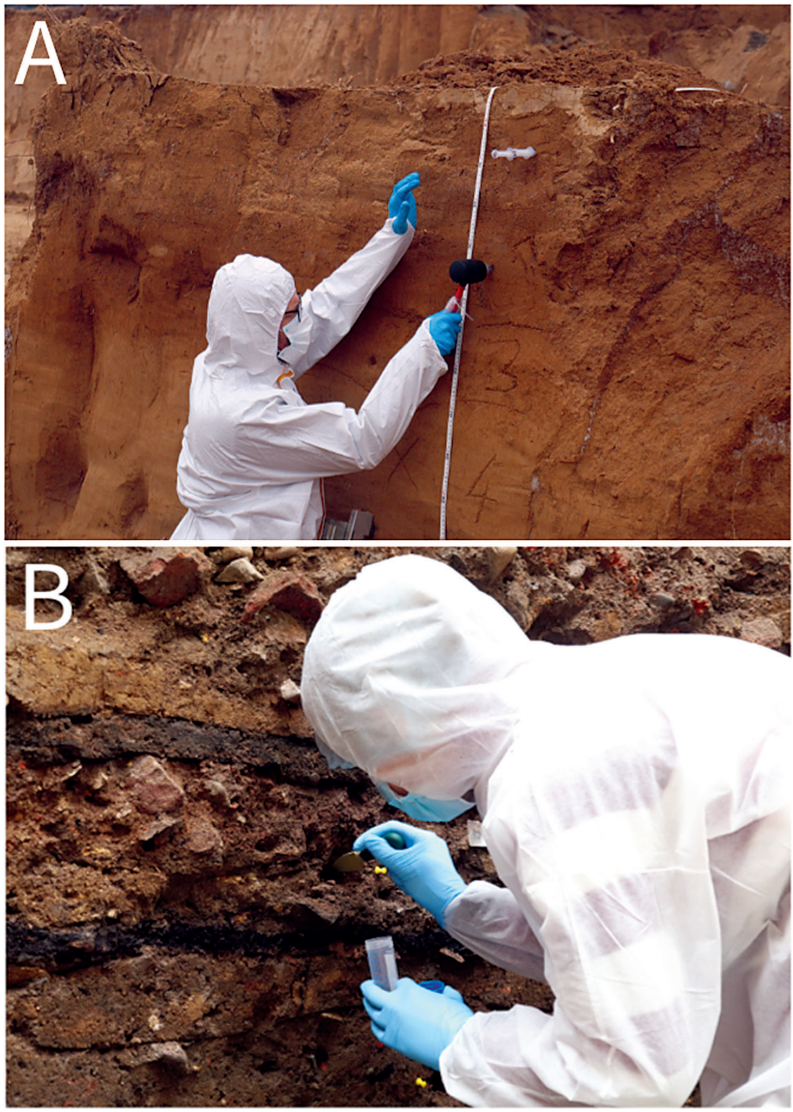



Fig. 1. Field sampling of different sedimentary contexts used in plant sedaDNA research: (A) Pleistocene loess-palaeosol deposits and (B) anthropogenic archaeological deposits within a medieval urban setting. The images highlight the contrast between relatively homogeneous natural sediments and the heterogeneous composition of archaeological deposits (photos: A – J. Zubalík; B – J. Čech).

(e.g. leaching) of DNA between horizons can be limited in certain contexts where stratigraphy is preserved. Additionally, compacted sediments, such as clay, or fine-grained sediments, such as loess, can reduce water leaching and permeability. However, DNA can leach from certain types of sediments, such as sand, and to a lesser extent from clay soils (*Andersen et al. 2012*). It should be noted that the problem of water leaching is not the only issue in terrestrial sediments, as roots, animal burrows, and frost fissures can also compli-

cate matters. Therefore, it is important to select sampling strategies tailored to site-specific conditions, integrate micromorphological and stratigraphic analyses, compare stratigraphic layers, and combine these methods with traditional approaches (*Han et al. 2025*).

When collecting sediment samples, it is essential to assess additional parameters that determine DNA preservation. pH conditions influence DNA stability in sediments, with neutral to slightly alkaline environments generally favouring preservation, while acidic conditions accelerate DNA degradation (*Jia et al. 2022*). Elemental composition analysis using XRF or gas spectrometry identifies the levels of phosphorus (P), carbon (C), nitrogen (N), calcium (Ca), and magnesium (Mg), which are critical for understanding DNA adsorption and diagenetic processes (*Freeman et al. 2023*; *Brown et al. 2025*). Grain-size distribution and clay content are vital factors, as finer particles and higher clay concentrations enhance DNA retention (*Freeman et al. 2023*). Furthermore, variations in clay mineralogy may influence these processes, making the characterisation of clay types through X-ray diffraction (XRD) an important component of sediment analysis. Organic and mineral composition analyses, such as loss on ignition (LOI) or total organic carbon (TOC) measurements, offer additional data on the capacity of the sediment matrix to protect DNA from degradation (*Heintzman et al. 2023*). Complementary analyses such as micromorphology, isotopic studies, and stratigraphically accurate sampling methods ensure a detailed geochemical and microstratigraphic understanding, which is critical for accurate sedaDNA analyses (*Massilani et al. 2022*; *Aldeias – Stahlschmidt 2024*). When combined with target enrichment and metagenomic methods (see below), these procedures enable comprehensive studies of sedaDNA and its environmental and archaeological contexts (*Heintzman et al. 2023*).

**The importance of aDNA damage patterns**

Despite the preservation of aDNA, characteristic damage patterns appear over time by which we can conclusively distinguish ancient DNA from recent molecules (*Hofreiter et al. 2001*; *Sawyer et al. 2012*). According to the study by *Orlando et al.* (*2021*), damage such as fragmentation and base substitution can be observed. First, ancient DNA is highly fragmented, with segments most often below 100 bp (*Armbrecht 2020*). Secondly, at the 5' end, cytosine becomes uracil due to deamination, which appears as thymine in the data (*Cribdon 2021*). These cumulative damages are further exploited for aDNA authentication (especially base substitutions) by bioinformatical tools such as MetaDamage (*Everett – Cribdon 2023*). In other words, thanks to this damage, we can check if our studied DNA is really old ('ancient') and not modern. Theory suggests that the deeper and older the sediment layer is, the more damaged the aDNA appears (*Zavala et al. 2021*). In a study of swamp sediments, *Dommain et al.* (*2020*) found that ancient DNA fragment length decreased exponentially with both age and depth, whereas terminal deamination increased with age. However, while post-mortem damage of aDNA generally accumulates with time (*Weiß et al. 2016*), *Dabney et al.* (*2013*) emphasise that the relationship between time since deposition and damage patterns in sedaDNA is highly context-dependent, being strongly influenced by the environmental factors mentioned above. Consequently, the degree of DNA damage (particularly fragmentation) may not always correlate with the chronological age of sediments. For example, in caves where calcite ($CaCO_3$) is a very common mineral, DNA is very well bound and preserved. The problem arises when calcite is repeatedly saturated, and the bound DNA is subsequently fragmented (*Freeman et al. 2023*).

## From fieldwork to laboratory and bioinformatic analyses

### Field

Field sampling procedures are determined by how sediments are exposed, whether through excavation profiles or coring. The surface of the excavated sediment profile must be carefully cleaned before direct sampling so that any contamination from recent exposure is avoided. During sediment sampling, it is important to be aware of potential contamination and mitigate these risks. Therefore, wearing a protective suit, especially gloves, a hair net, and a face mask is recommended (*Özdoğan et al. 2024*). Particular care should also be taken to avoid sampling sediments containing roots, sediment cracks, or animal burrows. Roots are particularly problematic because they constitute a direct source of modern plant DNA, while all three features may facilitate the post-depositional movement of DNA and organic material between stratigraphic units. Sediment samples should be collected separately from individual layers using sterile tools and transferred directly into sterile plastic tubes. The tubes are then given a proper description, so it is easy to store them in new sealable plastic bags without any confusion. Between each sampling step, it is necessary to sterilise the working instruments (ideally using DNA/RNA-ExitusPlus™ or, much more common and accessible ethanol or bleach) and use sterile DNA-free tubes. The collection of duplicate and control samples is strongly recommended, mainly in the case of rescue excavation or at sites where another archaeological excavation is not possible. Replicates can be stored in frozen condition for further analysis in the future.

Another option is to reach the sediment of interest via coring (e.g. lacustrine sediments, permafrost, infills of wells and cesspits) and take subsamples from the centre of a core using sterile tools in sterile laboratory conditions (see below; *Epp et al. 2019b*). Coring of permafrost or lake sediments does not generally require the use of protective clothing such as full-body suits or hair nets, provided that sampling is carried out carefully and contamination is minimised through proper handling.

Collected samples must be transported in cooling boxes to the laboratory for further processing and stored at 4°C or in a freezer for permafrost samples (be sure that plastic tubes are frost-resistant). The samples need to be cooled down for the following reasons: 1) lower temperature slows the activity of enzymes; 2) high temperature can trigger hydrolysis and oxidation; and 3) cooling can also help to prevent the growth of microorganisms (*Heintzman et al. 2023*).

### Laboratory

Once the material is transported to the laboratory, subsampling for DNA extraction must be conducted under strict sterile conditions to minimise the risk of contamination (*Orlando et al. 2021*). This requires a dedicated aDNA facility with clean rooms that ensure spatial separation of the different workflow steps, including the complete separation of pre-PCR and post-PCR work, ideally in different buildings. Such facilities are equipped with positive-pressure systems to prevent the entry of external contaminants, HEPA-filtered air circulation, and UV-C light; all surfaces are regularly decontaminated with bleach. Laboratory personnel must wear full protective clothing, including disposable suits, face masks, face shields, hairnets, and gloves. In addition, all reagents and solutions are

filtered and, where possible, UV-treated to further reduce the risk of contamination (*Knapp et al. 2012*).

The next step is the extraction of aDNA. New protocols for extracting aDNA from sediments are continuously being developed, focusing on the recovery of short DNA fragments (e.g. *Rohland et al. 2018*; *Epp et al. 2019b*; *Murchie et al. 2021b*). In general, the procedure involves several key steps: 1) the release of aDNA molecules from mineral surfaces; 2) the removal of enzymatic inhibitors such as humic acids, which may occur in high concentrations in sediments; and 3) the purification of aDNA from all the mentioned reagents, achieved by, e.g. binding to silica matrices (*Epp et al. 2019b*). Although many researchers continue to use commercially available extraction kits such as the PowerSoil DNA Isolation Kit, PowerMax Soil Kit, and UltraClean Soil Kit, these approaches can achieve performance comparable to other extraction methods when appropriately modified for ancient DNA applications (*Capo et al. 2021*). Rather than the choice of kit itself, protocol adaptations tailored to specific sediment characteristics may be particularly important. For example, *Capo et al.* (*2021*) demonstrated that the addition of an EDTA-based chelation step can improve the release of mineral-bound sedaDNA from calcitic sediments, resulting in higher plant taxonomic richness in calcite-rich deposits.

In the next phase, the main research question is what type of genetic information to focus on, given the benefits and drawbacks of each method. We can choose from three options—metagenomics, metabarcoding, or target enrichment. All these methods have their strengths and weaknesses, and each research team should choose the one that best suits their research questions/subjects.

Metagenomics is a method that sequences the entire set of DNA fragments present in the sample. The unquestionable advantages are the sequencing (shotgun high-throughput) of even very small fragments, the possibility to evaluate characteristic damage patterns of aDNA, and the possibility to investigate the total distribution of preserved aDNA. On the other hand, taxonomic assignment of very short fragments can lead to false positives (assignments of sequences to the wrong organisms). Such sequences are compared against DNA reference databases, which primarily contain modern sequences.

The shorter the fragment, the less information it carries, and thus the lower its specificity. This can result in false positives or, conversely, in very general and non-specific taxonomic classifications. In the case of plant sedaDNA, taxonomic assignment is further complicated by the high genetic similarity of closely related taxa and the incomplete representation of species in reference databases. Consequently, sequences can often only be assigned to higher taxonomic levels, such as genus or family, rather than to individual species. This limitation is particularly important in archaeobotanical studies, where distinguishing between species is often crucial for interpretation. Applying sequence filters and minimum length thresholds reduces the occurrence of false-positive identifications (*de Filippo et al. 2018*). However, such filtering entails a sensitivity–specificity trade-off and can lead to loss of low-abundance taxa from the dataset (*Alsos et al. 2024*). In practice, deep sequencing (sequencing multiple times to detect rare or low-abundance taxa) is employed only after verification that the sample contains an adequate amount of aDNA (*Armbrecht 2020*; *Cribdon et al. 2020*).

If the research is focused on a specific group (in our case plants), the researchers can also choose between metabarcoding and target enrichment of taxonomically informative regions. The common advantage of these methods is a smaller amount of output data

(reduction from hundreds of millions of reads to hundreds of thousands). In sedaDNA studies, the short and variable region trnL P6 loop (13–158 bp) is mainly used as a plant barcode (*Taberlet et al. 2007*), which allows identification of most genera (more than 75%) and one-third of species (*Sønstebø et al. 2010*). The main limitation of metabarcoding for sedaDNA studies is the difficulty of data authentication. This is because polymerases used in PCR tend to correct damaged bases, thereby removing the characteristic terminal substitutions that are typically used to verify aDNA authenticity. Consequently, it is not possible to determine whether the DNA is ancient. Despite this major obstacle, metabarcoding is the main method used by scientists for plant sedaDNA, also due to its cost. The target enrichment method (also known as the hybridisation enrichment approach), in which specially designed probes bind to target DNA, is also very promising for the authentication of aDNA. The designed probes could bind to, e.g. trnL, rbcL, and matK plant regions (*Murchie et al. 2021b*). This enrichment of a library can be performed in two rounds to increase coverage of target regions. However, target enrichment is less frequently used than previous methods because it is more expensive, and probes specifically designed for plants are still in development.

Despite the major use of metabarcoding (up to 75%) in plant sedaDNA studies, there is pressure on researchers to choose methods that can distinguish aDNA from recent contamination, as some authors strongly recommend (*Armbrecht 2020*; *Marchesini et al. 2022*). Ensuring the DNA is not modern contamination is critical, particularly in terrestrial sediments.

## Bioinformatics

The DNA fragments are sequenced using Illumina sequencing technology, generating raw sequencing data that are subsequently processed using dedicated bioinformatic pipelines specifically designed for analysing ancient environmental DNA sequences, such as the nf-core/eager (*Fellows Yates et al. 2021*) or NED-flow (*Nota 2026*). In general, the bioinformatic processing is highly demanding in terms of both time and computational resources, necessitating the completion of numerous steps, including quality control and the filtering of raw data before its mapping to reference genomes (*Orland et al. 2021*). Filtering improves the accuracy of sequencing data and reduces false positives (*Filippo et al. 2018*). It involves removing poor-quality reads, duplicates, reads with repetitive sequences, and other potential artefacts. In addition, negative controls (blanks) are sequenced alongside samples to detect contamination introduced during laboratory work, and any contaminant sequences identified are subsequently filtered from the dataset.

Fragmented and chemically modified plant sedaDNA resulting from post-mortem damage must be compared against large databases of available genetic data from various species and assigned to specific taxonomic groups. However, due to the specificity of some regions and DNA preservation, a species level is not always reached. At the end, the resulting sequences must be confirmed as sufficiently ‘ancient’ (not merely the result of contamination). Further details on the bioinformatics process can be found in various studies (e.g. *Pedersen et al. 2016*; *Heintzman et al. 2023*). Due to computational requirements on memory and speed, the entire process is usually conducted via high-throughput computing facilities.

## Applications of plant sedaDNA in environmental and archaeological research worldwide

The fact that aDNA is preserved the longest in cold regions has been the basis of almost all studies with sedaDNA. Lakes are the most common type of environment from which researchers obtain sedaDNA, with permafrost a close second. Studies from cold (even polar) regions include those from Siberia (*Zimmermann et al. 2017*; *Liu et al. 2020*; *Schulte et al. 2021*; *Huang et al. 2021*; *Courtin et al. 2022*; *Hippel et al. 2022*; *Stoof-Leichsenring et al. 2022*; *Perfumo et al. 2023*; etc.), Greenland (*Pedersen et al. 2013*; *Epp et al. 2015*), Canada (*Crump et al. 2021*; *Murchie et al. 2021a*), Alaska (*Wang et al. 2017*; *Edwards et al. 2021*; *Clarke et al. 2024*), Siberia and Alaska (*Courtin et al. 2025*), and the circumpolar Arctic (*Willerslev et al. 2014*). In addition to the lake sediments and permafrost, there are several studies of unique nature. For example, the studies that researched nunataks in Greenland (*Jørgensen et al. 2011*) or also the midden deposits from Greenland (*Seersholm et al. 2016*) should be mentioned. In cold regions outside the northern part of the world, studies come from mountain ranges and plateaus. Examples include studies on the Tibetan Plateau from both off-site lacustrine sediments (*Jia et al. 2022*; *Tian et al. 2024*) and on-site archaeological strata (*Gu et al. 2023*).

We encounter plant sedaDNA studies even in tropical environments, which are generally considered unfavourable for aDNA preservation. The main challenges include high temperatures, wet-dry cycles, and microbial activity. In addition, depositional environments that have been studied in tropical regions involved not only lake sediments but also swamps, which are problematic because fluctuating water tables, variable redox conditions, and organic-rich sediments typically promote aDNA degradation rather than preservation. Nevertheless, successful studies from lakes (*Bremond et al. 2017*; *Epp et al. 2019a*; *Tabares et al. 2020*), swamp and wetland deposits in Africa (*Dommain et al. 2020*; *Augustijns et al. 2022*) and Indonesia (*Ekram et al. 2021*) demonstrate that sedaDNA can still be preserved in a condition sufficient for analysis.

The aforementioned studies reconstruct vegetation dynamics and biodiversity for different time periods, especially the Last Glacial Period and the beginning of the Holocene. The research of *Ter Schure et al.* (*2022*) was able to reach even deeper into the past and provide insight on plant use in the Upper Palaeolithic during the 15,000 years of occupation of the Aghitu-3 cave in the Armenian Highlands. The authors were able to reconstruct plant-based food (e.g. *Pisum sativum* L.), medicinal plants (e.g. *Achillea pseudoaleppica*), sources of plant dyes (e.g. *Lithospermum officinale* L.), tools (e.g. *Cirsium* sp. as a tinder), and repellents (e.g. *Artemisia* sp.), among others.

European research on plant sedaDNA is diverse, both in the type of environment in which it is carried out and in the research questions. As in global studies, the most common sampling environments are lake sediments (*Alsos et al. 2021*; *Poliakova et al. 2021*; *Zetter et al. 2021*; *Brown et al. 2022*; *Van Vugt et al. 2022*; *Elliott et al. 2023*; *Harning et al. 2023*; *Morlock et al. 2023*; etc.). Other sites include open-air archaeological sites (*Hudson et al. 2022*; *2023*), caves and rock shelters (*Marchesini et al. 2022*; *Zampirolo et al. 2024*), palaeo-river sediments below current sea level (*Gaffney et al. 2020*; *Cribdon 2021*), pond (*Tams et al. 2018*), river and intertidal zones (*Everett 2021*), peatlands (*Parducci et al. 2015*), lynchets (*Pears et al. 2025*), and ancient human latrines (*Søe et al. 2018*).

European studies largely address the Last Glacial Period and the Holocene. Only a few of them focus on specific issues related to an archaeological context, with notable exceptions including studies conducted by *Zampirolo et al.* (*2024*), *Tams et al.* (*2018*), *Hudson et al.* (*2023*), and *La Torre et al.* (*2026*). *Zampirolo et al.* (*2024*) challenge the perception of the sandstone rock area as an untouched wilderness, revealing that the human impact on the forest landscape began as early as the Late Neolithic and Bronze Age. By analysing plant, animal, and microbial sedaDNA, they reveal that this transformation was driven by the systematic exploitation of forests through the grazing of domesticated animals. Human-nature interactions have been a focus of many more studies, with central motifs being various interventions in the landscape, either to lighten the forest and attract wildlife for hunting (*Hudson et al. 2022*) or deforestation associated with pastoralism and crop cultivation (*Pansu et al. 2015*; *Ter Schure et al. 2021*; *Zetter et al. 2021*; *Brown et al. 2022*; *Garcés-Pastor et al. 2022*; *Van Vugt et al. 2022*; *Messager et al. 2022*). Emphasis is also placed on the diet and health of people throughout history. Such studies draw information from somewhat atypical environments such as ancient latrines and wastes (*Søe et al. 2018*; *Tams et al. 2018*).

## Integrating plant sedaDNA into archaeobotanical research

To contextualise the application of sedaDNA in archaeobotanical studies, it is important to first outline the conventional analytical methods and consider how sedaDNA can complement and integrate with them. Accordingly, the following section provides an overview of palynology—regularly conducted in tandem with plant sedaDNA analyses—followed by sections on phytolith, charcoal, and plant macroremain studies (*Fig. 2*).

Pollen from some species can be transported over long distances by wind, meaning that its presence often reflects regional rather than local vegetation (*Sjögren et al. 2017*; *Wang et al. 2017*; *Liu et al. 2020*). The phenomenon of long-distance pollen transport has been extensively studied by palynologists, as pollen analysis has proven successful both in (a) reconstructing local and regional vegetation and (b) statistically distinguishing between local and regional signals (*Bradshaw 1991*; *Sugita 2007a, b*).

In contrast, sedaDNA analysis dominantly reflects the local presence of taxa (*Parducci et al. 2015*; *Van Vugt et al. 2022*). This distinction is clearly illustrated by *Wang et al.* (*2017*) in their study on St. Paul Island. Although pollen records indicated the presence of *Picea*, *Betula*, and *Alnus*, sedaDNA from the same site did not detect these genera, strongly suggesting that they never colonised the island during the studied period. It should be emphasised that sedaDNA is not derived from pollen (*Brown et al. 2025*). However, depending on taphonomic processes and laboratory procedures, there remains a small possibility that pollen may be the source of a certain aDNA fraction (*Parducci et al. 2005*).

While the ability of sedaDNA to capture only locally present taxa can be crucial in an archaeological context, it may represent a limitation in studies aiming to reconstruct regional vegetation dynamics or biodiversity. It is often the case that pollen and sedaDNA are used as complementary methods (*Zimmermann et al. 2017*; *Parducci et al. 2019*; *Tian et al. 2024*). This is exemplified in the study by *Messager et al.* (*2022*), which used pollen analysis to reconstruct the phases of deforestation and afforestation in the perialpine zone, while the composition of cultivated crops was determined with sedaDNA.

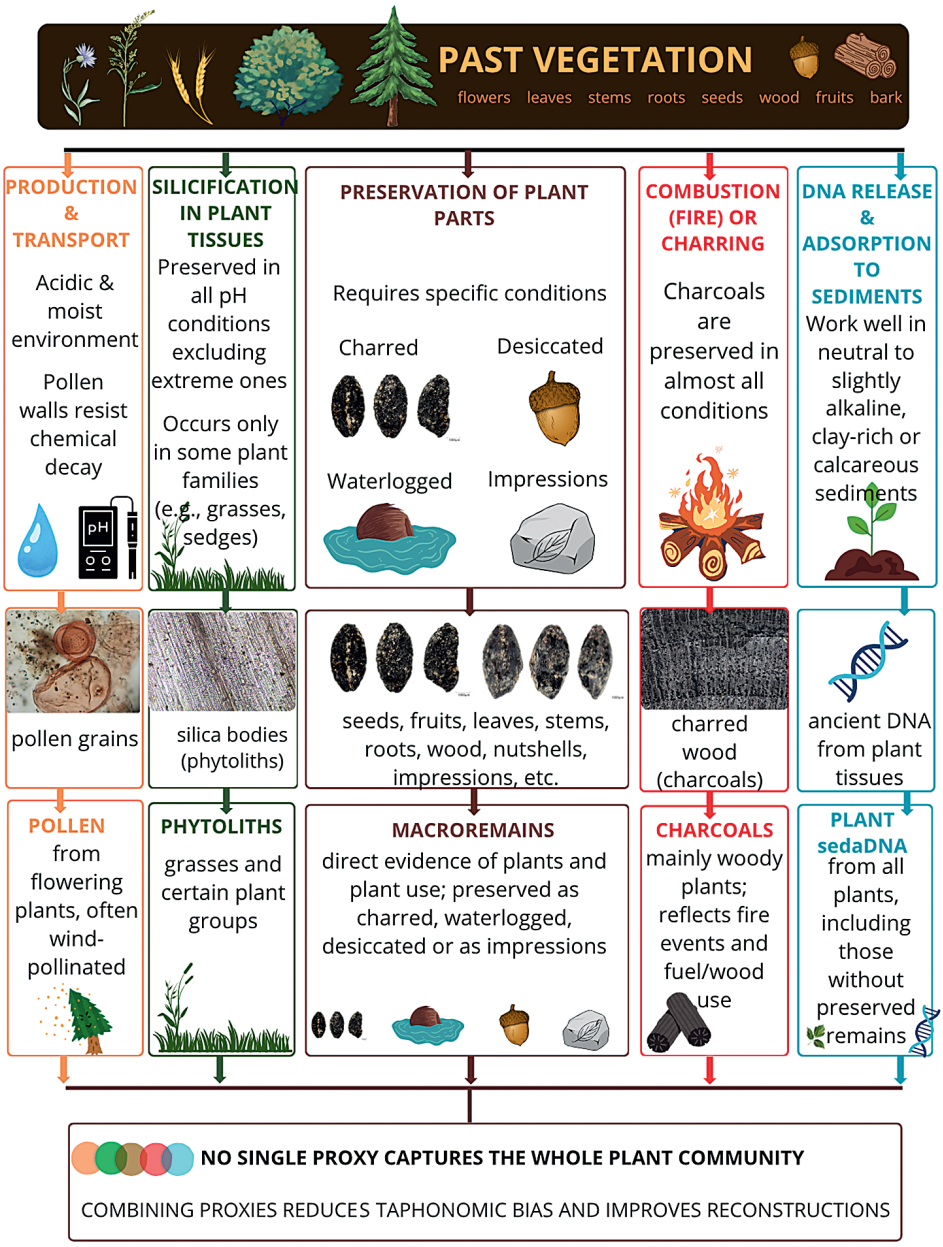



Fig. 2. Taphonomic pathways of plant material: Different processes and conditions preserve different traces of past vegetation (created by the authors using Canva).

Some studies point to the limited taxonomic resolution of pollen analysis (*Sønstebø et al. 2010*; *Pedersen et al. 2016*) and, conversely, better results of sedaDNA (*Sønstebø et al. 2010*; *Vugt et al. 2022*; *Tian et al. 2024*). However, this always depends on the type of sediment and the environmental conditions that allow good preservation of aDNA.

The results of *Hudson et al.* (*2023*) illustrate this point. Their data from a wetland show that sedaDNA reveals greater plant diversity (87:36 determination in favour of sedaDNA). The representation of trees in the study was almost comparable, in contrast to the representation of herbs, which differed markedly between the methods. SedaDNA analysis revealed up to four times more herbaceous taxa than pollen analysis. It is well known among palynologists that insect-pollinated plants are low pollen producers and are often underrepresented in pollen records (see, for example, *Broström et al. 2008*). These taxa can be more effectively detected through sedaDNA analysis (*Capo et al. 2021*). A clear example demonstrating both the improved taxonomic resolution of grasses and the enhanced detection of entomophilous flora is provided by *Clarke et al.* (*2024*).

In cases where palynological identification is limited by the morphological similarity of pollen grains or by poor preservation, sedaDNA can provide valuable complementary information. For instance, within families such as Poaceae, Cyperaceae, and Brassicaceae, distinguishing pollen grains with highly uniform morphology is particularly challenging (*Schüler – Behling 2011*; *Deza-Araujo et al. 2022*). Similarly, in the case of *Cannabis* and *Humulus*, which are usually classified together as the *Cannabis/Humulus* type in palynological studies (*Deza-Araujo et al. 2022*), sedaDNA can assist in improving taxonomic resolution. Pollen preservation is generally poor in alkaline (calcareous) and loess sediments but good in acidic ones (*Tomescu 2000*), showing the opposite pattern of aDNA. Thus, the two methods complement each other depending on the pH context.

In a similar vein, sedaDNA offers the potential to complement also the phytolith and starch grain analysis, but to the authors' knowledge, no comprehensive comparative studies of these three proxies have yet been conducted. Where wind transport may complicate the reconstruction of local vegetation through phytoliths, sedaDNA could establish evidence of local taxa. It could also overcome other issues. Similar phytolith morphotypes often occur across unrelated genera, making taxonomic identification difficult (*Shillito 2013*). Phytoliths are absent in certain taxa (e.g. *Taxus*, *Cedrus*, *Lilium*, *Vitis*; *Piperno 2006*), and in most assemblages, grass phytoliths tend to dominate, leading to potential overrepresentation of this group.

On the other hand, phytolith analysis provides relatively high taxonomic resolution among grasses, which is essential for studies focusing on grassland ecosystems (*Hošková 2025*). Phytoliths are often preserved in ash layers where the soft tissues of plants such as grasses or sedges have been burnt, and no other botanical remains can survive (*Shillito 2013*). However, unlike pollen, they rarely persist in acidic environments (*Croft et al. 2018*), similarly to aDNA.

Starch grain analysis can further provide direct evidence of the processing and use of starchy plant foods; it is mainly applied to stone artefacts (*Piperno et al. 2004*), ceramics, and dental calculus (*Barton – Torrence 2015*). However, the preservation and identification of starch grains in sediments are strongly influenced by taphonomic processes, including microbial degradation and soil properties (e.g. clay content; *Haslam 2004*). The overlap in taphonomic factors affecting starch and DNA preservation suggests that sediments preserving starch grains may also represent promising contexts for the preservation and recovery of plant sedaDNA.

Another significant method in archaeobotany is charcoal analysis, which reflects the vegetation of the immediate surroundings and can be used to reconstruct the vegetation development around archaeological sites (*Novák et al. 2017*). Nevertheless, the analysis

of charred wood remains reflects only a fraction of the actual plant spectra at the site, as it primarily includes material related to fuel use, waste, or wooden structures. Typically, only woody taxa are captured (*Ludemann – Nelle 2017*). Analysis of sedaDNA can extend the plant record beyond taxa preserved as charcoals and may capture a broader range of plants preserved at or introduced to a site.

Most of the plants used by humans in prehistory are not identified in the archaeological record because soft parts, which were utilised most often, were not preserved in sediments (*Behre 2008*). According to *Parducci et al.* (*2015*), aDNA can detect taxa with 'soft and easily degraded tissues that are often under-represented in fossil records'. However, plant macroremains (charred, desiccated, or waterlogged seeds, leaves, etc.) can provide valuable information in contexts where aDNA rapidly degrades and is poorly preserved. Consequently, these two methods are highly complementary. This is illustrated in the study by *Gu et al.* (*2023*), in which researchers reconstructed the subsistence economy at open-air archaeological Neolithic sites on the Tibetan Plateau. Both approaches detected *Hordeum vulgare* and *Triticum aestivum*. In addition, macroremains revealed *Chenopodium* and other Poaceae grains at the family level, whereas sedaDNA identified *Setaria italica*, *Setaria viridis*, *Digitaria*, and *Panicum*, but did not detect *Chenopodium*.

In addition, sedaDNA can be applied to the analysis of vessel infills. This approach is exemplified by *Kučera et al.* (*2019*), who presented the first evidence of broomcorn millet (*Panicum miliaceum*) in Central Europe, dating to the Eneolithic period. It was detected through the chemical marker miliacin. Their finding indicates a much earlier arrival of millet, previously thought to have arrived in the Bronze Age. However, these results are being questioned in the archaeobotanical community because of the methodology (*Filipović et al. 2020*), the fact that miliacin is also present in various other plants (*Bossard et al. 2013*), and because contamination by different sediments in this case cannot be ruled out. This puzzle could be solved with the help of sedaDNA analysis with the required authentication of the sequences. Moreover, sedaDNA analysis can be applied to grinding slabs as a complementary approach to phytolith and starch grain analysis, particularly when these remains are poorly preserved or occur in low quantities.

Similarly, to date, no known studies have specifically investigated the interaction between metal artefacts and the preservation of aDNA. However, research on the preservation of organic materials by metals (e.g. *Šálková et al. 2015*) suggests that metal objects may contribute to improved DNA preservation in their immediate vicinity. For instance, oxidised bronze surfaces can form metal oxides or hydroxides, which may theoretically interact with the negatively charged phosphate groups of DNA. This interaction raises the possibility that DNA could adhere to metal surfaces under specific conditions. Additionally, if textiles are present near these objects (or on them), their fibres could offer a certain degree of protection for DNA. Although recovering aDNA from metal objects presents significant challenges, further investigation into this potential preservation may be worthwhile.

## Conclusion

Sedimentary ancient DNA can serve as a bridge between traditional archaeobotanical and palaeoecological proxies, providing information not only on which plants were present but also on how they entered the local environment through natural processes, human

activities, or broader ecological change. Whereas sedaDNA from natural deposits primarily reflects natural transport and sedimentation processes, archaeological sediments often contain a stronger signal of human behaviour, including plant use, resource management, mobility, and land-use practices. When combined with animal and microbial aDNA, as well as conventional proxies such as pollen, charcoal, and faunal remains, sedaDNA can contribute to more detailed reconstructions of human-environmental interactions and landscape transformation. In addition, DNA damage patterns may provide valuable information on stratigraphic integrity and preservation biases.

To sum up, plant sedaDNA offers several advantages, including the ability to detect taxa without reliance on visible remains, generally good preservation in calcareous, clay-rich, sandy, and loess deposits, and the possibility of authenticating ancient DNA. When well-preserved, it may also provide higher taxonomic resolution than traditional proxies such as pollen or plant macroremains. However, its interpretation requires careful consideration of taphonomic processes, preservation biases, and the local nature of the sedaDNA signal. In addition, sedaDNA is poorly preserved in acidic environments and carries a risk of false positives due to short DNA fragment lengths. Therefore, plant sedaDNA cannot replace traditional archaeobotanical and palaeoecological methods; rather, it complements them by providing an additional line of evidence and enabling a more comprehensive understanding of past environments, human activities, and their interactions.

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