

The Implementation of SedaDNA Analysis in Roman Archaeology in the Netherlands

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Preface

Dear readers,

After a semester of hard work I am proud to present to you my master thesis in Roman archaeology. During this research I had the pleasure of collaborating with some of the researchers on the “Constructing the Limes”-team. They gave me this great thesis opportunity with a topic that I am very passionate about. SedaDNA research combines all the topics that I love, archaeology, biology, environmental studies, all mixed with a good amount of technical challenges. It was not an easy process to write this. Many of the research topics involved in this study were quite new to me, so obtaining in-depth knowledge of all the different elements of my research question was a time consuming and challenging process. Nonetheless, I feel like I have learned a lot in the past semester, not only within the contents of this thesis, but about the process as well.

I want to extend many thanks to everyone who has supported me during this process. I have had a lot of good feedback from my supervisors Astrid van Oyen and Arjen de Groot, assisted by Fabricio Furni and PhD-student Kadir Özdoğan. They have helped me throughout the whole process of my thesis, even if I was not always the easiest person to supervise. Additionally, I have met with three researchers, who have provided me with valuable information from their experience in the research field. I thank Laura Kooistra, Maaïke de Groot, and Fabricio Furni for their time and efforts.

Before you move on to reading the thesis, I want to say a last thank you to all of my friends and family members who have supported me throughout this whole process. I hope that you enjoy reading this thesis, the pleasure is all mine.

Much love,

Mirjam Rijpma

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Chapter 1 Introduction

The development of the analysis of sedimentary DNA (sedaDNA) has brought advances to a number of fields like ecology and paleoecology (Alsos et al., 2022; Brown et al., 2025). In Roman archaeology, however, this technique is not often used to research bioarchaeological material. Implementing sedaDNA analysis in Roman archaeology brings on a number of challenges, due to the degradation, migration and contamination of DNA molecules in the soil (Özdoğan et al., 2024). In order to combat this, many studies are currently done with the goal of improving sedaDNA analysis to make it applicable in Roman archaeology (Özdoğan et al., 2025). However, once the technique is ready to be implemented in bioarchaeological research, it is important as well that we know how this technique can fill research gaps currently found in Roman bioarchaeology. To research the possible advantages of sedaDNA, this paper focuses on the possibilities of current bioarchaeological techniques used in archaeobotany and archaeozoology, and its limitations. After establishing the current research gap, the ways in which sedaDNA could potentially help resolve those limitations are explored. To finish, current limitations of sedaDNA analysis and the steps that researchers need to take in order to resolve those limitations are established. With that information, this paper gives an overview of the current situation within Roman bioarchaeology, the potential of sedaDNA and the future steps needed to be able to implement the technique in the field.

Status Questionis

Archaeobotanical and archaeozoological research first started developing during the 19th century, but did not really gain popularity until the 20th century (Albarella, 2017; Lodwick & Rowan, 2022). Archaeozoology is defined as the discipline within archaeology that studies animal remains (Albarella, 2017). Archaeobotany is defined as the discipline within archaeology that studies plant remains (Lodwick & Rowan, 2022). Together, these two disciplines aim to reconstruct parts of the Roman world such as dietary practices, agriculture, and husbandry (Groot & Kooistra, 2009). In the Netherlands, Roman archaeobotany and archaeozoology have mostly been focussed on the southern half of the country, divided by the lower Rhine and the Germanic Limes (Groot & Kooistra, 2009). In order to research the developments in archaeological research techniques, I have first summarised the current reconstruction of Roman civilisation in the Netherlands.

Roman history in the Netherlands begins with Caesar's conquests of Northern Gaul during the Gallic wars around 50 BC (Mols & Polak, 2020; Osgood, 2009). After his victory over the Eburones in 54 BC, Caesar conquered the area below the Rhine in the south of the Netherlands (Van Enckevort & Heirbaut, 2015). In his *Commentarii de Bello Gallico* Caesar writes about his expeditions through the Netherlands, and he proclaims to have reached the Rhine area by 55 BC, where he later settled after failed attempts of crossing the river (Hunink, 2000). After the Romans came to the Netherlands various Roman settlements were formed (Mols & Polak, 2020). These settlements started out as military camps during Caesar's expedition, but over time, as more soldiers, retired soldiers and civilians found their place in the lower Rhine area, more towns started to form. The growth in this part of the empire was spurred by the establishment of Germania Inferior by emperor Domitian (Pellegrino, 2020). The area had periods of growth (1st century AD) and periods of defeat (4th century AD), but overall, it managed to stay the Northern border of the Roman empire until the conquest of the Franks in 450 AD (Mols & Polak, 2020).

Geographically, the Dutch sediment composition consists mostly of sand and clay (Habermehl, 2014). In the Roman period, the coast was characterised by large bogs, making the area mostly inhabitable. The central and eastern parts of the country were defined by a more diverse river landscape, which was more open to habitation (Habermehl, 2014). Agriculture there was still challenging, as the area was filled with sandy soils with limited fertility (Habermehl, 2014). Habitation in the lower Rhine region of the Netherlands consisted mostly of small towns and settlements with wooden houses, instead of the big towns and villas that are commonly seen in other parts of the Roman empire (Groot et al., 2009). Nonetheless, an extensive practice of husbandry and agriculture took place along the Northern Germanic Limes.

Archaeozoological research by Groot (2016) shows that the diet of early soldiers and inhabitants relied mostly on pigs, with cattle being the second most consumed animal. During the 1st century AD, when the population grew larger and Germania Inferior established the military and urban centre Ulpia Noviomagus Batavorum, consumption shifted towards a cattle-based diet, with pig becoming the second most consumed animal (Groot, 2016). There is evidence to suggest that the rural area could provide enough animal products to create a surplus that could be used to feed the urban population and the military (Groot et al., 2009; Groot, 2016). However, there is a possibility that part of the cattle was imported, for example by regions North of the Rhine (Groot, 2016). Apart from husbandry, wild animals were probably hunted for consumption as well (Groot & Kooistra, 2009). These animals could vary from wild cats or hares to brown bears (Groot & Kooistra, 2009). As populations grew and the market demanded more food, husbandry developed accordingly. There is no clear sign that farming became more specialised (Groot & Kooistra, 2009; Kooistra et al., 2013). However, to adhere to the growing demand, farming needed to be optimised (Groot & Kooistra, 2009). This meant an upscale in animals needed on the fields for ploughing and transport (Groot & Kooistra, 2009). It was not always necessary to breed more animals though, as animals that needed to be supplied to the army could first be used as plough animals (Van Dinter et al., 2014).

Archaeobotanical research shows that until the end of the 1st century, arable farmers mostly grew barley, wheats (bread, emmer and spelt), oats, and millet (Kooistra et al., 2013). Remains of nuts, fruits and herbs in forts along the Limes show that these were also consumed by Romans (Kooistra et al., 2013). Consumption in the military fort and that in settlements was probably similar, since they were both dependent on the supply of farmers in the region, and on import (Kooistra et al., 2013). There are some indications that urban settings allowed for small vegetable gardens, but nothing that could support a self-sufficient lifestyle (Kooistra et al., 2013). We know the Romans consumed vegetables as well, because of remains such as beet seeds, but vegetables and herbs used in the kitchen are difficult to analyse, because their remains are usually not well preserved (Groot & Kooistra, 2009). Based on the calculations done by Van Dinter et al. (2014) an estimated 50% of all cereals needed in the region could be produced by local farms. However, new calculations by De Kleijn et al. (2018) show that this hypothesis could probably not hold up from the end of the 1st century forwards. This means that a good portion of the cereals consumed in the Roman Netherlands were imported from other regions.

Apart from consumption, plants and animals were also used for other purposes, such as textiles and tools. Cows were used for leather, sheep were used for wool, wood was needed for the construction of buildings and for fuel, and reeds were used for basketry (Van Dinter et al., 2014; Van Enkevort et al., 2024). In the Netherlands, organic materials are generally well-preserved because of waterlogging (Van Dinter, 2013; Van Enkevort et al., 2024). Still, it is difficult to fully reconstruct the use of textiles and tools. Many resources in the Roman period were

recycled once they had fulfilled their original purpose. For example, wood was used to make fire, and wool and leather were often repurposed, thereby removing them from their original context (Van Enkevort et al., 2024). We can still reconstruct the use of such resources to a certain extent. For example, in Deurne leather pieces were retrieved during the excavation of a Roman settlement (Van Driel-Murray, 2000). The different pieces of leather turned out to be part of a Roman helmet, and a collection of shoes (Van Driel-Murray, 2000). These items indicate the presence of a military in the neighbourhood, but more specifically, the assembly in which the items were found could be an indication of a ritual related to the end of a military service (Van Driel-Murray, 2000).

Regarding religion and ritual, it is difficult to fully reconstruct the use of organic products in religious and sacrificial rituals. In Empel, at the temple complex, animal bones were found, which could suggest religious slaughter practices (Roymans & Derks, 1994). However, since many of the bones were found in a well quite far from the temple, they might also have been used for purposes outside of the temple (Roymans & Derks, 1994). Either way, it is probable that the Romans used to conduct some form of offering, where the slaughter and consumptions of animals played a big role.

In funerary practices, animals were also involved. In Groot (2018) a study is conducted where the animal remains in funerary contexts are analysed, for example in the burial site at Tiel-Passewaaij. There, various animal remains were found in cremation graves, although a decrease in material can be seen from the Iron Age on. The remains indicate rituals regarding food. In some places, unburned animal bones on plates were even found, which could mean food offerings were made to the deceased. Both for religion, and for funerary practices, it is difficult to say whether plants were also used in Roman rituals in the Netherlands, as studies on the topic are scarce (Groot, 2018).

Archaeozoological and archaeobotanical material alone is not enough to reconstruct Roman practices. When contextualising Roman bioarchaeology, it is important to look at find context as well. Find context includes the history of a site, both from ancient literary sources and from dating through material present on site. Furthermore, bioarchaeology can be understood by looking at depictions of food or agriculture in art, and with material findings on site that provide context for biological material. Material findings that are useful in that regard are cooking pots, farming tools, or larger structures such as villa structures (Groot, 2016; Habermehl, 2014).

Ancient literary sources and art from the Roman period have the ability to provide insight from contemporary figures regarding the Roman diet, or religious practices. A strong disadvantage of this is the fact that it is biased from the perspective of the creator and can be inaccurate to reality (Habinek, 2001). Literary pieces were often written by higher class members of Roman society with a specific goal in mind (Habinek, 2001). For example, one of the most extensive primary sources we have about Romans in the Netherlands is Caesar's *Commentarii de Bello Gallico*. Though this book provides insight in the history of the Northern part of the Roman empire, it is also heavily biased in order to make Germanic tribes and the Germanic landscape look barbaric and wild. Roman art in the Netherlands is scarce (Hunink, 2000). There are few distinguishable paintings or other art sculptures depicting food retrieved from Roman sites in the Netherlands. It is therefore difficult to reconstruct what Roman diets or rituals might have looked like through the use of art.

Roman sites in the Netherlands are usually rich in material findings that can help frame bioarchaeological material. Ceramics or metals are found often on site, and through analysing these materials it is possible to make estimations of a site's date and it can give more insight in trade patterns or imported goods, for example (Groot et al., 2009). Metal nails can also give context to leather findings, as they were used in the construction of shoes (Van Driel-Murray, 2000). Cooking pots containing grease can be analysed for cooking habits (Interview Kooistra, 2025). Structures found on site can help estimate if an area was used for farming, and if so, in which magnitude (Habermehl, 2014).

Finally, one research field that is closely connected with bioarchaeology is human archaeology. Though this is also a biological field, it differs from archaeobotanical and archaeozoological research in the fact that it focuses solely on human material. Recently many advances have been made in the field of human archaeology in the Netherlands. By using isotope analysis, cremated remains can be researched to look at changes in the human diet (Kootker et al., 2022). This could indicate mobility, or a change in imported foods. Either way, understanding the way humans moved or obtained their food can give us valuable insight into the way humans, animals and plants were distributed across the Roman empire (Kootker et al., 2022).

Both find context and the analysis of human material are important aspects in reconstruction the Roman practices. The compact overview I have given in the previous paragraphs show that it is essential to consider these aspects when conducting bioarchaeological research of the Roman period. However, these fall outside of the scope of this research paper. They will not be analysed in depth in this thesis, but I acknowledge their crucial function in Roman archaeology. With developments being made in all three research fields, archaeology of material culture, bioarchaeology and human archaeology can work together to provide the most detailed and accurate reconstruction of life in the Roman empire in the Netherlands. This is something that should be kept in mind when looking at archaeozoological and archaeobotanical research individually.

In general, organic material from the Roman period in the Netherlands is well preserved, thanks to the wetland areas along the Rhine that enable waterlogging (Van Dinter, 2013). Still, a lot of aspects of the Roman period in the Netherlands remain unsure. For example, rituals, migration patterns, and certain components of the Roman diet are understudied in the Netherlands, both due to a lack of preserved material and due to the limited potential of the material that is present on site (Van Enckevort et al., 2024).

A potential way to decrease the lack of available material on site is the retrieval of sedimentary DNA (sedaDNA) (Özdoğan, 2024). In archaeological context, sedaDNA is the retrieval of DNA from soil or sediments, which hold DNA from all organisms that have interacted with that soil (Özdoğan 2024). It is therefore both environmental DNA (eDNA) and ancient DNA (aDNA). The DNA found in sediments can provide a much more accurate and complete overview of life in the area than conventional bioarchaeological research could. While the technique is promising, it is not easily applied to Roman archaeology. There are still many questions surrounding sampling, analysing and interpreting sedaDNA. However, since the technique could potentially help with resolving some limitations currently found in archaeobotany and archaeozoology, in this paper I will be researching the possibilities, limitations and further steps required to integrate the use of sedaDNA into the field of bioarchaeological research.

Following this, I have constructed the following research question:

“To what extent can the implementation of sedaDNA analysis ensure a more accurate reconstruction of an archaeological site from the Roman period in the Netherlands, in relation to current bioarchaeological research methods?”.

In order to answer the main question, I have divided the research into four sub-questions as follows.

1. *What are the applications and limitations of current bioarchaeological research methods when researching different topics within Roman archaeology in the Netherlands?*
2. *How can the use of sedaDNA fill in the gaps left by current bioarchaeological research methods?*
3. *What are the current difficulties researchers face regarding the contamination and retrieval of sedaDNA?*
4. *Which further steps need to be taken in the current field of Roman archaeology in the Netherlands in order to be able to use sedaDNA in bioarchaeological research?*

Methods

In order to answer the main research question, I have conducted a qualitative research study divided by the four sub-questions, with both theoretical and applied research aspects. In this study there are different methods of data gathering. The literary study was done by analysing various research papers, which can be found in the bibliography. The aim of the literary review was to get an understanding of the status quo in Roman bioarchaeology, to research the possible applications of sedaDNA, and to identify limitations of sedaDNA analysis and how these could be resolved in the future, before integrating sedaDNA analysis as standard practice in Roman bioarchaeology.

In Chapter 2, the focus was to research the current bioarchaeological research techniques, in order to identify their limitations and their potential. Keywords I used for research queries are “archaeobotany”, “archaeozoology”, “overview”, “Roman”, and “reconstruction”. To properly show the process that happens to organic material after its usage, I designed a series of four models that illustrate each step of the process (see Figure 1 – 4). The first model shows the use of flora and fauna in the Roman period. The second model shows distinguishable features of organic material that can be found during excavation. The third model shows different analysis techniques of organic material in Roman archaeology. The fourth and final model shows the potential research applications of the analysis techniques in Roman bioarchaeology. All of the models were made using draw.io.

In Chapter 3, the aim was to research the potential applications of sedaDNA in Roman bioarchaeology. Keywords used in the query here are “sedaDNA”, “environmental DNA”, “ancient DNA”, “biodiversity”, “extraction”, “sequencing”, and “bioinformatics”.

The research goal in Chapter 4 was identifying the limitations and challenges currently found in sedaDNA analysis when applied in Roman bioarchaeology. Keywords I used are “sedaDNA”, “environmental DNA”, “ancient DNA”, “fragmentation”, “damage”, “contamination”, “PCR”, “NGS”, “costs”, and “database”.

In Chapter 5, the research objective was identifying the further steps that need to be taken to improve sedaDNA analysis for application in Roman bioarchaeology, based on the challenges found in Chapter 4. Keywords I used during the literature review are “occupancy detection models”, “quantification”, “qPCR”, “hybridisation capture”, “metagenomics”, “sampling”, and “nanopore sequencing”.

Additionally, I have also done oral data collection in the form of three expert interviews, in order to better understand the practical aspects of bioarchaeological research, and to get input from experienced researchers on the prospective of sedaDNA analysis as an archaeological research technique. The interviews were set up in a semi-structured way, so as not to steer the conversation too much with pre-determined questions. Instead, I decided on three or four general questions to ask the participants to encourage them to contribute their own input to the conversation as much as possible.

The first interview is with archaeobotanist Laura Kooistra, the transcript of which can be found in Appendix 1. Dr. Kooistra is an archaeobotanist who specialises in Roman paleoecology in relation to farming practices in Germania Inferior. She also strives to obtain a more multidisciplinary approach in archaeological research, especially within bioarchaeology, by collaborating with other specialists, such as the archaeozoologist dr. Groot.

The second interview is with Maaïke Groot herself. The transcript of this interview can be found in Appendix 2. Dr. Groot is archaeozoologist, who specialises in archaeozoological data in relation to husbandry practices in Germania Inferior. As both researchers are involved with the reconstruction of bioarchaeological aspects of the Roman world in Roman settlements in and near the Netherlands, and both advocate for multidisciplinary approaches to bioarchaeology, this made them good candidates to interview for this research. During the interview I discussed the status quo of archaeobotany and archaeozoology with them, both the possibilities and limitations. After that, I asked them about their perspective on the future directions of the field, and discussed how sedaDNA could form a possible addition to conventional research techniques.

The last interview is with geneticist Fabricio Furni, the transcript of this interview can be found in Appendix 3. Dr. Furni is a researcher for the “Constructing the Limes”-project (C-LIMES), where he is part of the research team currently working on the integration of sedaDNA in Roman archaeology. Since he specialises in genetic material and is actively working on sedaDNA analysis of Dutch sites, I found him a good candidate for the expert interview. During the interview, we discussed possible research topics that could be answered with the use of sedaDNA. I also asked him about the current potential that sedaDNA analysis techniques show regarding taxonomic resolution and accuracy, and what limitations researchers currently face with this developing technique.

The expert interviews were used as a framework for Chapters 3, 4 and 5. They gave me an introduction to the research topics within each chapter, but more importantly they provided me with insights from research experience in the field, a contribution that is difficult to get into proper perspective with just literary reviews. Since the aim of this study was to investigate how

sedaDNA could be integrated into bioarchaeological research in addition to conventional techniques to strengthen the potential research possibilities of the field, and the intention was not to replace conventional techniques as a whole, I thought it important to get input from experienced researchers in the field and work together with them to design what such research methods might look like.

The results of the literary review, combined with the expert interviews, can be found in the chapters 2 to 5. Each chapter discusses one of the sub questions of this research. In Chapter 6, the findings of all sub-questions are concluded to answer the main research question. This chapter will also provide the discussion, delving deeper into critical points of this research and addressing future directions.

Chapter 2 Applications and Limitations of Current Bioarchaeological Research Methods

In order to understand potential applications of bioarchaeological research, it is important to understand the journey of organic remains over time. In this chapter, I have created four models that schematically show the process that organic materials go through during their life, their decay, and ultimately during excavation. The models were constructed with the obtained knowledge in the Status Questionis. Specifically, the model in Figure 1 is in parts based on information that is difficult to retrieve from organic remains on site, and therefore uses knowledge gained from other sources such as Roman literary sources or depictions in art. Archaeobotanical and archaeozoological sources were used too, where possible. The rest of the models, shown in Figure 2 to 4, are primarily based on archaeozoological and archaeobotanical sources. These sources contain both literature analysing results from material data, and literature about the methodology of the two fields.

Ultimately, the goal was to schematically construct a representation of the many components that need to be accounted for when doing bioarchaeological research, in order to understand the depth and complexity of such research. Each of the models shows a step in the process of doing bioarchaeological research, which I have simplified into the key steps of material preservation. Therefore, certain aspects, like sampling methods, are not considered in this schematic visualisation. The first model, shown in Figure 1, focusses on the lifetime of organic materials, identifying key elements of the prevalence and use of flora and fauna in the Roman period. The second model, shown in Figure 2, shows the way in which organic material from the Roman period might be preserved on site, so all retrievable aspects of Roman biomaterial. The third model, shown in Figure 3, shows conventional analysis techniques suitable for organic material from the Roman period. The fourth and last model, shown in Figure 4, shows the reconstruction of the Roman period in the Netherlands in the way that we can currently reconstruct, using conventional research techniques. This way the first and fourth model are comparable to each other, to see how the potential reconstruction can differ from reality. If preservation and analysis processes were perfect, Figure 1 and Figure 4 would look the exact same. By comparing these it is possible to observe which aspects of the Roman world can be reconstructed with bioarchaeological research, and where limitations occur.

Use of Flora and Fauna in the Roman Period

The construction of Figure 1 is based on the Status Questionis of this research thesis (see Chapter 1). The left part of Figure 1 visualises the elements prominent in the natural landscape in and around a Roman settlement. Key factors that define the landscape are the soil, its vegetation, and the animals habiting the area. The right side shows the elements related to the production and consumption of a Roman settlement. To better focus on the individual aspects, this section is divided into the categories agriculture, husbandry, consumption and other usage. Key factors that define agriculture are manure and crops, with the use of manure/the former influencing the quality and quantity of the crops/the latter. Other factors that influence crop growth are the natural soil of an area, and its vegetation. Agriculture in turn influences the soil characteristics by taking up space for crops and changing the soil with manure.

Moving further right in the model, we can see how agriculture opens the possibility to husbandry by providing fodder for cattle. The model creates a loop, as cattle then turns this fodder into manure, feeding the land with nutrients beneficial to agriculture. The far right of

the model visualises the different products that come forth from either the land or from human production. Regarding animals, this can either be cattle or hunting products to eat. Additionally, animals were used to work on the land or for transportation, for fabrics like leather and wool, and worked bone was used for tools. Plants that came from both cultivation and foraging were used as food as well as reeds for basket weaving. In conclusion, this model gives us insight in the factors that come into play in biological processes in the Roman world and shows the materials that can be the origin of the remains we find today.

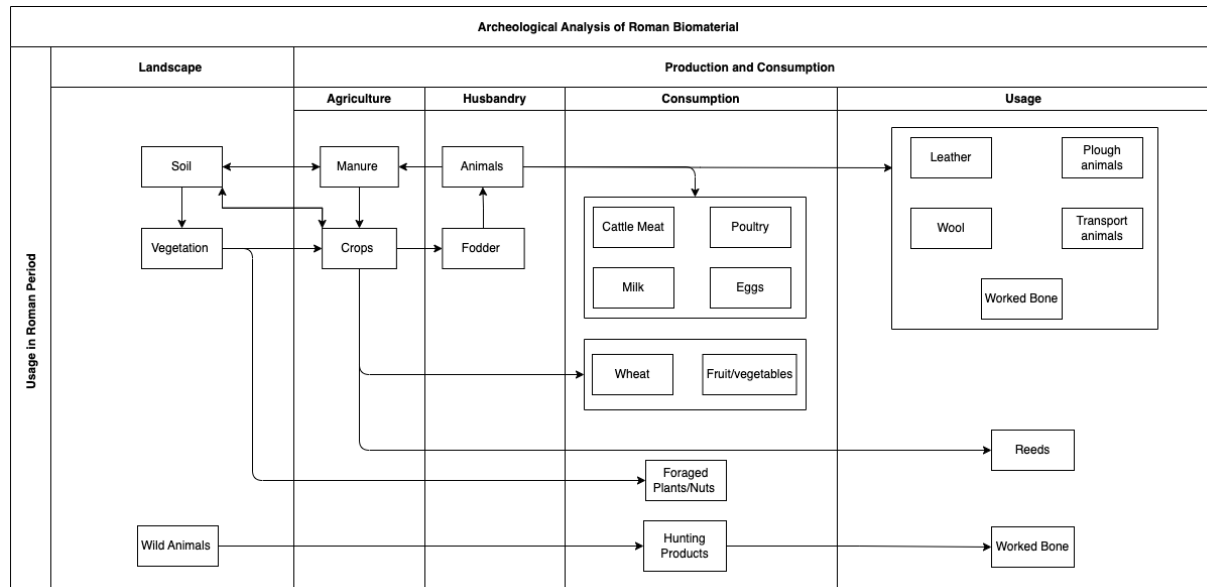


Figure 1: schematic model showing the use of flora and fauna in the Roman period

Aspects of Organic Archaeological Material

During excavation, all that we find from these elements of the Roman period are the parts of an organic structure that survive the decomposition process. The elements of each organic material shown in Figure 1 that we can still find during excavations today, are modelled in Figure 2.

Taphonomy

The model depicted in Figure 2 is constructed in the same way as the model in Figure 1. On the left we can see elements of the natural landscape, on the right we see elements of human practices. Animals, both wild and domesticated, leave their mark in the form of bones. Taphonomy determines how an animal gets preserved through time. Decomposition starts immediately after death, and follows a set pattern of putrefaction (rotting), liquefaction, disintegration, until finally skeletonisation occurs (Dent et al., 2004). Faunal remains from the Roman period are in the final stage of decomposition, meaning animal bones are the materials we find during excavations. The state and quality of these bones are dependent on the chemical processes that occur after skeletonisation, and factors such as the soil type and the burial depth of the bones influence those processes (Dent et al., 2004).

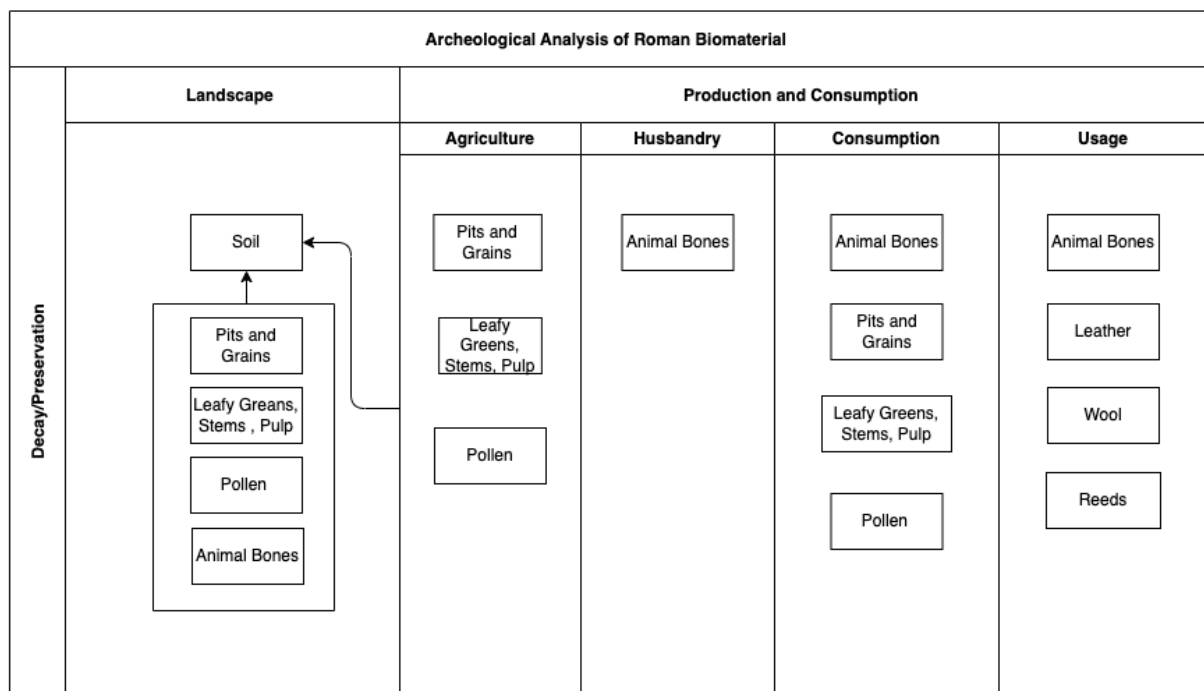


Figure 2: schematic model showing distinguishable features of organic material that can be found during excavation

Mineralisation

Floral remains have a more diverse preservation process. Roughly, their preservation can be divided into the processes of waterlogging, mineralisation, carbonisation, and desiccation, following the classification given by Lodwick & Rowan (2022). Mineralisation is a process that occurs when moist soil and material that is rich in phosphate (like bones) combine, causing an environment which is well suited for floral preservation. Mineralisation occurs most often in latrines and sewers, meaning that we mostly find fecal matter. Because our samples are collected from toilet components, we know that whatever organic material is found in these fecal samples was consumed by people at some point (Lodwick & Rowan, 2022).

Carbonisation

Carbonisation (charring) is a very common way of preservation. Through burning, plant remains get charred, discarding leafs and other parts in the process, but ensuring carbonisation of the sturdier parts of the plant, such as pits and grains. The best preservation of material is in places where a great fire occurred, which is most often cities (Lodwick & Rowan).

Waterlogging

Waterlogged material is most commonly found in areas that lie below the local water table, like ditches and wells, but can also be found in other places with rapid rises in the water table. Because these places form an anoxic environment, the absence of aerobic bacteria that feed off organic material prevents plant decay. This means that in waterlogged findings, more delicate parts of the plants are preserved. Because of this, waterlogging shows us information about other food sources that cannot be preserved through charring, like seedless fruits and leafy greens. This is the most common type of preservation found in the Netherlands. Textiles, such as wool and leather, can also be preserved through waterlogging (Van Enckevort et al., 2024). There are some sites in the Netherlands from which these materials have been retrieved. Wood can sometimes be found in waterlogged conditions as well, though wood was often repurposed

as fuel in the Roman times and therefore not always well-preserved (Van Enckevort et al., 2024).

Desiccation

Desiccation occurs only in areas where the soil is extremely dry, and is therefore most common in the parts of the Roman empire that were located in Northern Africa. Due to the aridity, there is an absence of plant-digesting bacteria, which enable the preservation of faunal material. The special circumstances that cause desiccation allow for very delicate parts of a plant to be preserved, like whole cloves of garlic. In the Netherlands, desiccation does not occur (Lodwick & Rowan, 2022).

Wild and Domesticated Remains

Animal remains can be difficult to categorise as wild or domesticated. Usually, faunal remains in a settlement context indicate domestication, as this is often a sign of husbandry. Animal bones found in dietary settings are most likely domesticated as well. However, hunting was an aspect of the Roman diet as well. Sometimes large collections of bones can be found, indicating hunt (Interview Groot, 2025).

Domesticated floral remains are more easily distinguishable from wild remains, as they have different processes of preservation. By rule of thumb, remains found in context of human settlement are always an indicator of human intervention (Interview Kooistra, 2025). Therefore, it is easy to determine that pits, grains, or leafy grains, all found in specific conditions such as waterlogging (water pits), mineralisation (latrines), or carbonisation (fire), are a product of vegetation. According to Kooistra (in interview, 2025), when excavating water pits, the determination of different phases of the pits can show whether material is domesticated or wild. Water pits in use were covered with a lid to keep the water clean. However, when the pit became out of use it would often be repurposed as a waste pit. Materials found from this phase are therefore a sign of human use, meaning plant materials are most likely domesticated or at least put there by humans.

Once the waste pit was abandoned, the place would be left alone. Over the course of time, the ground still compresses, which creates a deposition on top of the previous waste pit, where products of natural vegetation could gather via wind or pollinators. Understanding the different phases of use in connection to the different layers of the pit can therefore help make a distinction between domesticated and wild floral matter found on site. Additionally, it is difficult to find the remains of natural vegetation on site, although pollen are generally seen in situ. Agricultural materials sometimes contain weeds as well, showing what natural plants were found on the fields (Interview Kooistra, 2025).

Soil

The final type of material that remains of the Roman period is its soil. The soil contains many different types of microstructures, and gets influenced by the organic materials in it (Bhoyar et al., 2024). Worms migrate through the soil, mixing different layers with each other. Pollen or other material preserved in the ground can therefore migrate as well, getting removed from their original context in the process (Kooistra & Maas, 2008). Additionally, the decay of animal carcasses and plant rests give new organic materials to the soil (Dent et al., 2004). Understanding what type of material comes from which layer of the soil on site is an important

Microscopic Analysis of Archaeobotanical Material

To further analyse floral remains, a microscopic analysis is conducted. This allows the researcher to look further into the structures found on the exterior of the plant material and pollen. While this analysis can be done using a conventional electron microscope, in archaeology it is now becoming common practice to use an environmental scanning electron microscope (ESEM) (Lodwick & Rowan, 2022). The ESEM allows for scanning in more challenging environments, like water, meaning material doesn't have to go through an extensive sampling process, and thereby reduces the possibility to damage the material, or to alter the results by taking the material out of its environment (Primavera, 2017; Donald, 2003). Both macroscopic analysis and microscopic analysis of archaeobotanical material are common practice when doing archaeobotanical research in the Netherlands, as they are often used to research agricultural practices (Kooistra et al., 2013).

Skeletal Elements of Archaeozoological Material

The first step in analysing archaeozoological material is looking at skeletal elements of the material found on site (Groot, 2016). These skeletal features include: the fusion of epiphyses (the extremities of a mammal's long bones), the structure of the lower jaw (the mandibula), and its eruption or wear, and butchery marks (Groot, 2016). After inspecting the bones, they are then measured for their proportions using biometric analysis.

Biometric Analysis of Archaeozoological Material

Biometric analysis focuses on the physical aspects of an animal (Groot, 2016). The main technique used here is the withers height of an animal. This method uses the highest point of an animal, the withers (this technique is only relevant for four-leggeds), and measures its height to the ground. In archaeology, this method uses not the exterior of an animal, but its long bones to calculate the withers height. To expand this method to allow for an animal's width, the log-size index is applied (Wolfhagen, 2020). This technique takes several measurements of the bones and compares them to a reference measurement using Formula 1.

$$LSI_b = \log_b(x_{\text{measurement of material}}) - \log_b(x_{\text{measurement of reference}}) \quad \text{Formula 1}$$

In this formula, the base of the logarithm ("b") is free for the researcher to choose², as is the reference material, as long as all data are analysed with the same base and reference measurements (Wolfhagen, 2020). The LSI-index is a way to standardise data from different excavations, in order to make them comparable (Interview Groot, 2025; Wolfhagen, 2020). By plotting them all in relation to a common reference dataset, it is easier to see patterns and differences in different sites, time periods, or even between different subspecies of an animal (Interview Groot, 2025). It is also a way to make better use of smaller datasets, as the LSI-index collects information from all bones, meaning that datasets that would otherwise not be comparable (e.g. on one site a femur is found, and on another a tibia), can now be compared as well (Interview Groot, 2025; Wolfhagen, 2020). Both skeletal elements and biometric analysis are common practice in archaeozoological research in the Netherlands, often implemented to research cattle size or quantities (Groot et al., 2009; Groot, 2016).

² Most common bases chosen for the LSI-index are either base 10 or the natural base "e", for a more in-depth analysis regarding the impact of base-choice on the quality of LSI-analyses see "Wolfhagen, 2020"

Stable Isotope Analysis

A technique that can be applied to both floral and faunal remains is stable isotope analysis. There are four common types of isotope analysis used in Roman archaeology: nitrogen, carbon, oxygen and strontium. With nitrogen, we look at the ratio of ^{14}N and ^{15}N , otherwise known as $\delta^{15}\text{N}$ (Fraser, 2011). This can be studied in soil, plants, herbivores and even human remains (Fraser, 2011; Zangrando et al., 2014). The isotope composition of carbon that is used is the $^{12}\text{C}/^{13}\text{C}$ -ratio, otherwise known as $\delta^{13}\text{C}$ (Gorlova, 2015). This composition is mostly researched in plant, animal and human remains (Zangrando et al., 2014). The isotope composition of oxygen that is used in Roman archaeology is the $^{17}\text{O}/^{18}\text{O}$ -ratio, otherwise known as $\delta^{18}\text{O}$. Samples for this analysis are mostly taken from cattle teeth (Groot, 2021; Balasse, 2011). Lastly, for strontium, the isotope composition used in Roman archaeology is the $^{87}\text{Sr}/^{86}\text{Sr}$ -ratio (Budd et al., 2000). This is researched in animal and human remains, but not in plants (Budd et al., 2000; interview Kooistra, 2025). Stable isotope analysis is well implemented in archaeozoological research in the Netherlands. For example, in Groot et al. (2021) stable isotope analysis is used to research cattle management in the settlement at Houten-Castellum.

Ancient DNA (aDNA)

One technique I have left out of the model in figure 3 is ancient DNA (aDNA) analysis. The reason for this is the fact that it is not yet common practice in bioarchaeological research and therefore not part of the conventional techniques I have aimed to portray in this model. The technique is still developing, mainly because of retrieval problems (Brown et al., 2014; Lodwick & Rowan, 2022). However, since this upcoming technique is relevant for the second chapter of my research paper, I have briefly summarised the developments of this technique so far. Ancient DNA can be found in both floral and faunal material from the Roman period.

Because floral remains have so many different preservation possibilities, their potential for DNA extraction varies depending on how the remains are preserved. Desiccated or waterlogged materials lend itself best for DNA retrieval (Brown et al., 2014). Carbonised or mineralised materials are, in general, less usable for DNA sequencing (Brown et al., 2014). Within carbonised material, large, charred pieces are more likely to contain aDNA, while small grains often contain only short strands of aDNA, if any at all (Brown et al., 2014). As carbonised and mineralised material are usually more common on site than desiccated and waterlogged material, this can influence the possibilities for aDNA retrieval in botanical samples (Lodwick & Rowan, 2022). In recent years, there have been attempts to further develop aDNA analysis in archaeobotany, e.g. by researching which sequencing technique works best when working with differently preserved floral samples (Brown et al., 2014). Still, the application of aDNA analysis in archaeobotanical research remains challenging (Brown et al., 2014; Lodwick & Rowan, 2022).

Ancient DNA can also be retrieved from animal remains. Animal bones are often found on site and show good potential for the retrieval of aDNA material (Ottoni et al., 2009). One limitation found here, similar to archaeobotanical retrieval of DNA, is that cooked materials might have so much DNA damage that retrieval is no longer possible, or that DNA is not taxable anymore (Ottoni et al., 2009). Since a majority of bones found on site are indicators of consumption, often showing signs of processing, getting proper material to sample aDNA from can be challenging (Ottoni et al., 2009).

Research Applications

The last step after analysing the organic material, is to use the results to answer research questions about life in the Roman period. In Figure 4, the topics that can be answered with the analysis techniques from Figure 3 are modelled.

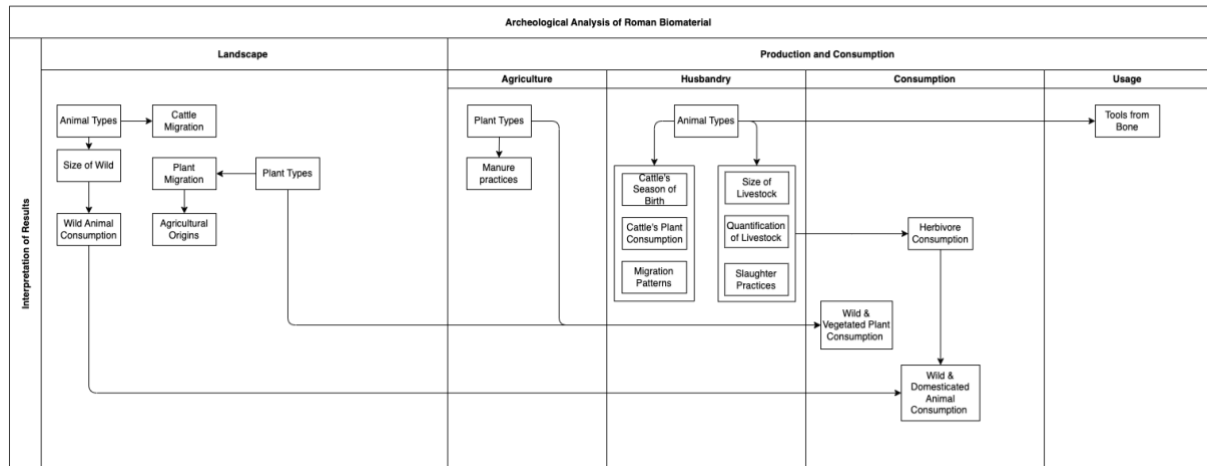


Figure 4: potential research applications of the analysis techniques in Roman bioarchaeology

Figure 4 visualises the research applications of Roman bioarchaeology. When looking at the landscape, the first step is to identify the plant and animal types of the floral and faunal remains found on site. Once the animal bones have been identified, they can be further analysed. For example, with the measurements of the bones (absolute and LSI), an estimation of the average size of a wild animal type can be made (Groot, 2016). This can help, along with other skeletal elements like hunting spores or butchery marks, to reconstruct the consumption of wild animals (Groot, 2016). Isotope analysis on faunal remains can then be done in order to research animal import practices. This is done by analysing the $^{87}\text{Sr}/^{86}\text{Sr}$ -ratio in animals (Budd et al., 2000; Interview Groot, 2025). In the case of wild plants, plant migration can be studied in order to reconstruct the agricultural origins of a settlement. This can be done by studying weeds found in an agricultural context, for example (Interview Kooistra, 2025).

The right side of the model again starts by identifying the plant and animal types of the floral and faunal remains found on site. From here on out, different aspects of agricultural and husbandry practices can be reconstructed. The number of bones found on site can give an idea of the number of livestock kept by farmers (Groot et al., 2009; Groot, 2016). The measurement of the individual bones (absolute and LSI) can give an estimation of the average size of cattle animals. Other skeletal elements, like butchery marks, can show slaughter practices and how animals were processed by humans for consumption, rituals or burial practices (Groot, 2016; Groot, 2018). Microscopically, isotope analysis can show some more details about Roman husbandry practices. For example, $\delta^{18}\text{O}$ -analysis can show a cattle's season of birth (Groot, 2021). Furthermore, $\delta^{15}\text{N}$ -analysis can indicate herbivore's plant consumption (Fraser, 2011). Lastly, migration patterns can be analysed, by analysing the Sr-isotope in cattle, and comparing that with the Sr-ratios in wild animals found in the area (Budd et al., 2000; Interview Groot, 2025). This can give insight into the origins of cattle, and therefore helps to reconstruct husbandry practices in the Roman period. Isotope analysis can also be done on plants. By looking at $\delta^{15}\text{N}$ -ratios, archaeobotanist can reconstruct manure practices on agricultural land (Fraser, 2011; Interview Kooistra, 2025). This gives insight into Roman agriculture, but provides context to cattle's plant consumption as well.

The final part of the reconstruction is to draw conclusions about the consumption and use of organic materials. The analysed remains of both wild and domesticated floral and faunal remains provide insight in the diet of the Romans that once inhabited the settlement. Along with the find context, the remains also enable archaeologists to reconstruct religious rituals like sacrificial slaughtering, and burial practices. The use of animals and plants for other purposes can be reconstructed as well, however limited. Processed animal bones, made into tools of sorts, can be identified.

Limitations

The models shown in Figure 1 to 4 show an important limitation in bioarchaeological research. Figure 1 shows a diverse range of different animals and plants present in the Roman period, and their different uses as food, tools, textiles and workforces. However, in Figure 2 a big contrast is visible in the materials that are preserved at the present time. Organic materials that remain today are scarce compared to the diversity of wildlife that was once roaming the grounds. When looking at animal bones for example, cattle is often well preserved, but other animals, like poultry, insects or even sheep and goats are difficult if not impossible to excavate (Interview Groot, 2025). The same limitation is seen in archaeobotany, because vegetables or herbs used in the kitchen are difficult to analyse, since their remains are usually not well preserved (Groot & Kooistra, 2009).

Additionally, the mobility of cattle and other animals in the Roman Netherlands is currently not well researched, though techniques like isotope analysis of DNA analysis show potential in this area (Groot, 2016). Plants are probably both imported and locally farmed as well, but how much is import and how much is local is difficult to establish with archaeological research (Groot & Kooistra, 2009). One way to tell when cereals or grains are imported is by looking at the weeds found among their remains, which can show the origin of the imported plants on site (Kooistra et al., 2013).

Furthermore, even though organic materials are usually quite well-preserved in the Netherlands due to the wet environment, resources like leather, wool and wood are still difficult to properly retrieve. They are not often found on site, and are not always retraceable to their original context because they often got recycled (Van Enckevort et al., 2024).

Lastly, in the model one thing stands out. The possible research applications are all based on human use, however with current archaeological research it is difficult to draw conclusions about the natural landscape of an area. Sometimes, pollen or natural weeds from an area can be recovered, but in general the materials that are preserved are indicative of human intervention of some sort (Interview Kooistra, 2025). It is difficult to research natural landscape and biodiversity in the Roman period using these conventional bioarchaeological research methods (Interview Groot, 2025; Interview Kooistra, 2025).

Now that the possibilities and limitations of conventional research methods within Roman bioarchaeology in the Netherlands are clear, it is time to focus on a potential solution to help resolve the limitations that are found in this chapter. To summarise, the main limitations with archaeozoological and archaeobotanical research are the lack of preserved material, difficulty in distinguishing between different species and different functions, analysing mobility practices, and the reconstruction of the natural landscape. Even with those limitations, archaeozoological and archaeobotanical research is able to make quite an extensive reconstruction about the Roman civilisation in the Netherlands (see Chapter 1). To further add

to this reconstruction, it is essential that bioarchaeological research keeps developing in order to reduce inaccurate or incomplete reconstructions of the Roman sites due to research limitations as much as possible. Therefore, the rest of this paper is focussed on integrating sedaDNA analysis into bioarchaeological research. In the next chapter, I have first given an overview of the sedaDNA analysis workflow, followed by a review of the potential research topics that sedaDNA could add value to.

Chapter 3 SedaDNA in Bioarchaeological Research

The limitations and difficulties that are currently found in Roman bioarchaeology prevent researchers from reconstructing the Roman period to its fullest potential. New techniques are still being developed in order to improve the possibilities of bioarchaeology (Özdoğan et al., 2024). One of the techniques that is in development right now is aDNA analysis, as described in Chapter 2. This technique however copes with the same retrieval problems as other methods do. If the organism is not physically preserved in some way, there is nothing to sample from. Another method that aims to resolve part of these retrieval problems is using sedimentary DNA (sedaDNA) to analyse bioarchaeological material (Özdoğan et al., 2024). In order to look at the way in which sedaDNA can combat current issues, it is crucial to understand the mechanism of preservation and the post-retrieval workflow of sedaDNA. This chapter will start by giving a theoretical overview of sedaDNA analysis.

To start, it is important to understand the basic principles of DNA. All organisms contain DNA, and leave traces of their DNA wherever they go (Thomsen & Willerslev, 2015). For animals, this is done both during their lives in forms of excretion, and after their death, when genetic material leaks into the soil while the body decomposes (Thomsen & Willerslev, 2015). Plants leave DNA behind as well, through their roots and leaves, as well as when they decay (Johnson et al., 2023). In other words, DNA that is left in the environment is preserved there (Thomas & Willerslev, 2015). How well this happens depends on various factors, including the type of material it is preserved in, how deep it is buried and what the climate is like in the region (Pietramellara et al., 2009). As explained in Özdoğan et al. (2024), when looking at sedaDNA from the Roman period, we are specifically looking at DNA left in soil, as opposed to DNA left in water or permafrost settings. On site, sampling is done in the soil from the Roman settlement. This is then transported to a lab, where DNA is extracted from the samples. This DNA needs to be sequenced, in order to multiply the number of strands available for analysis and to determine their genome sequence (Özdoğan et al., 2024). Lastly, the sequenced DNA can then be analysed by comparing it with databases of reference DNA (Nguyen et al., 2023). Now that the general workflow of sedaDNA analysis is clear, let's look at each step individually.

DNA

Genetic material of organisms is stored in DNA, deoxyribonucleic acid (Alberts et al., 2015). Every (eukaryotic) cell contains information about its properties in DNA in the nucleus of the cell (Alberts et al., 2015). The DNA, carefully packed up into chromosomes, makes it possible for cells to replicate and create proteins. To understand DNA analysis, it is crucial to understand the biochemical properties of a DNA molecule. Each molecule contains two chains that are built from four different nucleotide subunits: adenine (A), cytosine (C), guanine (G), and thymine (T) (Alberts et al., 2015). The nucleotide subunits form two sets of base pairs, meaning that when one strand contains the base G it will bond with the base C on the other strand, and when one strand contains the base A it will bond the base T on the other strand. Between these base pairs a hydrogen bond forms, which keeps the DNA molecule together (Alberts et al., 2015).

Environmental DNA (eDNA)

Environmental DNA, or sedaDNA refers to DNA that is not found within organisms, but in the environment (Brown, et al., 2025). There are two types of DNA: intracellular and extracellular DNA (Brown et al., 2025). When organisms die, their cells break down, thereby releasing the DNA that was stored in the nucleus (Brown et al., 2025). This makes the DNA molecules vulnerable to decay, not only by microorganisms in the ground, but also by nuclease that is released into the ground after cells break down (Dabney et al., 2013). The rate at which such structures break down is dependent on the soil type they are preserved in (Pietramellara et al., 2009). Important characteristics of the soil that determine DNA degradation are the pH of the soil, mineral and ionic compositions, and hydrophobicity of the soil (Pietramellara et al., 2009). The characteristics of the soil, along with the release of DNA altering enzymes, and the absence of DNA repair mechanisms, cause fragmentation of DNA. (Dabney et al., 2013; Brown et al., 2025). The main reason for fragmentation is depurination, otherwise known as the deletion of A- and G-bases (Dabney et al., 2013; Brown et al., 2025). DNA extracted from ancient remains will therefore usually be fragmented into pieces of 40-500 base pairs (bp) (Dabney et al., 2013). DNA degradation is further discussed in Chapter 4.

Sampling

If we want to use sedaDNA to analyse the past, it is crucial that contamination with other DNA is minimised, as this can influence the sequencing and analysing processes (Fulton & Shapiro, 2019; Özdoğan et al., 2025). It is therefore important that extraction is done properly, with attention to possible contamination. There are two types of contamination: modern contamination and ancient cross-contamination. Modern contamination can occur on site, when researchers or materials come in contact with the soil. Ancient cross-contamination can happen when soils from different contexts mix during the excavation, or when samples come in contact with each other during storage or in the lab (Fulton & Shapiro, 2019; Özdoğan et al., 2025). To avoid cross-contamination, protocols have been developed, with step-by-step guidance for archaeologists in the field to follow. These protocols focus on sanitation of equipment and of the conducting researcher, and explain exactly how to collect the proper part of the soil (Özdoğan et al., 2025).

After taking samples, it is important to store them correctly in order to avoid cross contamination or DNA damage (Fulton & Shapiro, 2019; Heintzman et al., 2023). Like sampling, storing the samples needs to be done in a sterile space with sterile equipment, so that the ancient DNA does not come in contact with modern or other ancient DNA (Fulton & Shapiro, 2019). To avoid damage or fragmentation, it is important that DNA is stored in a dry, cold environment (Fulton & Shapiro, 2019; Heintzman et al., 2023). The storage temperature should ideally be about the same as the soil the DNA was preserved in (Fulton & Shapiro, 2019). If the storage room does not have the right conditions, mould can grow on the sediment samples, affecting the ancient DNA in the samples (Seeber et al., 2022).

Extraction

When it is time for DNA analysis, subsamples are taken from the larger samples to extract DNA (Heintzman et al., 2023). The extraction consists of three steps, that are all focussed on isolating DNA fragments from sediment particles and debris. First, disintegration is done in order to break up soil and other organic structures (Heintzman et al., 2023). Then, inhibitor removal is done by purification (Heintzman et al., 2023). Inhibitors are structures that may interfere with DNA sequencing, like phenolic compounds found in soil (Kemp et al., 2006).

This can be done with silica extraction (Heintzman et al., 2023; Kemp et al., 2006). DNA binds to the silica, while other structures are washed away, leaving behind purified DNA (Heintzman et al., 2023). The extracted DNA should then be stored at -20 °C, before beginning the sequencing process (Heintzman et al., 2023).

Sequencing

Before sampled DNA can be researched with a database, researchers first have to expose its sequence. This is done after DNA is extracted from a sample, and is ready for sequencing. Nowadays, the technique most often used is Next Generation Sequencing (NGS) (Heintzman et al., 2023). This technique is good for sedaDNA because it works well with small base pairs (about 300 bp), and sedaDNA usually contains sequences of about 40-500 bp (Taberlet et al., 2018; Dabney et al., 2013). Another big advantage of this technique over others, like Sanger, is that multiple strands of DNA can be sequenced at the same time, instead of doing them one by one (Qin, 2019). This also decreases the sample size needed for sequencing, meaning smaller samples can be taken in the field (Qin, 2019). NGS starts by binding the DNA fragments to a primer on a flow cell, where they can then be duplicated to form clusters (Taberlet et al., 2018). The forward strand (5' to 3') is then sequenced by flushing the flow cell with nucleotides (dNTP's), each dNTP type tagged with a different colour fluorescent tag, of which one of them will bind to the complementary base (Taberlet et al., 2018). So for example: the forward strand starts with A, a T-nucleotide with a fluorescent tag will bind that A. A camera then records the colour of each cluster, before moving on to the next base in the sequence (Taberlet et al., 2018). This is done until all bases in the sequence have been photographed, leaving behind a series of photographs that show the colour order of each cluster sequence (Taberlet et al., 2018). The data are then filtered to clean up areas where there is overlap of clusters, or areas of low intensity (Taberlet et al., 2018). What is left is a representation of all DNA strands present in the DNA sample. This dataset is then used for DNA analysis.

Bioinformatics

After DNA sequences are determined they can be analysed to find out which species can be found in the soil on site. To do so, a reference strand of DNA is needed, to which the sample DNA can be compared (Pochon et al., 2023). Without a reference we cannot identify similar patterns in DNA samples, so we cannot determine which species' DNA is in the soil. In the past sedaDNA analysis was often done with a technique called metabarcoding (Ambrecht et al., 2021). However, this technique is not ideal, since it does not work well with short and fragmented DNA strands (Ambrecht et al., 2021)³. A more recent technique is metagenomics (Nguyen et al., 2023). Metagenomics is a tool that uses centralised databases of DNA sequences, allowing the collective sample DNA to be compared to not one taxon, but a large selection of different taxa (Pochon et al., 2023). In the Netherlands, the databases that are used for this are the “National Centre for Biotechnology Information”-database (NCBI) and the “European Nucleotide Archive”-database (ENA) (Interview Furni, 2025).

³ Short bp form a problem in sedaDNA barcoding because it prevents PCR primers from binding to the sequence, and because target sequences are sometimes longer than the DNA fragments itself, resulting in bias towards better preserved sequences; for a more in-depth analysis regarding metabarcoding see “*Ambrecht et al., 2021*”.

Research Applications

The goal of sedaDNA analysis is to use the information to research the presence and use of organic materials in Roman period. Data sustained through bioinformatics serve three purposes: to check for contamination, perform authentication, and classify different taxa present in the soil samples (Özdoğan et al., 2024). The last one, the identification of different taxa, is the crucial part of this research, as it allows us to answer different research questions. In this paragraph I have made an overview of some possible research topics that can be answered with the use of sedaDNA, and how this may add to the research techniques discussed in Chapter 2.

As seen in Chapter 2, limitations with conventional techniques are often related to scarcity in preserved material. With animals, material found on site usually originates from bigger animals like cattle or horses, as smaller animals like chickens or even mice are less well-preserved. As well as that, distinction between certain species, like sheep and goats, can be difficult. The same goes for plants. In areas where human settlements are found, most floral material comes from carbonisation. This method of preservation does not preserve leafy greens, and therefore plants like cabbage are often not found on site. Ancient DNA from macroremains can help to determine some floral and faunal species, but this technique is again reliant on material remains available on site. This is where sedaDNA shows potential. Because it takes samples from soil, it can show species that are not preserved through materials remains (Özdoğan et al., 2024). This has potential in many areas of Roman archaeology.

Biodiversity

A big research gap in current Roman bioarchaeology is natural vegetation and the environment. Humans leave traces wherever they go, and therefore floral and faunal remains on site can usually be tied to human involvement (Interview Kooistra, 2025; Interview Furni, 2025). However, the environment around settlements can be harder to reconstruct, as we don't have much material to research (Interview Furni, 2025). SedaDNA can help solve this problem, as the soil is full of organisms that existed during the Roman period (Özdoğan et al., 2024). This way it becomes possible to reconstruct biodiversity and climate in the Roman period. Since sedaDNA sampling picks up whatever forms of sedaDNA can be found, there is no distinction between DNA from species that were and were not exposed to human contact (Özdoğan et al., 2024). SedaDNA analysis is therefore mainly a way to reconstruct biodiversity.

After detecting which species were present in the environment surrounding a Roman landscape, we can then look further into human involvement. To research this a mixture of taxonomical detection of species and further context from archaeological material is needed. If there is a specific research question, for example whether cows were present on site, sedaDNA analysis can then be used to confirm the hypothesis, or not. The different research topics that sedaDNA analysis could help answering are summarised in the following paragraphs, according to Özdoğan et al. (2024).

Diet

Regarding diet, sedaDNA can give a more taxonomically diverse overview of what Romans used to eat. For example, as discussed with dr. Kooistra, it could be interesting to use sedaDNA analysis in latrines to research the consumption of cabbage (Interview Kooistra, 2025). Cabbage is a plant often seen in Roman art, but is rarely found on site. Cabbage doesn't leave a lot of remains, because it is a plant that is consumed before seeds are spread around, which can explain why we find little evidence of cabbage consumption, despite seeing it in Roman

art (Cheung et al. 2021; Interview Kooistra, 2025). SedaDNA could provide more insight into this. This goes for faunal remains as well, as for example chickens usually leave very little evidence on site because their bones are so small, even though we do expect they were part of the Roman diet (in form of meat or eggs) (Interview Groot, 2025).

Farming Practices

SedaDNA can help distinguish between wild and domesticated species. This is currently not always possible to determine, because of macroscopical and microscopical similarities in floral and faunal remains (Özdoğan et al., 2024). Ancient DNA from macroremains (e.g. bones) could also help with this, although this technique is dependent on available remains from both wild and domesticated species. SedaDNA could help make the distinction between wild and domesticated species. However, taking this one step further, sedaDNA analysis could be used to research the domestication of certain species (Özdoğan et al., 2024). This would be possible by analysing the evolutionary split between wild and domesticated subspecies of the same species (Özdoğan et al., 2024). Apart from the distinction between wild and domesticated, sedaDNA can also be used to distinguish between native and imported species (Özdoğan et al., 2024). This does not always have to be concentrated only on diets but is also interesting when researching which species migrated with Romans to the Netherlands. For example, we know rats are not native to the Netherlands, and they most likely were introduced when the Romans came here (Interview Furni, 2025).

Understanding the process of domestication and migration of certain species can help us better understand Roman farming practices. If sedaDNA data are used in addition to results from bioarchaeological research of macroscopical remains, and find context on site, it could help further improve our understanding of the way that agriculture and husbandry was conducted in the Roman period in the Netherlands.

Human Mobility

The mobility of humans can also be researched more extensively with the use of sedaDNA. Currently, mobility is studied by isotope analysis, specifically the ratio of Sr-isotopes (Kootker et al., 2022). This technique has some limitations. The retrieval of material for isotope analysis is dependent on cremated human remains, since inhumated remains are susceptible to changes in isotope ratios due to the chemical components of the soil they are buried in. Another limitation is that the standard Sr-ratios of different areas can be difficult to distinguish. Furthermore, the changes in isotope ratios among individuals do not always have to indicate mobility, but can also be explained by changes in diet, for example (Kootker et al., 2022). SedaDNA data can indicate the presence of humans on site, to provide context for the interpretation of isotope analysis. Additionally, when sedaDNA is used to better understand species migration (e.g. the import of certain wheats), that can help to understand where certain changes in isotope ratios originate from. For example, if isotope analysis shows changes in Sr-ratios for a number of individuals, but sedaDNA analysis shows the presence on site of a plant that matches that Sr-ratio, it is more likely that new imported plants were integrated as part of a standard diet for humans on that site, than it is likely that they were very mobile.

Health

SedaDNA can also help research topics about health in the Roman period. Recently, a study was published in which parasite infections in the Roman period were researched using sedaDNA (Ledger et al., 2025). Before the use of sedaDNA, health research was often limited to microscopical analysis of material from latrines (faecal matter) of human remains (Ledger

et al., 2025). However, as those are not always present on site, sedaDNA analysis could extend the possibilities of researching health in the Roman period (Özdoğan et al., 2024). Specifically, parasitic, bacterial or viral infections could be an interesting research topic, I think, as these type of health issues are closely related to DNA.

Status Quo

Now that the research possibilities with sedaDNA are established, it is important to look at the status quo of sedaDNA analysis in Roman archaeology. Using sedaDNA in the field of Roman archaeology is relatively new, even though sedaDNA has been well applied in other fields like paleoecology and contemporary biology/ecology (Alsos et al., 2022; Brown et al., 2025). When applying sedaDNA to Roman archaeology, it can be more difficult to handle sampling and contamination. In paleoecology, samples are often taken stratigraphically, so that sub-samples can be taken at intervals in different layers throughout time (De Schepper et al., 2019; Li et al., 2023; Pansu et al., 2015). This makes ancient contamination and dating less problematic, as researchers are not focussed on one specific time period, but rather gradual changes over time (Li et al., 2023; Özdoğan et al., 2024). Furthermore, palaeoecological research mostly takes samples from permafrost or deep lake environments, which preserve DNA much better than the soil in which sedaDNA from the Roman period is contained (Özdoğan et al., 2024). In modern ecology, sedaDNA poses less problems in terms of fragmentation, as the DNA is less old and therefore less degraded. DNA from the Roman period is both very fragmented and sensitive to contamination from other DNA in the soil because it is bound to a specific time period, which makes it challenging to implement sedaDNA in Roman archaeology.

Still, sedaDNA is an upcoming technique in Roman archaeology. In the Netherlands the implementation of sedaDNA in Roman archaeology is currently researched. Here, researchers have analysed the current ability of sedaDNA analysis in the Netherlands when used to reconstruct the Roman period (Özdoğan et al., 2024; Özdoğan et al., 2025). This forms a baseline that shows what sedaDNA is capable of in Roman bioarchaeology, before research application-specific confinements are made. What they found was that right now, sedaDNA is able to reconstruct the Roman period about as well as conventional research methods, meaning that this technique is at least on par with current research (Özdoğan et al., 2024; Furni, 2025).

Research done by Özdoğan et al. (2024) analysed the performance of sedaDNA analysis when compared to conventional bioarchaeological research techniques, for different research topics (Özdoğan et al., 2024). On the topic of diet, it performed as well as conventional methods, except for food preparation procedures, which is a research topic that has limited potential with sedaDNA. The topic of health was about equal when comparing conventional methods with sedaDNA. Domestication also showed similar results, sedaDNA even showed more potential when looking specifically at the use of wild animals. Environmental aspects analysed with sedaDNA scored the same as those analysed with microscopical inspection. Lastly, when looking at mobility and migration, the use of DNA scored better than conventional methods, although DNA from macroscopical remains seems to have an advantage over DNA from microscopical remains (Özdoğan et al., 2024).

The research currently done on sedaDNA shows promising perspectives for future applications. However, to be able to implement sedaDNA as a standard technique in the field of Roman archaeology, more work needs to be done. The next chapter focuses on the current limitations of sedaDNA analysis. Establishing these limitations enables us to look at the further steps that need to be taken before being able to implement sedaDNA in the field.

Chapter 4 Current Difficulties Regarding the Retrieval and Analysis of SedaDNA

Integrating sedaDNA into the field of Roman archaeology comes with many limitations. As promising as the potential applications of sedaDNA look, the technique first needs to be further developed before it can become an addition to current techniques. This chapter aims to establish the most prominent pitfalls and limitations of sedaDNA analysis in Roman archaeology, in order to determine future directions of the technique. In short, these limitations can be divided into two different categories: DNA specific, and methodological. First, this chapter focuses on DNA specific limitations.

DNA specific Challenges

DNA Degradation

In theory, all organisms leave DNA in the habitats they come into contact with, which means that traces of all organisms that once existed in a certain place can be retrieved. In reality this works differently. As shortly discussed in Chapter 3, DNA that is left in soil is susceptible to fragmentation and decay, as result of the surrounding enzymes, organisms and soil-types (Dabney et al., 2013; Pietramellara et al., 2009). Because of this, sedaDNA found on site is often heavily fragmented (40-500 bp) (Dabney et al., 2013). One article by Bhoyar et al. (2024) summarised the different types of DNA degradation after death. One of these types is enzymatic degradation. Once an organism dies, and thereby the cells within it, breakdown is started by endonucleases. This enzyme breaks down phosphodiester bonds between bases, which results in the DNA breaking down into smaller fragments. Then exonucleases cut nucleotides from the end of the DNA molecule. This can be done from both the 5'-end and the 3'-end, depending on the type of exonuclease. So instead of fragmentation, exonucleases cause overall shortening of the DNA molecules. Apart from enzymatic degradation, non-enzymatic processes also play a role in DNA degradation. Non-enzymatic degradation consists of oxidative damage, and the influence of chemical substances (Bhoyar et al., 2024). Oxidative damage can be caused by reactive oxygen species (ROS) which can result in fragmentation of DNA or denaturation, the process in which double stranded DNA becomes separated into two single strands (Jakubczyk et al., 2020). Chemical substances, such as radiation, can also cause DNA damage in a number of ways (Bhoyar et al., 2024).

The damage pathways can be affected by different environmental factors (Bhoyar et al., 2024). For example, temperature plays an important role in DNA degradation. With high heat, hydrogen bonds between DNA strands will break apart, resulting in separation of the double stranded helix into two separate single strands. Humidity affects DNA degradation processes as well. When DNA comes in contact with water, hydrolysis can occur, a process that can cause depurination (deletion of adenine and guanine), or depyrimidination (deletion of thymine and cytosine). As discussed in the previous paragraph, oxygen levels are an important factor in the process of DNA degradation, as higher oxygen levels cause more oxidative damage. Furthermore, the pH of the environment of the DNA influences damage processes. DNA is best preserved in neutral environments, as both acidic or alkaline environments make DNA susceptible to more damage. Lastly, microbial activity surrounding the DNA can cause DNA breakdown. There are bacteria that produce the enzyme nuclease, an enzyme that can make breaks in DNA strands (Bhoyar et al., 2024).

Soil Types

All of the factors affecting DNA degradation that are mentioned above are characteristics that define different soil compositions. Therefore, the soil type that the DNA is preserved in is a big contributing factor to the levels of DNA degradation found in sedaDNA samples. Certain soil types are better for DNA preservation than others. According to the environmental factors listed in the previous paragraph, an ideal soil type for sedaDNA to be preserved in would be a soil with a neutral pH, with low oxygen levels, that is dry and cold. An experiment conducted by Sirois & Buckley (2019) shows that DNA degradation rates are indeed higher in soils with a higher temperature and moisture level. They found that DNA degraded slowest in forest soils and was most stable in meadow soils. Ground that had been ploughed through showed more degraded DNA (Sirois & Buckley, 2019).

Another problem that occurs in soil is the migration of DNA particles (Aldeais & Stahlschmidt, 2024). Just as we see in palynology, where pollen gets removed from their original context through stratigraphical changes throughout time, the same problem occurs with sedaDNA. In order to distinguish modern DNA from ancient DNA, it is possible to analyse the damage in the DNA fragments based on patterns of deamination. However, when trying to differentiate ancient DNA from varying time periods, issues arise, as damage to the fragments does not always happen linear over time (Aldeais & Stahlschmidt, 2024). It is therefore possible that ancient DNA from different time periods gets mixed up, without researchers being able to distinguish them biochemically. Again, the soil composition plays a role in the degree to which migration will occur. Sand has large soil particles (2 – 0,06 mm), meaning there is a lot of air space between particles. This enables DNA particles to move between different soil particles. Silt has smaller soil particles (0,06 – 0,002 mm), and clay has even smaller soil particles (< 0,002 mm), so the air space in between particles is tighter, allowing less room for movement (Blott & Pye, 2012).

Understanding the stratigraphy of the soil is a key component in locating the original context of DNA fragments, and to help date them (Aldeais & Stahlschmidt, 2024). Soil patterns can be analysed through micromorphological analysis (Aldeais & Stahlschmidt, 2024). On site, samples are taken from undisturbed ground and preserved in resin in laboratory environments. The cleaned and polished resin slab shows the layers and composition of the ground, which can then be analysed through microscopical analysis (Goldberg & Berna, 2010). This can help researchers identify and date different layers in the soil. This provides valuable context to the DNA that is preserved in those layers.

Modern Contamination

Apart from forms of ancient sedimentary DNA mixing in the ground, the contamination of modern DNA on site or in the lab poses problems as well. Even though it is possible to make a distinction between modern and ancient DNA, this only works to a certain extent. In order to distinguish modern DNA from the original DNA in the find-context, authentication needs to be done (Özdoğan et al., 2024). The distinction between modern and ancient DNA is done by analysing damage patterns of the DNA (Hübler et al., 2019). Besides contamination, inhibitors can also stick to the DNA fragments. Inhibitors, such as complex proteins, can disrupt the analysis of sedaDNA (Moreno & McCord, 2016; Özdoğan et al., 2024). It is therefore important to work as sterile as possible, and to adhere to a protocol of DNA purification during DNA extraction, but even then inhibitors can remain problematic (Moreno & McCord, 2016; Özdoğan et al., 2024).

Amplification

When sequencing the extracted DNA fragments, the level of degradation of the DNA molecules poses some challenges. First of all, if data could benefit from amplification to increase the sample size, it might be difficult. Amplification is commonly done with polymerase chain reaction (PCR). This amplification technique works by taking a primer (a short DNA fragment) and binding it to the DNA strands in the sample (Alberts et al., 2015). The primer will then bind to the enzyme polymerase, connecting the enzyme to the DNA strand (Alberts et al., 2015). Polymerase causes DNA synthesis, a process in which the DNA strands are duplicated (Alberts et al., 2015). This can then be repeated until a sufficient sample size is reached.

The degradation of DNA molecules makes sedaDNA less suitable for amplification techniques like PCR, because PCR does not work well with short, fragmented DNA pieces, resulting in a bias towards longer, better preserved DNA strands (Armbrecht et al., 2021). As a result, false negatives of less well-preserved taxa can occur, meaning that the surviving DNA fragments are not representative of the DNA in the environment (Fulton & Shapiro, 2019). Furthermore, PCR poses problems to the authentication of sedaDNA. (Fulton & Stiller, 2011). When primers bind to a DNA strand, and polymerase rebuilds the congruent sequence, ancient damage to the DNA strands becomes less visible (Golenberg et al., 1996). In other words, this means that ancient DNA strands are more difficult to distinguish from modern DNA based on damage patterns, which poses problems for the authentication of the DNA sequences (Fulton & Stiller, 2011). There are some studies that do suggest that PCR can play a valuable role in the authentication of ancient DNA by picking up false positives (Bunce et al., 2011). If amplification of a sequence is high despite a low number of starting templates, there is a higher chance that this sequence is a modern DNA contaminant, instead of ancient sedimentary DNA (Bunce et al., 2011). Either way, there is still debate about the authentication of ancient sedaDNA when using PCR, and whether damage patterns like miscoding lesions are actual DNA damage patterns or PCR amplification errors (Fulton & Stiller, 2011; Gilbert et al., 2007).

Quantification

Another form of PCR, real-time quantitative PCR (qPCR) is a tool used to quantify DNA fragments in sample. In the case of ancient sedaDNA, this can then be used to estimate species abundance (Bunce et al., 2011). qPCR combines amplification with quantification because of real-time monitoring that uses a fluorescent binder that can show the amplification process (Kubista et al., 2006). After a round of PCR, the DNA molecules that were initially present can be calculated, because the amount of DNA molecules present after amplification, that can be seen due to the fluorescence, are about double of the initial amount (Kubista et al., 2006). qPCR can detect both relative abundance between two or more samples, and absolute quantities of a sample in reference to a standard curve (Bunce et al., 2011). Even though qPCR is, to an extent, able to quantify the DNA of single species, this becomes more challenging when working with multi-species samples from a biodiverse environment, meaning this technique becomes extremely costly when working with multi-species samples (Thomsen & Willerslev, 2015). Because of this cost-inefficiency, and the limitations of PCR listed in the previous paragraph, amplification and quantification via PCR are generally avoided. Instead, amplification is done straight away with NGS. This is more cost efficient than PCR, and preserves ancient damage patterns better, meaning it is more suitable to perform authentication (Armbrecht et al., 2021).

Bioinformatics

Multi-species detection through NGS is then done either with metabarcoding or metagenomics (Özdoğan et al., 2024). Metabarcoding is done by selecting a region of the DNA sequence, which is known to differ from DNA sequences of other taxa (Özdoğan et al., 2024). The selected region is then amplified by PCR and sequenced (Özdoğan et al., 2024). Due to the use of specific primers, metabarcoding creates a bias in primer-affinitive DNA, and thus results from metabarcoding are not necessarily representative for all species in the sample (Thomsen & Willerslev, 2015). It is also difficult to perform authentication, because of the challenges we saw with PCR authentication as well (Fulton & Stiller, 2011; Özdoğan et al., 2024). For this reason, bioinformatics for sedaDNA is progressing towards metagenomics (Armbrecht et al., 2021). Metagenomics still poses other taxonomic issues, which are summarised in the next few paragraphs (Thomsen & Willerslev, 2015).

The first issue with metagenomics arises, again, when encountering short sequences. These fragmented DNA sequences can be difficult to identify due to their poor taxonomic resolution, which can result in false negatives of taxa in a data sample (Thomsen & Willerslev, 2015). This illustrates once again the way in which longer, less fragmented sequences can be overrepresented in sedaDNA analysis due to taxonomic bias. Apart from this, distinctions between species cause problems as well. In order to better identify specific taxa in a sediment sample, a proper understanding of interspecific sequence diversity is needed (Thomsen & Willerslev, 2015).

Additionally, sequence gaps also occur in databases, meaning not every species or subspecies has adequate reference sequences in metagenomic databases (Özdoğan et al., 2024). This complicates the identification of different taxa and sub taxa (Özdoğan et al., 2024). For example, this limits our ability to make a distinction between wild and domesticated animals, as this distinction requires both a reference for the wild species as for the domesticated species. This is another result of research bias, because the focus on identifying sequence diversity and building reference databases is often set on animals relevant to consumption, like cattle (Interview Furni, 2025).

The last DNA specific limitation that occurs, is the limited ability to trace back specific functions of faunal and floral remains. Plants and animals were used for a wide variety of things, not only as part of the Roman diet but as tools, textiles or workforces as well (see Figure 1). In order to make a distinction between a plough animal, and a piece of leather, find context on site would be needed to fit the species correctly into the reconstruction of the Roman landscape. Apart from that, a poor taxonomic resolution also prevents researchers from investigating specific traits in species, like its origin or quality for a certain purpose (e.g. wool quality in sheep subspecies) (Interview Groot, 2025). If there is no clear find context, it will be very difficult to make any conclusions about the organism apart from its presence on site.

Methodological Challenges

Apart from DNA specific challenges, the methodology of sedaDNA is complicated as well. To better understand the way in which sedaDNA analysis is done in the Netherlands, I have spoken with post-doc Furni, a researcher for the “Constructing the Limes”-project (Interview Furni, 2025). In the Netherlands, specialists who are able to work with sedaDNA are scarce. Implementing the use of sedaDNA as a research technique is therefore difficult in the

Netherlands, as there is only a limited number of researchers available who are able to work with sedaDNA and properly set up the bioinformatics needed to analyse extracted samples. Therefore, work sometimes gets exported to facilities elsewhere, to run analyses there, thereby increasing the cost and energy needed for analysis due to transportation and outsourcing.

Even when sedaDNA analysis is done in the Netherlands, the technique is still very time and energy consuming. Sequencing done with NGS is quite expensive, especially when working with sediment samples, since they contain a lot of different DNA molecules that all need to be sequenced (Gasc et al., 2016; Sun et al., 2020). A less expensive option is nanopore sequencing (Brown et al., 2025; Sun et al., 2020), but this sequencing technique is not yet widely applied to ancient or sedimentary DNA. The use of nanopore sequencing is further discussed in Chapter 5 of this paper. Apart from costs, sedaDNA analysis uses a lot of energy, because it requires large bioinformatical data centres with reference sequences, which use a lot of computational energy (Lannelongue et al., 2021; Niewenhuis et al., 2024). This leaves an environmental impact as well, as data centres are responsible for about 1% of emission of greenhouse gasses (Niewenhuis et al., 2024). Although this is not specific to genomic databases, it is definitely a limitation to note down, especially since the rising use of large data centres (Steinhardt, 2024; Niewenhuis et al., 2024).

Chapter 5 Further Steps to Implement SedaDNA in Bioarchaeological Research

The limitations described in Chapter 4 make it difficult to implement sedaDNA analysis in Roman archaeology at the moment. In order to optimise this technique, these limitations need to be further resolved so that sedaDNA might become a valuable addition to Roman bioarchaeology in the future. The steps that need to be taken can be divided into DNA specific developments and methodological developments, following the limitations established in Chapter 4. This chapter will first address DNA specific steps.

DNA specific Developments

Soil Types

When looking at soil, one of the things that can be developed further is research on the effects of different soil types on the preservation of ancient sedaDNA. Since degradation of DNA is affected by certain characteristics in the soil, it is important to understand how different soils cause different damage patterns. Currently, there is not yet a lot of research on the soil types that affect sedaDNA from the Roman period specifically. If we can establish more data on these effects of soil on DNA, we could research which soil type preserves DNA best. Furthermore, it would be possible to get a deeper understanding of how damage patterns in DNA are caused, which is important for the interpretation of the find-context of DNA fragments in the soil.

Additionally, getting a better understanding of micromorphology is crucial to interpreting different soil layers, and therefore migration patterns of DNA. Sufficient knowledge of the micromorphology of a sample site can show researchers where to sample when wanting to sample material from the Roman period. It can also help gain insight in which layers might cause disturbance or contamination of the sampled layer, so that researchers can keep this in mind when doing analyses.

Contamination

To further reduce modern DNA contamination it is essential that proper protocols are put in place when working with sedaDNA. In the field, this means sampling with gloves, and preferably a clean suit, avoiding cleaning with water, using bleach instead, and storing the samples in a dry, cooled environment (Pérez et al., 2022; Özdoğan et al., 2025). When preparing the soil samples for extraction, the outer first centimetre of soil should be removed, whereafter the inner soil can be mixed, and sampled for extraction (Pérez et al., 2022). Furthermore, sedaDNA samples should be processed in specialized aDNA facilities, where no modern DNA is processed in order to avoid cross-contamination (Pérez et al., 2022). Additionally, it is important that controls are done during each step of the process in order to identify possible contamination (Özdoğan et al., 2025). We should further extend our current knowledge on the effect that inhibitors have on the analysis of sedaDNA. There should be more research done on the ways in which to remove these sufficiently, in order to minimise disruption during analysis.

The authentication of ancient sedaDNA can still be improved as well. In Bhoyar et al. (2024) fourteen different methods to assess DNA degradation are listed. Further research into these

methods could show which techniques work well with ancient sedaDNA. If we improve the analysis of DNA degradation it will become easier to assess ancient sedaDNA damage patterns in order to authenticate sedaDNA samples (Bhoyar et al., 2024). This can help researchers to gain more insight into how specific damage patterns occur, so that the effects of soil types on DNA degradation can be studied accordingly. It can also improve our possibilities to distinguish between ancient and modern DNA and thereby detect modern DNA contamination.

Taxonomic Resolution

Another challenge seen with sedaDNA analysis is the limited ability to differentiate and identify species within a sedaDNA sample. While metagenomics is a good tool to compare a large number of DNA fragments with different references at once, it is less suited to identify very specific DNA matches with complementary (sub)species. This is an issue, specifically because sedaDNA contains DNA fragments from species such as the soil microbiome and other bacteria, which disturb the bioinformatic process when using metagenomics (Paijmans et al., 2019). An upcoming technique designed to resolve this issue is capturing (Jones & Good, 2016; Paijmans et al., 2019). There are different capturing techniques, but one that is currently researched for possible applications in ancient sedaDNA analysis is hybridisation capturing (Burbano, 2010; Soares, 2019). To target specific DNA molecules in larger samples from extracted DNA fragments, an RNA bait molecule is used to capture the desired DNA molecules (Soares, 2019). This technique is often done before NGS, to further optimise and speed up the sequencing process (Jones & Good, 2016; Paijmans et al., 2019). Currently, the lack of genomic material makes it difficult to create accurate capture probes for species that are not thoroughly studied yet. In order to implement capturing to research specific species or sub-species, it is therefore important that researchers keep working on expanding the genomic library for all taxa (Özdoğan et al., 2024).

When specifically looking at the resolution of the bioinformatic analysis, it could be an idea to perform capturing after the initial round of metagenomics. By analysing the full sample of extracted material first, and obtaining a general idea of the species found within the sample, it could then be an option to further finetune the read by performing capturing with targets to specific species, and running the metagenomics again. In theory, by running this cycle numerous times, it could be possible to identify very specific organisms present in the sample set. If we could optimise the capturing technique, this could help resolve the resolution issue currently found with sedaDNA and might even create the opportunity for very specific research question to be answered (Jones & Good, 2016; Özdoğan et al., 2024). Questions that might be researchable when taxonomic resolution is improved are for example, the origin of cattle in a Roman settlement, or specific traits like the quality of wool a subspecies of sheep might give (Interview Groot, 2025).

The second way to increase taxonomic resolution of sedimentary DNA is the further expansion of genomic databases (Özdoğan et al., 2024). In order to identify species, a reference sequence is needed to match the sample to, as a result of that researchers can only identify species that can be recognized in the database (Orlando & Cooper, 2014). Even though the databases are expanding constantly, making identification of species more precise, there is still work to be done regarding the collection of new genomic sequences. A research bias is present here, since the amount of representation a species gets in the genomic library is dependent on researchers collecting materials and identifying sequences for different organisms (Özdoğan et al., 2024; Interview Furni, 2025). It is therefore important that researchers focus on collecting genomic

material from species that are underrepresented in the databases right now (Özdoğan et al., 2024).

Function

Apart from taxonomic resolution, the function of different species on site can be researched by combining data from sedaDNA analysis with find context. We should aim to combine sedaDNA with conventional research techniques within Roman bioarchaeology, to create the most extensive and accurate reconstruction of the Roman period. As goes for conventional bioarchaeology, sedaDNA is also much better interpreted when combined with other material findings on site. SedaDNA analysis might detect certain species, but other material on site could help determine the function of the species in that place. For example, if cow DNA is found in the soil, and during excavation the structure of a farm is found, it is likely that the cow was part of husbandry practices.

Statistical Analysis

Like discussed in Chapter 4, fragmentation of DNA complicates sequencing procedures, resulting in a bias towards better preserved DNA fragments and therefore results that are not representative for reality. To combat this, the standard sequencing technique currently used for sedaDNA is NGS. Even though this technique is compatible with short base pairs, there is a minimum limit of about 250 bp, though some studies say it can sequence as short as tens of basepairs (Hu et al., 2021; Zhang et al., 202). Short reads can therefore still be missed. This can result in false negatives of certain species in the DNA analysis, which would create an inaccurate reconstruction of reality (Ficotela et al., 2015). Furthermore, false positives can occur due to contamination of DNA (Ficotela et al., 2015). A way to account for this, that is currently not often applied in ancient sedaDNA research, is by making use of statistical analyses (Burial et al., 2021; Chen & Ficotela, 2020). Numerical analysis is a method that we should look into further if we want to improve accuracy and reliability of sedaDNA analysis results.

A possible numerical method for sedaDNA data processing is the use of site occupancy detection models (SODMs) (Chen & Ficotela, 2020). SODMs make statistical probability analyses of the reliability of sedaDNA data (Chen & Ficotela, 2020). The model works by quantifying the probability that a species at a site is detected based on the data collected during DNA analysis. It is a binary model, meaning that there is a possibility of either a true or a false outcome, otherwise known as the Bernoulli distribution (Burial et al., 2021). Based on this, it is possible to construct some statements. A positive taxon detection can be detected in the sample and present in reality (true) or can be detected in the sample but not present in reality (false). A negative taxon detection can be absent in the sample and absent in reality (true) or can be absent in the sample but be present in reality (false) (Burial et al., 2021). This is combined into an uncertainty factor, p . When p equals one, it means that detection of a taxon in the sample is a perfect representation of reality. In practice, p is almost always lower than one (Burial et al., 2021; Ficotela et al., 2015).

In other words, the model can help account for false positives and false negatives of different taxa in sedaDNA (Chen & Ficotela, 2020). This way it can account for false positives, and help identify and correct contamination of DNA samples (Ficotela et al., 2015). Constructing an SODM requires some calibration steps. One requirement is a survey method that can provide

data without false positives, which is difficult (if not impossible) to acquire when working with sedaDNA (Burial et al., 2021; Chen & Ficotela, 2020). Furthermore, calibrations are needed for each step of the analysis to estimate false positives and negatives. This can be done by taking blank extractions or using control groups during the process (Chen & Ficotela, 2020). Despite these requirements, which can be difficult to meet, the use of SODMs can be a way to account for an uneven distribution of taxa in a sample due to DNA degradation, or for autocorrelation between datasets, both of which could lead to false positives and false negatives of certain taxa in a dataset (Chen & Ficotela, 2020).

To deal with quantification issues SODMs shows potential as well. The occupancy models can be defined in such a way that it can measure abundance (Chen & Ficotela, 2020). The amount of DNA present in a sample seems to be significantly correlated with the amount of a species present in the sample area, permitting a prediction of species abundance in terms of biomass based on their occurrence in a sample (Chen & Ficotela, 2020). This is done with a Poisson distribution. This model predicts the events in a fixed interval, e.g. the number of species on site. To implement this, qPCR data on species abundance could be used. qPCR is currently difficult to implement in ancient sedaDNA analysis, because of the likelihood of false positive and false negative outcomes, but when the qPCR data are combined with statistical models that account for those factors, data become more reliable.

Since it is difficult to obtain a calibration data set without false positives, other steps can be taken to ensure maximum validity of the statistical analysis. By conducting repeated survey, thereby taking multiple samples, the data become more reliable than when only one sample is used (Burial et al., 2021; Valente et al., 2024). It is still unclear what number of samples is an optimal amount for both data accuracy and time efficiency (Chen & Ficotela, 2020). Additionally, it is important that sampling improvements continue to be made, to further increase the accuracy with which samples can be taken (Valente et al., 2024). Some suggestion on how to improve sampling are made in this paper. For example, we can further explore the use of soil micromorphology to better understand time periods in the soil and migration patterns. It is important as well that we minimise modern contamination, by further evaluating sampling and processing protocols, as seen in Özdoğan et al. (2025).

Even though I recognise the importance of applying statistical models to sedaDNA data, I by no means pretend to be an expert in statistics. To further look into the possibilities of numerical methods for sedaDNA analysis, I suggest that moving forward, sedaDNA researchers seek collaboration with biometrists (biological data scientist). By combining knowledge on statistical models and ancient sedaDNA, we could optimise the use of statistics to improve accuracy and reliability of sedaDNA data.

Methodological Developments

One of the challenges currently found with sedaDNA analysis is the limited availability of specialists. In order to integrate this technique in the field of Roman bioarchaeology without the need for specialists present for every step along the way the research team of Constructing the Limes has recently experimented with the possibility of having sampling in the field done by conventional archaeologists (Özdoğan et al., 2025). In this study, an experiment was done where experts and non-experts took some sediment samples, which were then compared with regards to contamination levels found inside the soil samples. There was no significant difference in contamination levels found between experts and non-experts. This is an interesting result, because it shows the potential to make sedaDNA analysis more accessible by

making use of non-expert archaeologists to perform sediment sampling. This would reduce the need for a specialist during sampling, thereby further reducing time and costs needed to deploy DNA experts in the field (Özdoğan et al., 2025).

Additionally, the study by Özdoğan et al. (2025) researched the possibilities to take samples from previously collected sediments. This option is advantageous because it extends the research possibilities to a whole range of existing sediment samples. If it is possible to use previously collected soil samples, it would decrease the initial process of collecting samples. Furthermore, it would allow researchers to analyse sites that have been excavated in the past, when sedaDNA was not yet sampled.

Recently, there has been debate about the option of using nanopore sequencing instead of NGS as a way to reduce costs during sequencing (Brown et al., 2025; Sun et al., 2020). Nanopore sequencing is a technique that uses nanopores on a flow cell, through which single-stranded DNA fragments pass. During the process, each nucleotide that passes through the nanopore disturbs the current of the membrane of the pore in a nucleotide-specific way. These current changes are measured and used to reconstruct the sequence of the DNA fragment (Zhang et al., 2024). The advantage of this technique is that it is able to sequence ultra-long DNA fragments (10 kb to >1 Mb) and that it can sequence in real-time (Pagano et al., 2025). Real-time sequencing allows for the process to be stopped at any time. This is mainly an advantage when working through long reads that are focussed on finding, for example, one specific genomic mutation. It can also help reduce run-time because it enables researchers to stop sequencing when it becomes clear that the sample quality is not high enough.

Most advantages of nanopore sequencing techniques cater to long reads, and the costs are still higher than NGS, even when costs decrease with the number of samples sequenced per run (Cuber et al., 2023; Wydro, 2022; Zhang et al., 2024). Since sedaDNA from the Roman period is often very fragmented, nanopore sequencing will currently not bring many advantages to the table. Instead, I think for now it is best to stick with NGS, although further improvements of the nanopore sequencing technique might make it more economically viable in the future.

Chapter 6 Conclusion

The goal of this research was to find an answer to the research question, “*To what extent can the implementation of sedaDNA analysis ensure a more accurate reconstruction of an archaeological site from the Roman period in the Netherlands, in relation to current bioarchaeological research methods?*”. To summarise the research done in this paper, the results are concluded in this final chapter. By presenting my findings I can answer the main research question of this paper as follows.

SedaDNA is a promising tool for Roman bioarchaeology. Conventional current techniques, like macroscopical and microscopical analysis or isotope analysis, have shown great applications so far, but have their complications as well. The main problem with conventional techniques is the reliability on floral and faunal material. What is no longer present on site during excavation cannot be reconstructed. Therefore sedaDNA can become a great tool in the future for the reconstruction of biodiversity and the environment, as this is difficult to do with conventional techniques. The detection of different species that are present on site can then be used to answer different research topics about human practices in the Roman period. Research topics regarding human practices that can be explored using sedaDNA are diet, agriculture and husbandry, mobility, and even health.

While the implementation of sedaDNA analysis is a great prospective in theory, current research shows that sedaDNA analysis is so far on par with conventional research techniques, and does not far exceed their potential. The reason for this is a number of limitations. First of all, DNA in the soil is exposed to degradation and migration. This can cause DNA molecules to become heavily fragmented, which makes it harder to read them during sequencing, possibly resulting in false negatives and thereby the underrepresentation of the species on site. Along with this, migration can cause DNA molecules to get removed from their original context, making it hard to interpret the data found with DNA analysis. It is therefore important to work with sedaDNA alongside pedologists, in order to sufficiently understand the micromorphology of soil sediments. Apart from these issues that arise in situ, when excavating the material and taking soil samples, contamination is an important risk to be aware of. In order to avoid contamination, archaeologists should be taking samples sterile, with properly cleaned equipment. Samples should be handled sterile as well, and should only be processed in ancient DNA labs to avoid contamination with modern DNA. When doing sedaDNA analysis, it is important to always perform authentication to check for modern DNA contamination. When we reach the stage of bioinformatics, we see limitations with the taxonomic resolution of sedaDNA. This is due to short reads that are difficult to identify, but it is also an effect of the limited available genomic sequences in DNA databases. Along with this, it is difficult to quantify groups of species on site based on sedaDNA data alone. Lastly, an organism's function is not retraceable using sedaDNA alone, if there is no find context to interpret the data with.

To resolve these issues, there is a number of developments that could be further investigated in the future. First, more research should be done on the effects that different soil types have on the degradation and migration of sedaDNA fragments. In order to do this, I see potential in working together with pedologists, as they have a thorough understanding of soil micromorphology. Modern day contamination should be minimised by adhering to sterile protocols and working with control measurements. To improve taxonomic abilities of bioinformatics, hybridisation capturing could be applied during the sequencing process in order to target specific genomic sequences. This could be done after a general overview of present

species in a sediment sample has been identified, to make an even more specific analysis of which species or even subspecies are present in the sample. Additionally, it is important that researchers keep working on adding genomic sequences to databases, to create a more detailed and inclusive reference library. Furthermore, we should combine sedaDNA data with archaeozoological and archaeobotanical data, to better understand find context. This way material from conventional methods and sedaDNA can complement each other, thereby filling potential gaps the other left or validating their results. Finally, the use of statistical analysis is not used to its fullest potential. In order to deal with short reads, it could be a good idea to work on the widespread application of occupancy modelling when working with sedaDNA, as to limit false negatives or false positives, and to improve the resolution of sedaDNA analysis. This can also help to quantify sedaDNA in a sample, to estimate species abundance. To further implement this, I think it would be beneficial to collaborate with biological statisticians.

Additionally, a few methodological challenges occur as well. Right now, sedaDNA analysis is still relatively expensive, and consumes a lot of energy. This is amplified by the fact that in the Netherlands there is a limited number of sedaDNA specialists, meaning that materials are outsourced to specialised labs elsewhere. To make this technique more cost and energy effective, it is important to recruit more specialised sedaDNA analysts. Furthermore, looking into new techniques, like nanopore sequencing, could help reduce the costs of the overall process. Though I do not see any advantage of nanopore sequencing right now, it is important to keep evaluating whether the use of different techniques might be able to help reduce costs or time. The energy consumption is a more complicated problem, as this is not specific to sedaDNA analysis, but rather a general problem of the use of large data centres.

Even though sedaDNA shows great potential for the future, there are some research topics that it is not suitable for. Quantification of species remains difficult. Additionally, sedaDNA alone can say little about the function that an organism served in Roman times. It is therefore important that we see sedaDNA not as a new golden standard for bioarchaeology, but as a technique that can add to existing techniques to ensure more accurate data when reconstructing Roman sites. It can help detect species when floral and faunal remains are hard to recover, but it is best interpreted when combined with other findings from archaeozoological and archaeobotanical material, or when compared to the general historical or geographical context of a site. There are a lot of steps to be taken in the future in order to optimise sedaDNA. However, once the technique gets more integrated as standard practice in bioarchaeological research, I am sure it can help reconstruct an even more accurate and precise picture of Roman settlements and Roman landscapes in the Netherlands.

Discussion

Bioarchaeological Advances

There is a wide application of sedaDNA for Roman bioarchaeology, but when we truly want to improve the field as a whole, we must look into future developments beyond sedaDNA as well. Like discussed with dr. Groot and dr. Kooistra, methodological shortcomings in the field still provide an important limitation in current research. For archaeobotany, this has to do with the fact that implementation of archaeobotanical research on site is not yet standard practice. In other words, botanical material is not sampled by specialised researchers, if at all (interview Kooistra, 2025). This can lead to wrong or suboptimal sampling, where material on site might be missed as well. Along with that, sampling is not always done with a specific research objective in mind (Interview Kooistra, 2025). This means that research is often done

retrospective, which is counterproductive considering that data are analysed based only on available samples, instead of contextualised based on question-led sampling approaches (Lodwick & Rowan, 2022). Lodwick and Rowan suggested therefore that steps must be taken to integrate archaeobotany further into Roman archaeology, finding a more multidisciplinary approach together with general Roman archaeology and archaeozoology. They recognise the progress being made in the field of archaeozoology with the formation of the International Council for Archaeozoology (ICAZ) and suggest a research council like that be created for archaeobotany as well, in order to establish a research community and find an integral agenda for archaeobotanical research (Lodwick & Rowan, 2022). However, a large part of the shortcomings in archaeobotany are also caused by a lack of time and funding, which is an issue that sedaDNA could potentially help resolve (Lodwick & Rowan, 2022; Interview Kooistra, 2025). SedaDNA utilises small sample-sizes and shows potential to be performed during excavation by archaeologists without the presence of a specialist on site (Özdoğan et al., 2025; Interview Kooistra, 2025). This could reduce the costs and time needed to sample archaeobotanical material (Interview Kooistra, 2025).

Much like archaeobotany, archaeozoology also advocates for a more multidisciplinary approach to Roman archaeology (Interview Groot, 2025). According to Groot, archaeozoological research is commonly applied in Roman archaeology, and is well-developed as a research field in the Netherlands. Improvement lies in the standardisation of the field, as data are often not standardised, leading to a lot of variability among research papers. Where one paper may publish all data acquired, another may publish only the polished data in form of graphs, making it difficult to validate the results (Interview Groot, 2025). Standardising not only the data publication, but also data processing in general can help generate a higher comparability among different studies. The LSI, for example, is an important tool in archaeozoology, but it is not always clearly specified which logarithmic base is chosen for calculations, or which reference animal is used (Wolfhagen, 2020). It is important to note these choices, but taking it one step further, it might even be beneficial to further standardise those choices. Currently, there is not a lot of research done on the interpretative effects that these variations may have, although some research suggests that the choice of reference animal and picking the proper logarithmic base is essential for LSI-based research (Wolfhagen, 2020). Researching these variables in data analysis can help to standardise the field of archaeozoology and thereby make data more valuable to the research field.

When trying to improve the accuracy of bioarchaeological data within Roman archaeology it is crucial to evaluate these systematic methodological challenges. If we do not work on establishing a solid base for the field of Roman bioarchaeology in the Netherlands, then integrating new techniques into the field will not be as successful. Even though sedaDNA shows potential solutions to some research issues, we must not forget that if we do not advocate for imbedding, funding, and standardising archaeobotany and archaeozoology in Roman archaeology, the accuracy and validity of the research field as a whole will fail to actually strive forward. SedaDNA should always work alongside conventional techniques, and add to them, not replace them. By solidifying the base of Roman archaeology we can uplift conventional techniques and integrate sedaDNA in order to ensure more accurate data when reconstructing archaeological sites from the Roman period in the Netherlands.

Ethics

With the rise of sedaDNA, the ethical debate surrounding genetic material also grows (Heintzman et al., 2023). This debate is focussed not only on the laws that describe the handling

and export of genetic material, but on moral complications regarding ancient DNA as well. One of the questions that arises here, is how to handle potential recovery of human DNA (Heintzman et al., 2023). When this outcome is possible, it is important to consult with local communities during the design stages of the research process, in order to find a consensus on how to handle potential recovery of human material. According to the article of Heintzman et al., another way to work around this would be to apply bioinformatic filtering before running analyses (Heintzman et al., 2023). I do, however, not completely agree with this solution. Filtering such information might prevent it from ending up in the research results, but the genetic material will still have been excavated and extracted, meaning it is taken out of its natural habitat. On top of that, it is at that point already stored somewhere in a lab, where it could still be used in other research projects.

Apart from the extraction of human DNA, the use of environmental DNA raises questions in general. The excavation of sediment samples is a disruption of local ecosystems. Even though this is true for most of archaeology, it is still an important issue to keep in mind, especially because the research applications of sedaDNA are often focussed on climate, biodiversity and ecology (Heintzman et al., 2023). I think, when trying to reconstruct important aspects of the Roman landscape, in general you could say that the benefits outweigh the costs. However, much like the excavation of human DNA, researchers are not the only stakeholders in the process.

Lastly, an ethical concern that I have so far missed in most other research is the environmental impact of bioinformatics (Lannelongue et al., 2021). This is of course, not specific to genomic data, but rather applies to large databases and datacentres in general (Niewenhuis et al., 2024). As we progress in the age of data, the use of large datacentres will only increase (Steinhardt, 2024; Niewenhuis et al., 2024). Especially with the rise of generative AI-models, such as Large Language Models, the carbon footprint of datacentres will continue to grow (Niewenhuis et al., 2024). When working with sedaDNA, I do consider this an important ethical concern, as sedaDNA analysis is focussed on the reconstruction of biodiversity and other environmental aspects. I think it goes without saying that, when researching environmental material, the impacts of this research process on the environment should be considered. Though there is not a direct solution to this problem, a cost-benefit analysis of this technique should be reviewed. Being aware of the problem alone will already help shape research design in a way that keeps the ulterior motive of such landscape and settlement reconstruction in Roman archaeology in mind. Again, I think that here the benefits outweigh the costs, because it is important that biodiversity research is done. It is still good to keep the environmental impact in mind when doing research, though.

I think when ethics come in the picture, it is always best to consult a diverse group of people, and most importantly it is crucial to connect with local communities and people outside of the academic world who do not stand direct gain from such excavations. This can generate mutual respect between stakeholders in the process and will benefit the research design process. After all, what value does research hold if it is not done ethically?

Future Directions

Extending Research Applications

SedaDNA shows valuable potential for future research. Along with the ways in which sedaDNA can add to conventional techniques in order to research specific research topics on site, I think it shows broader applications for the field of archaeology. Instead of researching

one site in the frame of one time period, we can extend bioarchaeological research both spatially and temporally. One of the limitations of archaeobotanical material, for example, is that it can be preserved in different ways throughout the Roman empire, depending on the climate of an area. This means that one area might have a lot of leafy greens, and another might not, resulting in very different datasets of material. Because of this, it is difficult to compare different sites across the Roman empire. SedaDNA is available everywhere, so by using sedaDNA we might be able to create more standardised datasets that allow for comparability. DNA is still susceptible to damage depending on the climate it is preserved in though, so there might still be some difficulty in comparing different areas. However, if we combine conventional bioarchaeological material and sedaDNA data, we could increase the research possibilities across different sites.

SedaDNA shows the potential for longitudinal research as well. If we can reconstruct biodiversity, we gain the possibility of reconstructing human activity throughout time in order to research the impact of human activity on their surroundings (Interview Furni, 2025). If we can sample one site for multiple different time periods, we can explore changes in biodiversity and climate over time (Lodwick & Rowan, 2022).

Both of these research applications are prospectives for far into the future. Not only are they dependent on the further development of sedaDNA analysis as a research technique, but they require a consistent effort of data gathering. Bioarchaeological data are definitely growing as more and more samples are collected and analysed (Lodwick & Rowan, 2022). The increase in bioarchaeological data can give us more options for research across different time periods and different areas. Even though this is an application that does not lie in the near future yet, it is interesting to consider that such research could become a possibility further down the line.

Methodological Developments

The field of sedaDNA has made big steps towards becoming a valuable tool for Roman archaeology. Still, limitations and future improvements need to be considered. In this paper I have aimed to highlight the most important challenges of the application of sedaDNA in Roman bioarchaeology, as well as that I have tried to provide future research steps that should be taken in order to resolve the biggest challenges. Currently, a team of researchers at the C-LIMES project is working towards perfecting the technical aspects of sedaDNA analysis. In a recent study, the team experimented with sediment sampling done by archaeologists who are not specialised in working with DNA, to test if they could set up a followable protocol that minimises contamination (Özdoğan et al., 2025). I think this multidisciplinary approach, using specialists as well as general field archaeologists, is a good direction for the future. This makes bioarchaeological research more accessible and cost-effective, while also promoting the integration of bioarchaeology into Roman archaeology as a more standard practice during excavations. On top of that, I think another important step is to train more sedaDNA specialists and to create more accessible DNA facilities in the Netherlands. This will open more research possibilities, accelerating the process of resolving current issues regarding sedaDNA analysis. Like discussed with Furni, training specialists starts by making biomolecular archaeology a standard part of the archaeology university curriculum, to get students familiar with the basics of biomolecular archaeology (Interview Furni, 2025). While that is not easy to execute, it is important to advocate for it.

Multidisciplinary Approach

In the process of developing sedaDNA as a tool for Roman bioarchaeology, I think it is important to involve other archaeologists, like archaeobotanists and archaeozoologists, in the research process. I have experienced in this paper, how valuable the insights from experienced archaeologists are when focussing on the research applications of sedaDNA analysis. Consulting other fields can also help with the further improvements of sedaDNA analysis techniques. For example, by consulting biological statisticians, we could research the potential that statistical analysis holds for sedaDNA data. In future research, it is important to take on this multidisciplinary approach, much like bioarchaeologist themselves advocate for (Interview Groot, 2025; Interview Kooistra, 2025).

Lastly, ethics are always an important part of research, and as we enter an age where the world is stored in data, the environmental and moral impacts of that should not be neglected (Steinhardt, 2024). In this paper I briefly touched the topics of ethics in genomic research, but I think it is important to delve further into the ethical component of the use and storage of genomic data. Apart from ethical discussions about this, I think it would be good to do more research about the energy use and environmental impact of bioinformatics and large genomic databases, as limited research is currently available on this topic. This does not mean that all of the research needs to be done by sedaDNA researchers themselves. Much like within archaeology, it is important to adapt a multidisciplinary approach outside of archaeology as well. By consulting data scientist, ethicists and local communities, sedaDNA could become a well-designed tool beneficial to archaeologists, environmentalists and locals alike. We still have a long journey ahead of us, but Rome was not built in a day either.

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Statement of AI

The author declares that no form of generative artificial intelligence, such as ChatGPT, was used in the literature review, construction of the paper, or any further parts of this thesis.

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Appendix 1 Interview with Archaeobotanist Laura Kooistra, PhD

24-04-2025, translated from Dutch

What are current limitations in the field of archaeobotany?

An important issue in archaeobotany is dating. Archaeobotanical material needs to be dated in order to be relevant for research, preferably in a closed context. In the case of Roman archaeology, pottery dating is more accurate than C14-dating. So archaeobotany needs this find context to determine the period where the material came from. Especially the early Roman period (10 BC – 140 AD) is rich in pottery, and can therefore be used as a pretty accurate dating tool in archaeobotany.

The distinction between consumption and production is difficult to establish. When we find material from a Roman settlement this always indicates human intervention. However, it is hard to tell if this was from consumption of production, or perhaps both. When looking at water wells, we can occasionally find some products from surrounding nature in a human context. In order to distinguish between human products and nature here, it is important to understand the structure of the well, as this provides important find-context for your archaeobotanical material. When water wells are in use, they are normally covered, meaning that it is not possible for surrounding material to enter the well (apart from pollen, those always get into the well). After water wells get out of use, they usually get repurposed as waste pits. Then, when they are also discarded as waste pits, the organic material in the pits shrinks a bit, leaving a small well at the top for organic material from the surroundings to collect in. Archaeobotanical material found in the last phase of this process is usually material directly from nature, not from human intervention. Apart from this, it is difficult to distinguish material from nature in the context of human settlements.

In order to further distinguish between wild plants and agriculture, it is also important to look at other aspects of the find context. For example, acorns found in high concentrations in the same area are collected by humans from the wild, so they are not cultivated.

Which parts of archaeobotany are researchable well with current techniques?

Deposits on tools for cooking can say a lot about plant based and animal based fats present in the Roman diet, this is often researched in combination with the environmental scanning electron microscope (ESEM).

Isotope analysis is used to research manure practices on Roman farms. This is done by analysing nitrogen ratios in floral remains. Nitrogen has a heavy and a light isotope, plants containing more heavy nitrogen isotopes indicate the presence of nutrient-dense and manured soil. Other isotopes, like strontium, are used more in humans and animals, not so much in plants. It is therefore difficult to use isotope analysis to say something about migration of plant species.

For botanical research it is important to do culture research, but it is even more important to research field flora. Wild plants on the field indicate how plants grow in a natural environment and form an important reference frame for agriculture. Wild plants found on the fields can also help to clarify the origin of certain species. Sometimes, when excavating floral remains, we

find weeds that are not natural to the site, which means that we can see that the cultivated plants found along those weeds originate from a different area.

In which ways do you think that sedaDNA analysis could contribute to the field of archaeobotany?

One of the advantages of sedaDNA is that it enables field archaeologist to take much smaller samples than is currently needed, and that it can potentially show a much more diverse range of DNA. It is also cheaper, which is a big advantage for archaeobotany. In Roman archaeology there is not a large budget for archaeobotany available, meaning that archaeobotanical sampling is not part of the standard excavation procedure, and when it is done it is not always done well. In that case, sampling is not done purposefully with a specific research question in mind and not always by expert, this can result in the wrong samples being taken or the overall quality of the archaeobotanical analysis decreasing.

It is interesting to look at the Roman diet using sedaDNA. For example, on images from the Roman period we can often see cabbage, but we rarely ever find it on site. Based on the images you would expect it to be much more present than it currently is. You could look at material from latrines to see if you can identify more cabbage in there using sedaDNA analysis. It could also be an interesting technique to analyse pollen found in latrines, to see if we can get more details about the exotic plants the pollen originated from. This way we could research the use of spices in the Roman diet.

Ancient DNA could potentially tell us more about the origin of certain plants. For example, we know that beets and celery grew on the coast of the Netherlands during the Iron age. In the Roman period, such vegetables could also be found land inwards. What is thought however, is that the vegetables found in the inward parts of the Netherlands originate from the Mediterranean, not from the Dutch coastline. By comparing DNA you could identify which plants are related to each other, and thereby trace back the origin of certain plants used in the Roman period in the Netherlands.

SedaDNA still copes with the same migration problem as conventional archaeobotanical material. When materials get mixed, due to migration in the soil because of worms for example, the DNA is taken out of its original context. In the field of palynology we therefore use the soil micromorphology to better understand the layers of the soil, and to identify migration. You could apply the same technique to sedaDNA samples to better understand their context and origin. This problem usually occurs most in sand, and less in moors.

Appendix 2 Interview with Archaeozoologist Maaïke Groot, PhD

01-05-2025, translated from Dutch

What are current limitations in the field of archaeozoology?

The biggest limitation right now is the fact that there is not a standard method for archaeozoological research. This creates different ways of data publishing. For example, the level of detail in data publications varies among different papers. Some papers publish only the final results, shown in graphs and conclusions, but do not add their rough data to the papers. This makes it difficult to validate the conclusions, as you cannot look at the data they came from.

It is difficult to distinguish between consumption and production of zoological finds, unless there is a very clear find context. Wild animals are usually also only found in the context of humans, when wild animals are found this often indicates hunting practices, meaning that these animals were usually consumed, unless you find antlers.

Which parts of archaeozoology are researchable well with current techniques?

In the Netherlands, archaeozoological research is executed as part of the standard excavation procedure, and we have a lot of skilled experts. There is a lot of data, many sites have been analysed using archaeozoological data.

With zoological remains there is a lot we can research. We can analyse weight and age of an animal, slaughter, burn or bite marks, or ratios of different species. Measurement can be researched using the LSI, which is increasingly applied in the field. Isotope analysis is less integrated into archaeozoological research. At first it was a little unclear what the potential of isotope analysis was for the field of archaeozoology, and it had to be fit in our budget as well. Nowadays we do use it, for example to look at cattle's season of birth. In animal bones, isotope analysis can be done on nitrogen, carbon, oxygen and strontium.

In which ways do you think that sedaDNA analysis could contribute to the field of archaeozoology?

For the future directions of archaeozoology, we are mostly focused on the standardisation of the field. Sample methods during excavations can still be perfected, for example by sifting faunal remains to look for fish or other small bones. Along with that, data publications need to be more standardised. Furthermore, it is important that archaeozoology becomes more integrated with archaeobotany to better analyse Roman sites. For example, when archaeozoologist suspects an increase of cattle throughout the years, an archaeobotanist can look at whether the size of grass fields also increased.

To answer more specific questions we could potentially implement ancient DNA or sedaDNA. This could be used to research migration patterns among animals, or the origin of certain species. It can also be used to look into specific traits of certain animals, like the quality of wool of different sheep subspecies. Lastly, sedaDNA could also give insight in the animals that occurred in the wild landscape surrounding a Roman settlement, and could help identify naturally occurring animals like different insect species.

Appendix 3 Interview with Geneticist Fabrício Furni, PhD

13-05-2025

What are possible applications of sedaDNA analysis in Roman archaeology?

SedaDNA can be used to reconstruct biodiversity, in order to get insight into surroundings, landscapes, and past climates, in other words things that are not preserved through human interference. These are often difficult to reconstruct with conventional methods, because it is time consuming and might neglect species that are not well preserved. Species that are not preserved through conventional methods can leave traces in the soil we sample for sedaDNA. With the reconstruction of biodiversity comes the possibility of reconstructing human activity throughout time in order to research the impact of humans on their surroundings. This also includes human activity that is not directly related to diet, such as the introduction of rats by Romans in the Netherlands.

In relation to human diet, it is also interesting to research species that are known to be consumed by Romans, but are not often found on site. The point that dr. Kooistra made in regard to cabbage is a good example, as cabbage is consumed before it produces seeds, so it often leaves no traces on site, but could in theory be found with sedaDNA. This goes for animals as well, for example chickens, rats, or insects.

What are the current limitations regarding the application of sedaDNA analysis in Roman archaeology?

Migration of DNA in soil poses a problem when dating DNA fragments. This is less of an issue as is seen with pollen in palynology, for example, because sedaDNA can be dated to a certain extent. When DNA is in the soil for a long time, damage and fragmentation occur, which makes ancient DNA distinguishable from modern DNA. However, we can not make a distinction between DNA from the early Roman period and the late Roman period. Therefore, in the soil DNA from different time periods can get mixed up. In general, DNA contamination is important to be aware of, both ancient contamination and modern contamination.

Right now, sedaDNA analysis is expensive. Over time we do expect it to become cheaper, but for now it remains an expensive procedure. The facilities for data analysis use a lot of computing power and electricity, because we work with databases of 30-40 TB in order to compare the samples to reference material from all over the world. So, not only is analysis expensive, but it is also not environmentally friendly. In the Netherlands, we currently use a number of databases, like the NCBI and ENA as reference databases. These do not contain material from all organisms as sequences need to be determined before being able to upload them to a database, and we see a strong research bias in human related species as opposed to environmental species. Nevertheless, new species are added by the month, so we do see a continuous improvement of taxonomic databases.

Another issue in the Netherlands is the lack of specialist and facilities. We do not have enough specialist who are able to analyse sedaDNA, neither do we have the proper facilities in the country. Material therefore has to be sent to labs outside of the Netherlands. Not having the proper facilities and specialist makes it more difficult to integrate sedaDNA analysis into bioarchaeological research. To fix this, we should not only look at specialist training, but we

should also work on implementing biomolecular archaeology in university programs, so scholars get familiar with this part of archaeology at the start of their career already.

What is the current reach of sedaDNA analysis of the Roman period in the Netherlands?

Right now, the results of sedaDNA analysis show the potential to reconstruct a broad view on the behaviour of the Romans in the Netherlands. We can even reconstruct their diet. The technique is not yet developed enough to show specific organisms. All in all, we can say that the technique is at least on par with conventional techniques, so it is good enough to reconstruct the same level of depth and detail as we have done with conventional techniques.

Appendix 4 List of Figures

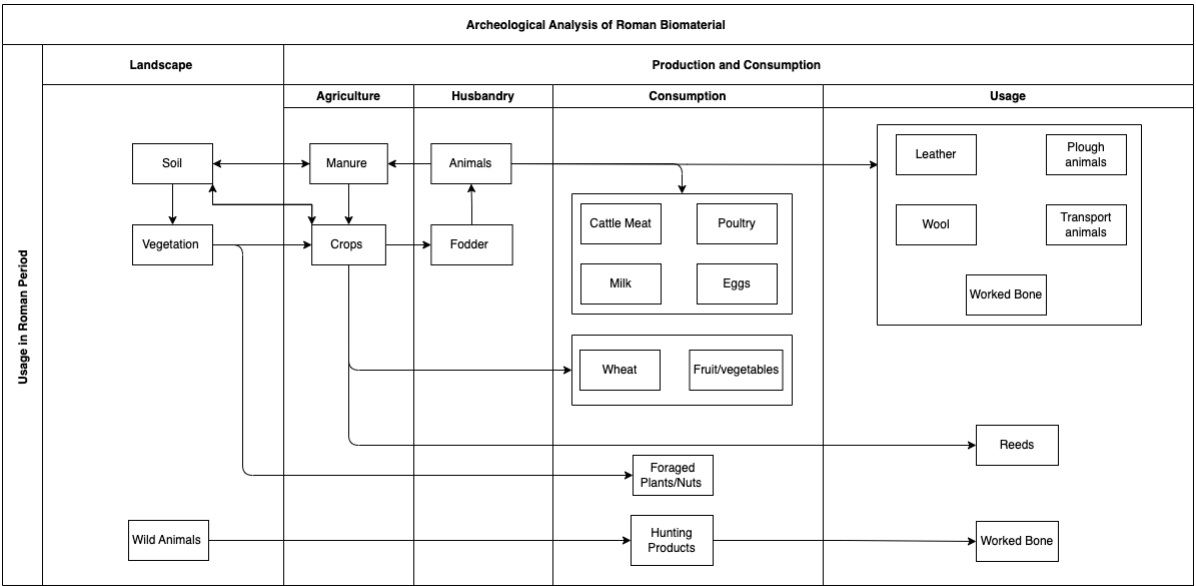


Figure 1: schematic model showing the use of flora and fauna in the Roman period

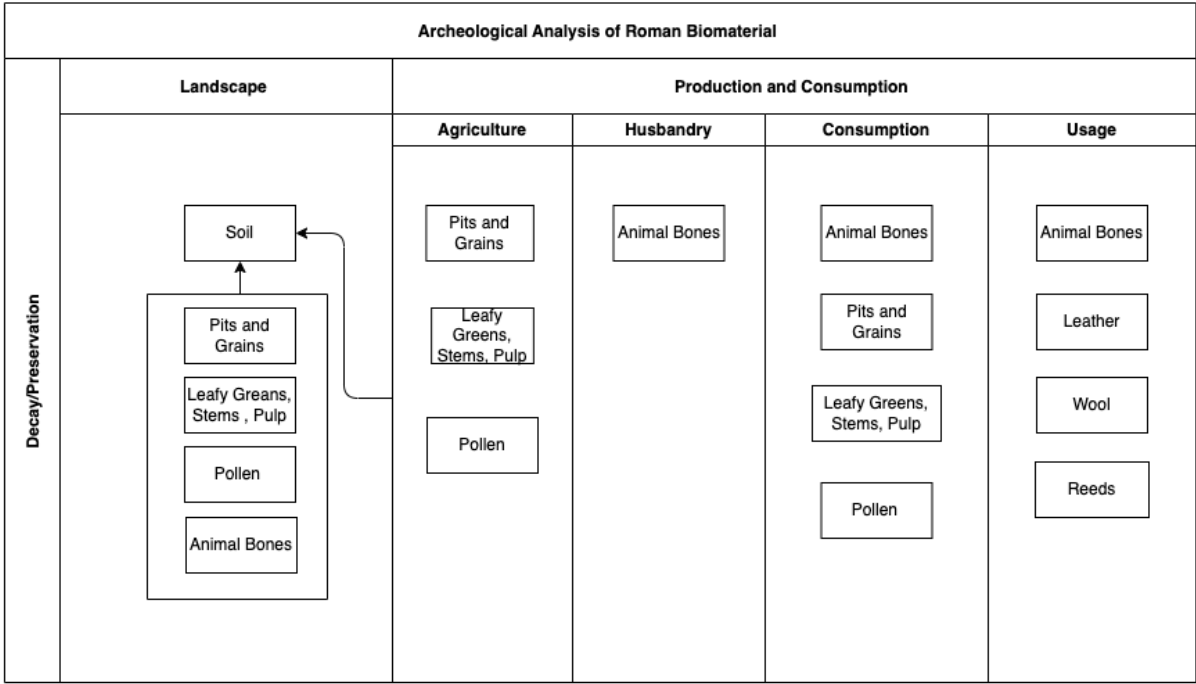


Figure 2: schematic model showing distinguishable features of organic material that can be found during excavation

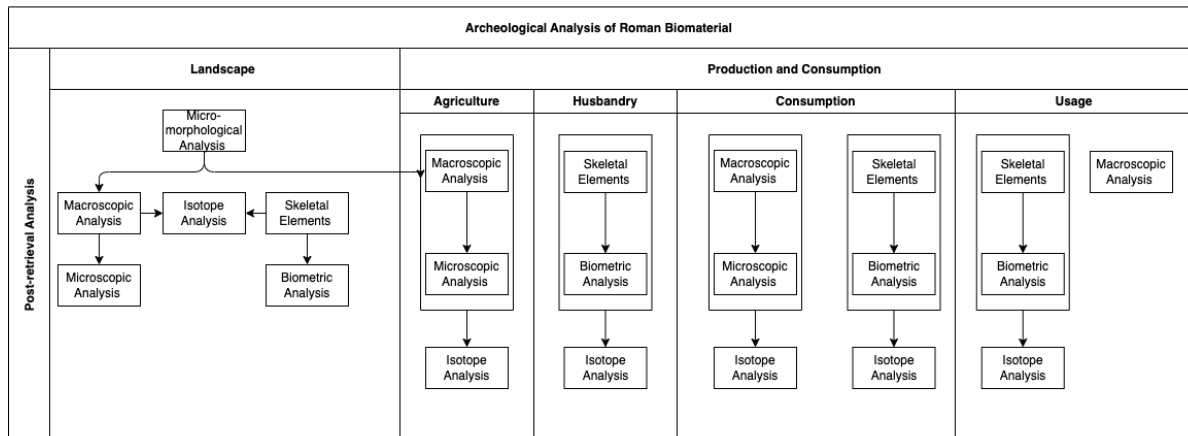


Figure 3: schematic model showing the different analysis techniques of organic material in Roman archaeology

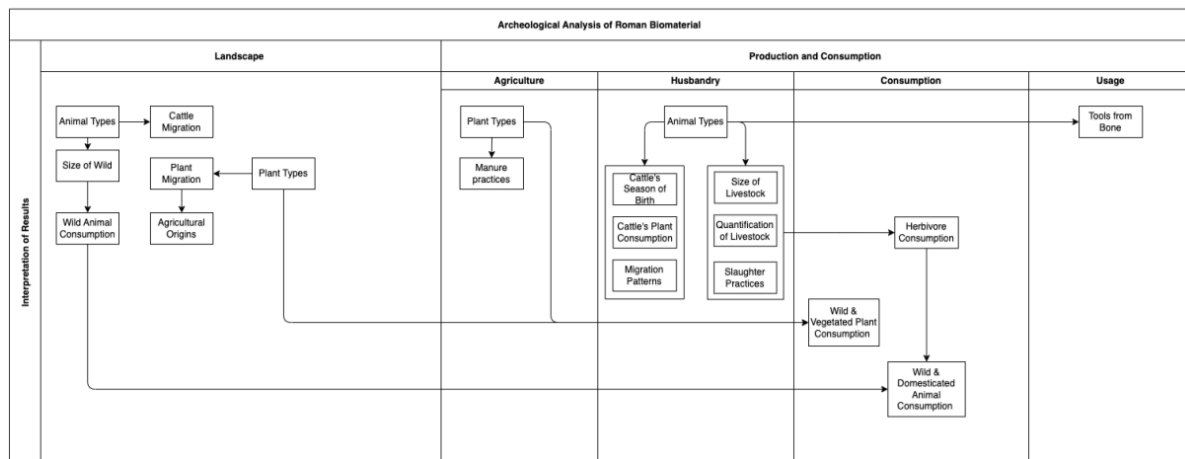


Figure 4: potential research applications of the analysis techniques in Roman bioarchaeology