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Reconstructing bat diet and insect biodiversity from guano sedimentary DNA

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European insectivorous bats predominantly prey on beetles, flies, moths, and other insects. However, insect biodiversity and biomass have dramatically declined in the 20th century due to habitat loss, agricultural intensification, pollution, biological invasions, and climate change. These declines likely impact bat populations by altering prey availability and foraging habitats.

The REPAST project used guano cores as sedimentary DNA (sedaDNA) archives to investigate historical changes in bat diet and insect biodiversity. We analyzed insect DNA from guano using high-throughput sequencing of the mitochondrial COI gene, a method adapted from DNA barcoding of arthropod prey in bat feces. This approach allowed us to identify prey species and assess changes in insect species richness and diet composition over time. Results indicate temporal shifts in diet, with declines in specific richness that likely reflect changes in landscape composition and exposure to environmental stressors.

By linking temporal changes in bat diet to human-induced pressures, such as land-use changes, pesticide use, and climate variations, this study provides new insights into the drivers of insectivorous bat population dynamics. These findings highlight the potential of guano sedaDNA for understanding past ecosystems and guiding conservation strategies in the face of ongoing biodiversity loss.

Presentation of MAGIC2 project: Mountain AGro-ecosystems past trajectories developed by different societies under different bioclimatic conditions

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One of the challenges facing societies in the 21st century is how to feed a growing population against a backdrop of resource depletion and climate change. This will require a rethink of our production methods and a more sustainable use of our environment. Studying the interactions between society, climate and the environment since the birth of agriculture can provide food for thought by showing how humans have profoundly altered ecosystems over the millennia and how they have sometimes had to change the way they farm. In this general context, the aim of our project is to document the complex interactions between agro-sylvo-pastoralism (activities and practices), the environment (biotic and abiotic), societies and climate in the Western Alps. The project will focus on three-time windows (4th century BC to 7th century AD, 11th-15th century and 8th-20th century) that show bifurcations in the trajectories of socio-ecosystems. We propose to integrate data from lake sedimentary archives (6 new and 6 old sites), historical and archaeological archives to provide a better understanding of the origins of these bifurcations. Landscape changes at different spatial scales, soil erosion dynamics, plant pest dynamics, fertilisation practices and changes in the intensity and nature of agro-pastoral activities will be documented using a multi-indicator approach. Here we will present the preliminary results of the 3-year project that has just started.

Plant sedaDNA from the Highlands of Ecuador

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Historically, analysis of sedimentary ancient DNA (sedaDNA) was thought to be restricted to freshly-collected sediment cores from cold environments, primarily the high latitudes with some reported success in sediments from alpine lakes. In recent years, however, advances in theory and laboratory methods have yielded a proliferation of studies using sedaDNA from temperate and tropical environments (e.g. Giguët-Covex et al., 2014; Dommain et al., 2020), as well as from cores that have been removed from situ for ≥ 20 years (Seeber et al., 2022). Our work on sediment cores collected in 2003 from the Ecuadorian highland lake Laguna Culebrillas (3900 m a.s.l.; 2.4232 °S, 78.8603 °W) is one such study. We extracted and prepared sedaDNA for plants from this lake with an extraction protocol specified for ancient materials by Rohland et al., (2008), the Santa Cruz Reaction (Kapp et al., 2021) for single-stranded library preparation, and the Angio353 kit by Arbor Biosciences for hybridization capture. Our results show recovery of authentic ancient plant DNA from sediments as old as 4400 cal. yr BP, which included reads classified as grasses (e.g. *Paspalum*, *Calamagrostis*, and *Brachypodium*), sedges (e.g. *Carex*), and rushes (e.g. *Juncus*), which are otherwise difficult to identify to the genus level using classical proxies such as pollen analysis. As the first study from the Ecuadorian highlands, this represents a promising start for research using sedaDNA from páramo lakes.

Environmental salinity and pH determines DNA-mineral interactions

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Despite the recent advances within sedimentary environmental DNA (SedaDNA), we still have much to learn about the taphonomic processes leading to DNA preservation in sediments. In the environment DNA can be degraded by physical, chemical and biological factors. DNA degradation in an aqueous suspension is well understood, however, the nano level mechanisms of DNA adsorbed on mineral surfaces are still unclear. The DNA adsorption capacity of minerals depends mainly on the mineral surface charge and surface charge densities, which are strongly influenced by aqueous environmental conditions. Consequently, a shift in e.g. pH or salinity can influence the DNA-mineral bond leading to poorer or enhanced preservation.

In this work, we used Chemical Force Microscopy (CFM) to measure adhesion forces between DNA backbone phosphate groups and the iron(III) oxide-hydroxide mineral goethite. We probed the association strength during varying pH (pH 5 and 7) and salt composition (10mM NaCl and 10mM MgCl₂).

Our results show that DNA are stronger adsorbed to goethite under the higher saline conditions (MgCl₂>NaCl>H₂O). Our findings comply with principles of interfacial geochemistry and can be explained by Na and Mg decreasing the thickness of the electric double layer surrounding the mineral surfaces. Our results highlight that geochemical properties of depositional environments are a crucial step for DNA preservation and can be used to inform design of targeted eDNA extraction protocols.

Distinct marine eukaryote sedaDNA signatures characterise East and West Antarctic ecosystems

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Antarctica is highly vulnerable to climate change and studying past ecosystem responses to environmental change, such as through sedimentary ancient DNA (sedaDNA), is a matter of urgency. We extracted sedaDNA from different Antarctic locations, including the West Antarctic Scotia Sea (IODP Exp. 382 Site U1536) and the East Antarctic Totten Glacier region (IN2017_V01 Sites KC02 and KC14). A metagenomic shotgun approach was applied to investigate marine eukaryote composition over the last 45ka, complemented by the targeted capture of plankton-derived sedaDNA ('Planktonbaits1'). In the Scotia Sea, the shotgun data revealed a clear shift from a *Phaeocystis*-rich to a *Chaetoceros*-rich community starting at ~15.8ka, with *Chaetoceros* becoming the dominant marine eukaryote from 11.75ka to present. In the Totten Glacier region, marine eukaryote communities were much more diverse and fluctuating over the last 22ka, with a short-term dominance of *Chaetoceros* at KC02 from 10.3ka to 8.2ka, and at KC14 from 9ka to 5.3ka. Our study shows that there are significant differences between the marine paleo-ecosystem structure of East and West Antarctica, and also on relatively small spatial scales such as the neighboring Totten Glacier sites (~50km apart). Considering additional lithological observations from the same sediment cores showed that differing oceanographic conditions influenced these paleo-ecosystems, warranting further research into the population dynamics around the continent.

Reconstructing Vegetation and Agricultural Dynamics Over 9,500 Years: Insights into DNA Deposition in Northern Norway

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The analysis of ancient sedimentary DNA (sedaDNA) from lake sediments is an established method for investigating past biodiversity changes influenced by climatic and anthropogenic factors. In this study, we analyzed a sediment core from Stabbevatnet, a small lake in northern Norway, spanning 9,500 years. Using metabarcoding with 16S (mammal) and trnL (plant) primers, we identified 217 taxa. The results reveal the introduction and increased prevalence of agriculturally associated plant taxa, such as *Avena* (oats), *Vicia* (legumes), and Solanoideae, beginning around 2,000 years ago, with intensification around 1,000 years ago. These taxa co-occur with agriculturally significant mammals like *Capra* (goat), *Bos taurus* (cattle), and *Ovis* (sheep), highlighting the link between plant introductions and pastoral land use. The study is supported by two vegetation surveys and 84 surface sediment samples across the lake. Additionally, the top 4.48% of taxa, consistently detected in most surface sediments, were dominant throughout the core, particularly during the last millennium. This alignment of modern and ancient sedaDNA with vegetation surveys reinforces the reliability of sedaDNA for detecting dominant vegetation over different time periods and addressing taphonomic questions regarding DNA deposition. By tracking the disappearance and persistence of taxa, this research provides a narrative of vegetation changes shaped by climatic variability and human activity.

Reconstructing beaver-modified Holocene ecosystems with sedimentary ancient DNA

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Ecosystem engineering by beavers profoundly impacts the hydrology, geomorphology, and ecological communities of riparian environments. However, relatively little is known about how beavers in North America were temporally and spatially distributed prior to their near-extirpation by the European-American fur trade. Similarly, our understanding of how beaver ecosystem engineering alters the local environment on long (beyond decadal) time scales is limited.

Here we present lake core sedaDNA from two alpine ecosystems in North America – the Sierra Nevada range in California and the Grand Teton range in Wyoming – to investigate beaver presence and vegetation dynamics over the Holocene.

Our results indicate that beavers established in the Grand Teton range in the mid-Holocene, coinciding with increased regional precipitation and a shift in vegetation regimes. We documented 5 ka of continuous beaver presence in one lake catchment, suggesting that this important ecosystem engineer is an established and integral part of the local landscape and may be able to maintain wetlands during periods of extended drought.

The second phase of this project is focused on the Sierra Nevada range in California, further testing the hypothesis that beaver presence is locally dynamic, impacts vegetation trends, and is modulated by regional climate. By assessing these two regions together, we can begin to infer larger-scale patterns of beaver engineering and landscape response throughout the Holocene.

Unraveling Historical Dynamics of Marine Biological Invasions: A sedaDNA study of Intertidal Ecosystems

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Understanding the historical dynamics of biological invasions is key for unravelling population dynamics of invasive species and informing biodiversity managers. However, little work has been conducted in this research area and no study has used sedimentary DNA (sedaDNA) for this purpose. Here, we studied the intertidal zone of the Solent Estuary on the south coast of the UK, using a paleogenomic approach integrating genetic (metabarcoding), paleoecology, and geochemistry data to advance our understanding of community changes during biological invasions, as well as the timing and patterns of species introduction.

We performed metabarcoding of sedaDNA on COI and 18S genes to detect non-indigenous species (NIS) of ecological and socioeconomic importance and correlated native and NIS richness with trace elements from XRF analysis. Sediment cores, dated with Pb210, revealed two peaks of pollutants likely corresponding to increased marine transit, facilitating NIS introductions. Our results showed significant increases in NIS abundance since the 1960s, coinciding with historical anthropogenic activities, such as container dock construction. Different NIS exhibited distinct introduction patterns, and we observed community shifts, with NIS often outcompeting native species. By detecting historical community changes, our work contributes to forecasting future ecosystem shifts driven by NIS, offering insights into key ecological concepts such as biotic resistance and priority effects.

Overview of eukaryotic succession in Gdansk Bay over the last 5000 years BP

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The growing use of sedimentary ancient DNA (sedaDNA) has significantly enhanced our ability to assess anthropogenic impacts on coastal marine environments, particularly in regions affected by industrialization (Barrenechea et al., 2023; Siano et al., 2021). Gdansk Bay (Baltic Sea, Poland) is a heavily polluted area due to its enclosed nature and slow water renewal, making it vulnerable to land-based pollution from industrial activities, rapid urbanization of towns such as Gdańsk and Gdynia, as well as the extensive drainage basin of the Vistula River. This study utilizes the V9 region of the 18S rRNA gene, a common marker for assessing marine biodiversity, to explore eukaryotic succession in the bay over the last 500 years BP. Our objective is to analyze the community composition and identify key drivers, both climatic and anthropogenic, that influence biodiversity in this region. The study will incorporate additional data, including chloroplast DNA (TrnL), to provide a comprehensive view of terrestrial biodiversity and the resulting ecological dynamics.

This research aims to enhance our understanding of historical biodiversity patterns and inform future biomonitoring and conservation efforts in Gdansk Bay.

Process-based modelling of Fennoscandic plant communities using sedimentary ancient DNA

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Future trajectories of plant communities under climatic warming are difficult to predict, in part due to our inability to account for mediation from community interactions. Sedimentary ancient DNA provides taxonomically rich data to explore the nature of relationships between plant and animal taxa over relevant temporal and climatic gradients. Focusing on 17 key plant taxa and one mammal taxon in ten Fennoscandic lakes over the Holocene period, we evaluated the addition of interspecific competition, tree and shrub shading, herbivory, and temperature forcing to logistic growth model fitting using a scientific machine learning parameter retrieval approach. Community interactions (specifically herbivory and shading) or temperature forcing provided better model fits in most lakes, but more complex model structures did not consistently reduce residual prediction error. At the scale of the watershed, consideration of ecological interactions is most beneficial in communities with complex temporal population trends.

Using palaeoecological off-grid genomics to understand the effects of wildfire on freshwater ecosystems

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Wildfires are increasing and threaten many fire-sensitive ecosystems, including freshwater environments; however, very little is known about how fire impacts freshwater systems. Sediment archives provide the opportunity to observe past terrestrial-aquatic environmental change and its ability to recover from disturbance. Sedimentary ancient DNA has revolutionised how we interpret past environmental change, providing more detail on complex systems, even with old and degraded DNA. This work aims to 1. use off-grid genomics to enhance techniques used on sediment archives and 2. determine if fires alter freshwater environments. In 2024, three >50cm long sediment cores were collected from Paddy's Lake, Forgotten Lake, and Lake Osborne in Tasmania, Australia. These cores were subsampled for sedaDNA extraction at ~1cm intervals in the field during extruding. DNA extractions and sequencing was performed off-grid using Oxford Nanopore technology. DNA preservation varied across the sites likely due to differing geography, from the 20m deep oligotrophic, dystrophic alpine Paddy's Lake in the northwest to the 8m deep acidic, ultra-oligotrophic subalpine Lake Osborne in the south. Preliminary results indicate that palaeoecological metagenomic sequencing can be performed off-grid, within 48 hours of sediment collection. We anticipate the results our will be the first to utilise off-grid field techniques on sedimentary archives and elevate our understanding of fire impacts on freshwaters.

Holocene climate warming and coastal ecosystem dynamics in northern Norway: mapping marine metazoan diversity using sedaDNA

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Over the past century, Arctic coastal regions have experienced rapid climate change, resulting in major shifts in ecosystem composition and function. Marine Arctic food webs and community dynamics are changing as boreal species migrate northward in response to ocean warming. Despite these changes, many implications remain unknown due to the largely unmapped marine biodiversity. Advancements in paleogenomic techniques and improved resolution of spatiotemporal paleoenvironmental data are providing new insights. The last glacial-interglacial transition, around 11,700 cal. years BP, featured some of the most rapid warming events, leading to extinctions and community changes within just 1,600 years.

This study aims to map coastal metazoan diversity, using ancient environmental DNA extracted from sediments (sedaDNA) providing a DNA record dating back to around 11,700 years BP during the Holocene postglacial warming period near the Lyngen peninsula in Northern Norway. We use metabarcoding with the eukaryote 'allshorts' 18S primer V7 SSU rDNA and shotgun sequencing to assess changes in biodiversity over time. This study contributes base-level insights necessary to understand changes in marine species composition in Northern Norway. Integrating high quality past and present community information has the potential of enabling better models of marine coastal ecosystem responses to projected climate scenarios and thereby, expanding the frontiers of ecological theory.

Genetic insights into the occupational history of El Castillo Cave over time

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The transition from the Middle to the Upper Paleolithic, the extinction of Neanderthals and the arrival of Anatomically Modern Humans (AMH) are complex topics in European archaeology. One key site for studying this time period is El Castillo cave in Puente Viesgo, Cantabria, Spain.

Here we take advantage of the potential of sedimentary aDNA analysis for reconstructing the occupational histories of Palaeolithic archaeological sites. For this purpose, we collected 384 sediment samples from El Castillo, spanning from unit 16 (dated to >41 kya) to unit XXV (dated to 141 kya). In an initial screening for aDNA preservation we analyzed 218 samples, 56 of which yielded ancient hominin DNA. The analysis of diagnostic positions in the mtDNA genome that distinguish different groups of hominins revealed significant support for AMH in unit 17, and Neanderthals in units 17, 18b, 18c, XXab, XXc, XXd, XXe, XXf and XXh.

Using the software Kallisto to compare the hominin mtDNA fragments to published mtDNA genomes of Neanderthals we found similarities to Mezmaiskaya 2, a late Neandertal from the Caucasus, in samples from units 18b to XXc, and significant similarities to Mezmaiskaya 1, a Neandertal dated to 70-60 kya, in samples between units XXf and XXh. However, further nuclear DNA data is required to determine if this indicates a population turnover in Neanderthals at the site.

Biodiversity impacts of environmental changes in the Canadian Beaufort Sea

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Arctic coastal environments are some of Earth's most vulnerable regions to global climate change. Warming leads to permafrost degradation, changes in sea ice conditions, and increasing land-ocean fluxes which alter coastal biogeochemistry and reshape the ecosystem. Severe biological and socioeconomic consequences of ecosystem shifts are evident today, yet the dynamics and driving forces of ecosystem response to climate change impacts in nearshore Arctic regions remain understudied. We use marine sedimentary ancient DNA from the Mackenzie Delta region of the Beaufort Sea, Canada, to provide long-term context for biodiversity and ecosystem shifts over the past millennium, by combining state-of-the-art metabarcoding and shotgun metagenomics. The aims of this project are to create baseline data of past coastal biodiversity for key taxa in the Beaufort-Mackenzie region and to compare historical and modern biodiversity and ecosystem dynamics. Further, we assess links between specific climate change impacts, such as sea ice dynamics and land-ocean organic matter fluxes, and ecosystem and diversity responses. The outcomes of this study will offer perspective on ecosystem dynamics over the past millennium in the Canadian Beaufort Sea, and provide a framework for understanding current changes to and potential future consequences for Arctic coastal environments, their management and conservation.

SedaDNA and Phytoliths provide a 700-year record of multi-cultural plant biodiversity in the Central Mediterranean

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Ancient sedimentary DNA (sedaDNA) and phytoliths from agricultural terrace sediments are a rich new source of evidence of human-driven multi-cultural biodiversity change during the Anthropocene. This first use of sedaDNA at a dry-land Medieval archaeological site reveals polyculture with 134 plant taxa (including 20 crops/medicinal plants), 9 animal taxa in the central Mediterranean (Sicily). Archaeobotanical analyses revealed 29 taxa of which 15 were also found in the sedaDNA. The chronology spans the 11th Century CE to modern times and is consistent with the expansion of the 'Norman' castle in the 14th–15th centuries CE. Although the castle is a militaristic response to Arabic influence, the plant assemblage revealed by the sedaDNA has strong Arabic elements. Many of the plants have strong affinities with Arabic gardens and so although constructed under the Norman/Angevin elite the terraces within the town were probably modelled on earlier Islamic plots and reveal a continuity of cultivars and agricultural practice. The remarkably high plant diversity had many societal benefits; nutritional breadth, drought resistance and strong cultural associations. The high-preservation of sedaDNA is also investigated revealing the presence of swelling-clays (including smectite), allowing DNA preservation above 10°C. This application of sedaDNA and phytoliths opens a new window on ancient agriculture which has relevance to agricultural sustainability today.

Virus-host interactions throughout the Late Quaternary inferred from lake and marine sedimentary DNA

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Viruses play a critical role in biogeochemical cycling, host evolution and population control, impacting the functional potential of ecosystems. Understanding these impacts requires a paleoenvironmental framework. We used sedimentary ancient DNA from lake and marine cores to infer past DNA virus taxa from metagenomic sequences. Supporting our hypothesis that sedaDNA is a viable proxy for ancient virus communities, lake and marine virus assemblages were distinct, with transitions observed from the last glacial to the current interglacial. Lake virus communities were dominated by bacteria-infecting Caudoviricetes, whereas marine communities featured Caudoviricetes, Phycodnaviridae and Mimiviridae, which infect bacteria, algae and protists, respectively.

We identified 209 virus-host pairs based on published literature and observed positive correlations. However, we also uncovered pairs with non-correlated abundance patterns, such as increased Arctic *Pelagibacter* abundances in the Bering Sea after the last Bering Strait opening, likely due to reduced viral infection. Negative correlations between bacteriophages and hosts suggest shifts between life cycles - where either viruses coexist with or lyse their host - driven by environmental changes.

This novel study explores virus-host interactions since the late Pleistocene, highlighting the potential of sedaDNA for studying ancient viruses in lake and marine ecosystems.

Quality Control of Ancient Environmental Genomic Data and Reference Databases for improved Taxa Profiling

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Shotgun-sequenced ancient environmental data have been increasingly used in recent years for past ecosystem taxonomic profiling. Despite their advantages over other approaches, two major drawbacks of ancient metagenome data analysis hinder its broader applications: namely, the high rate of false-positive taxa identifications and low analysis efficiency. In this project, we developed the following schemes to address these two issues: 1) exploring the factors and variables of ancient metagenome sequencing data, and thus identifying different data quality control approaches that affect the rate of false-positive identifications; 2) investigating the redundancy rate of commonly used reference databases and benchmarking its effects on the efficiency and accuracy of ancient metagenome-based taxa identifications; 3) standardizing the taxonomy of different databases; and, 4) testing various methods for decontaminating reference databases. Combining these approaches, we developed a pipeline for quality controlling ancient metagenome data and preparing reference databases, which significantly improves efficiency and accuracy for shotgun-sequenced ancient environmental data taxonomic profiling.

Integrating modern occurrence records and sedaDNA data to improve Environmental Niche Models

Fiona Callahan; Rasmus Nielsen

Understanding how species interact with their environment is a central objective in community ecology. Environmental niche models (ENMs) have been widely used to examine species distributions and their associations with environmental factors. Traditionally, these models rely on modern occurrence and climate data, which are readily accessible through web-based repositories for many species. Recently, ENMs have been extended to incorporate long time series of community occurrence data derived from sedimentary ancient DNA (sedaDNA). We are developing niche modeling methods that integrate both modern occurrence data and sedaDNA time series into a unified framework. This dual approach harnesses the temporal depth provided by sedaDNA while leveraging the statistical power of extensive modern data. By combining these datasets, our methods may improve the accuracy of inferred associations between taxa and climatic or environmental variables, offering new insights into species-environment relationships over time.

Developing a Standardized Protocol for Sediment Sampling in Greek Archaeological Sites for Ancient DNA Analysis

Aurora, Campo; Eugenia, Tabakaki; Viviane, Slon

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The rich historical tapestry of Greece, characterized by its numerous archaeological sites, presents unique opportunities for ancient DNA (aDNA) research. However, the lack of a standardized sediment sampling protocol can lead to inconsistent data quality and comparability across studies. This abstract details the development of an official protocol for sediment sampling specifically tailored for Greek archaeological contexts. Initiated as part of a collaborative postdoctoral project, this protocol aims to standardize the collection, storage, and processing of sediment samples to maximize the recovery of aDNA. We discuss the interdisciplinary approach involving molecular biologists, archaeologists, and geologists to address the specific challenges posed by Greek sites, such as varied sediment compositions and climatic conditions. The protocol encompasses guidelines for site selection, sample collection depth and volume, contamination control, and sample storage, ensuring reproducibility and reliability in aDNA yield. This standardized approach not only enhances the comparability of genetic data across different sites within Greece but also contributes to the broader field of aDNA research by providing a replicable model adaptable to other archaeological settings.

A Snakemake Pipeline for SNP Calling in Ancient DNA: Identifying Heterozygous Sites for Positive Selection Analysis

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The study of positive selection in ancient populations provides insights into the evolutionary pressures that shaped human genetic diversity. A critical challenge in ancient DNA (aDNA) analysis is the accurate identification of single nucleotide polymorphisms (SNPs), particularly heterozygous sites that may indicate adaptive genetic traits. This abstract introduces a robust Snakemake pipeline designed to streamline the SNP calling process in aDNA samples, focusing on the detection of heterozygous sites. The pipeline integrates state-of-the-art bioinformatics tools and bespoke scripts to manage the complexities associated with degraded and contaminated aDNA, ensuring high-fidelity SNP identification. We describe the pipeline's workflow, which includes read alignment, quality control, post-mortem damage filtering, and SNP calling, with specific adjustments to optimize for the low coverage and fragmented nature of ancient samples. The objective is to provide a reliable and reproducible means to investigate heterozygosity patterns across genomic data, facilitating studies on positive selection. The effectiveness of the pipeline is demonstrated through case studies where heterozygous sites linked to adaptive traits were successfully identified in ancient genomes, suggesting potential areas of positive selection that contributed to survival and adaptation in historical human populations.

Protecting the future by learning from the past: Salmon nursery lake ecosystem reconstruction through sedimentary DNA

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Sedimentary DNA (sedDNA) has emerged as a powerful proxy in paleoecology, offering expanded taxonomic scope compared to traditional methods. In this study, we used sedDNA to reconstruct ecosystem changes in salmon nursery lakes in British Columbia, Canada, spanning centuries to millennia. Using digital PCR (dPCR), we present the first DNA-based reconstruction of Sockeye Salmon biomass dynamics across the study lakes. DNA metabarcoding of the 18S ribosomal RNA gene tracked shifts in micro-eukaryote communities, including phytoplankton, zooplankton, and other groups. Most lakes exhibited notable changes in algal communities, with increased diatom abundance, declining chlorophytes, and fluctuating chrysophyte and dinoflagellate populations. We observed a pronounced increase in planktonic diatom species in surface sediments, likely reflecting longer open-water periods and enhanced thermal stratification. Additionally, we detected dynamic changes in potential fish parasites, with nematode reads showing consistent increases across lakes, possibly indicating higher fish infection rates. Network analysis identified novel indicator taxa, including Pirsoniales, Desmodesmus, and Glissomodida. Our findings demonstrate that sedDNA enables comprehensive ecosystem reconstructions, offering critical insights into aquatic ecosystem health. In collaboration with Fisheries and Oceans Canada and Indigenous Peoples, these results inform conservation strategies for these vital nursery lakes.

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Long-term ecological data are essential for understanding past environmental changes, establishing restoration baselines, and identifying ecosystem stressors. Here, we use sedimentary DNA (sedDNA) to reconstruct ecosystem changes spanning centuries to millennia in salmon nursery lakes across British Columbia, Canada. Using digital PCR (dPCR), we present the first DNA-based reconstruction of Sockeye Salmon population dynamics, revealing distinct temporal trends across study lakes. We also applied DNA metabarcoding targeting the 18S ribosomal RNA gene V7 region to track shifts in eukaryotic communities, including phytoplankton, zooplankton, and other taxonomic groups. Most lakes exhibited notable changes in algal communities: increasing diatom abundance coupled with declining green algae. Among diatoms, we observed pronounced increases in small planktonic species in surface sediments, likely reflecting longer open-water periods and enhanced thermal stratification. Dinoflagellates increased in the early 20th century, followed by declines in recent decades, potentially reflecting rising water turbidity that disadvantages photosynthesis-dependent taxa such as green algae. Ordination analysis integrated with the large-scale LakePulse dataset indicates that, land-use changes are likely the primary driver of recent biological shifts in salmon nursery lakes. Our findings demonstrate that sedDNA captures taxonomically diverse communities, enabling comprehensive ecosystem reconstructions that provide critical insights into aquatic ecosystem health. These results can inform conservation strategies for protecting salmon nursery lakes, including efforts to mitigate carbon emissions and manage watershed land-use practices.

A Sequence of Southern Sweden: Sedimentary Ancient DNA Analysis of Environmental Change on the Kullen Peninsula

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The Kullen Peninsula in the northwestern corner of Skåne county in southern Sweden was amongst the first land areas in Sweden to become ice free following the most recent deglaciation. Previous investigations of the small lake of Kullatorpssjön, situated on the peninsula, demonstrate that sedimentation began 17,000-18,000 cal BP into what was then a brackish lagoon before isostatic rebound resulted in the isolation of the lake from the neighboring Kattegat sea 1000-1500 years later. Given the unique geographical position of Kullatorpssjön in southern Sweden and its dynamic palaeoecological history from a glaciated to marine to terrestrial environment, it is of great interest to further elucidate the timing and composition of postglacial colonising floral and faunal communities, as well as the timing of early humans, on the peninsula. Here, we use sedimentary ancient DNA (sedaDNA) shotgun metagenomic and metabarcoding approaches, applied to ~80 samples from a 4-meter core collected from Kullatorpssjön in 2024, to investigate the critical shifts in the coastal environment of the Kullen peninsula during the Late Weichselian.

Exploring Arctic ground squirrel evolutionary history with ancient (environmental) DNA

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Quaternary studies of Beringian megafauna have long overshadowed small mammals, despite the latter's ecological importance. Arctic ground squirrels (*Urocitellus parryi*) are a prime example. This study explores the evolutionary history and population dynamics of Arctic ground squirrels from east Beringia using ancient DNA from bones and faecal pellets. By analysing these two sources of DNA, we aim to reconstruct the evolutionary history of ground squirrels from two main time periods, marine isotope stage (MIS) 4 (~80,000 years BP) and MIS 2/3 (~30,000 years BP). We aim to identify the influence of climate on ground squirrels over the last 80,000 years and to understand the relationship between ancient and modern ground squirrel populations in the region. Lastly, we focus on ancient environmental DNA from faecal pellets associated with the Gold Run tephra which dates to ~690,000 years BP. These middle Pleistocene data provide our earliest understanding of the evolutionary roots of ground squirrels in east Beringia. Our data suggest that the evolutionary history of *Urocitellus* spp. is significantly more complex than previously thought and that currently available mitochondrial reference genomes of Arctic and Richardson's ground squirrels provide an insufficient window into the genetic diversity of Quaternary ground squirrels.

Benchmarking transfer function's calibration approaches for sedimentary ancient DNA

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Analysing past ecosystems can improve our understanding of the mechanisms linking biodiversity with environmental changes. Sedimentary ancient DNA (sedaDNA) opens a window to past biodiversity, beyond the fossil record, that could be used to reconstruct ancient environments. To this end, paleoecologists often calibrate transfer functions on modern biodiversity patterns and environmental conditions, and then apply them to past biodiversity data to reconstruct past environments. Doing this with sedaDNA has some challenges, because sedaDNA is often obtained in limited quantities and fragmented into small fragments. This often leads to noisy datasets, with a low alpha diversity relative to modern environments, patchy taxa detection patterns and/or distorted relative abundance profiles through time. How this affects past environmental reconstructions remain untested. Here we simulated modern and ancient sedaDNA reads counts matrices, and tested hundreds of combinations of counts transformations, beta-diversity metrics, and ordinations methods, in order to assess their performance in i) separating the ecological signal from the noise related to ancient DNA degradation and in ii) classifying ancient DNA samples to the correct associated environment. We will present preliminary results of this work that may ultimately improve our capacity to reconstruct ancient environments with sedaDNA data.

Unlocking Arctic Marine Biodiversity: Enhancing Taxonomic Resolution with Sedimentary Ancient DNA

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Greenland's west coast is highly vulnerable to climate change, yet uncertainties remain regarding the arctic marine ecosystem responses. Understanding past climate impacts on marine species can help predict future changes. Traditional microfossil records, while valuable for reconstructing past ecosystems, are biased toward hard-bodied species and lack comprehensive temporal and spatial coverage. To address this, we use sedimentary ancient DNA to track Holocene marine biodiversity, enabling detection of a wider range of organisms, including soft-bodied species absent from the fossil record.

To enhance the taxonomic resolution of marine eukaryotes across all trophic levels, from primary producers to marine mammals, we developed custom hybridization capture probes targeting barcoding regions. We (1) compiled databases to identify knowledge gaps in Arctic marine biodiversity and (2) evaluated various barcoding genes for taxonomic resolution and reference availability. We developed 46,804 custom probes targeting barcoding regions of 11,389 species, focusing on the V7 ribosomal RNA region as a universal marker and applying alternative markers for groups with lower resolution. We validated these probes both in silico and on marine surface sediment samples from 25 Greenland sites.

This approach improves Arctic species identification, offering critical insights into ecosystem dynamics and supporting strategies for long-term ecological sustainability amid climate change.

Investigating 250 Years of Cyanobacterial Community Dynamics in a Currently Eutrophic Lake Using Sedimentary DNA

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Lake Żabińskie in north-east Poland is a postglacial, eutrophic lake with algal blooms during the growing season and low water transparency. There is an extensive body of paleolimnological research from this lake as a result of its calcium rich epilimnion and a seasonally anoxic hypolimnion; which provide ideal conditions for biogenic varve formation and proxy preservation, including sedimentary DNA and algal pigments.

We applied a metabarcoding approach to reconstruct the history of cyanobacteria using 16S rRNA amplicons and algal pigments from a sediment core covering the past ~250 years. In combination with our previously submitted research - using the HEP primer pair to amplify, sequence, and assign taxa to *mcyE/ndaF* genes involved in the biosynthesis of microcystin and nodularins - we attributed changes in toxigenic cyanobacteria and the cyanobacteria community to reconstructed environmental and anthropogenic variables.

Developing a compact field kit for the rapid detection of ancient DNA preserved in sediments

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Since the early 2000's, technological advancements have significantly enhanced the quantity and quality of genetic data retrievable from sediments. However, there has been relatively little innovation in sampling methods. The potential effects of different archaeological features on DNA preservation, and the exact origins of DNA retrieved from sediments remain unclear. As a result, sampling continues to rely on luck when attempting to recover aDNA from the few samples typically collected for screening. While it may take several months to obtain the sequencing results, the probability of finding preserved aDNA can be quite low, especially in warmer climates. This can negatively impact project timelines, therefore, ensuring an informed sampling is critical. Here, we aimed to develop a field kit capable of quickly detecting preserved aDNA in sediments using minimal equipment. First, we compared three rapid DNA extraction protocols against the 'gold standard' for aDNA recovery from sediments, in terms of DNA quality, field applicability and time required. Subsequently, the extracts were subjected to a qPCR assay to estimate whether uracils - the most common type of postmortem DNA damage and a hallmark of preserved aDNA - are present in the samples. Our results point to a promising strategy to quickly extract DNA from sediments and to detect where aDNA is more likely to be preserved, thereby providing researchers with the means to perform informed sampling for downstream analyses.

Exploring Southern Ocean diversity: sedaDNA Bioinformatic Pipeline benchmarking for rare eukaryotic taxa

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Marine sedimentary ancient DNA (sedaDNA) provides critical insights into past community compositions. This is vital for understanding how ecological communities may respond to environmental changes, particularly in vulnerable regions like the Southern Ocean and Antarctica. Despite many advancements, challenges persist in accurately verifying ancient DNA from low-abundance taxa in sediment archives, including rare and ecologically significant vertebrates.

This study re-analyses metagenomic marine sedaDNA data from the Scotia Sea (IODP Exp. 382, site U1538), focusing on three objectives:

1. Testing precision of taxonomic assignments for rare marine eukaryotic taxa using simulated datasets, including iconic Southern Ocean vertebrates and complex empirical datasets (Exp. 382 data)
2. Evaluating assignment sensitivity across sediment ages
3. Establishing the minimum sequence quantity threshold necessary for robust identification of low-abundance taxa

This study evaluates the reliability of sedaDNA assignments to rarely represented Southern Ocean species. It also explores the potential for identifying a broader array of rare taxa, such as marine invertebrates and pathogens, to reconstruct ancient biodiversity and ecosystem dynamics. These findings may improve rare taxa detection in ancient metagenomic datasets or identify targets for secondary sequencing, offering pathways to deeper investigations of Southern Ocean biodiversity and its responses to environmental change over time."

Ancient microbial metagenomics of subglacial precipitates from beneath the Laurentide and Antarctic ice sheets

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Subglacial precipitates are layered accumulations of minerals that form from subglacial waters beneath glaciers and ice sheets. While formation occurs beneath the ice, they can be exposed onto the surface by ice retreat or by being frozen into basal ice before being transported upwards. Their layered composition of calcite and opal has recently been linked to the melt-freeze cycles of ice sheets, providing valuable insights into the ice sheet response to climate change. Calcite and silica (opal) in a cold climate may also form an ideal environment for sedaDNA preservation. Here we use shotgun metagenomics to study the microbial communities of these precipitates, using 26 samples from across Antarctica and Baffin Island in Northern Canada between 16 and 550 thousand years old. We show that the extent of DNA damage can be used to roughly differentiate between the microbial communities which lived in the rocks during their formation in the subglacial water, and those which live in the rocks once exposed on the ice sheet surface. These results are consistent in samples from the Arctic and Antarctic, although some ancient microbial phyla are significantly more abundant in the Baffin Island communities. Combining metagenomics with isotope records from the precipitates suggests that ancient microbial communities actively influenced the chemical composition of subglacial waters.

Past Winter conditions reconstructed from microeukaryote sedaDNA: A Siberian study case

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Winter conditions are regressing in time and space due to Global Warming. Nevertheless, both the ecological consequences and the spatiotemporal history of vanishing Winters in lakes are poorly understood. In this study, we attempted to identify past changes in Winter conditions in a Siberian Mountain Lake. To do so, we used a V7 18S metabarcoding approach to characterize contemporary microeukaryote communities at different stages of the Winter planktonic succession, in summer plankton and summer epilimnion. Then, we looked for Winter representatives in a sedaDNA core spanning to about 2500 y BP.

The four kinds of samples showed clearly distinguishable community profiles. Taxa developing in Summer dominated the sedaDNA record. Nevertheless, some low-abundance taxa developing in winter were successfully detected across the sedimentary record. This was the case of some cryophiles such as *Geminigera cryophila* and a representative of Pseudodendromonadales. They were detected in the sediment core from c. 2500 y BP to 1989-2001 CE, but absent from that point until 2017. This roughly agrees with the relatively stable cold conditions in the region until 1970s-80s as described in the literature. In conclusion, the current approach successfully reconstructed a part of the past Winter microplanktonic communities, captured a recent decrease in Winter conditions severity and described compositional changes in Winter communities during a millennial hard Winter period.

Tracing the invasion: Using eDNA to track the spread of Dreissena mussels

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Human activities have significantly increased the translocation of species, driving biological invasions that impact ecosystems, often with negative effects on biodiversity, particularly in aquatic habitats. Sedimentary DNA (sedDNA) from perialpine lakes offers a powerful tool to trace the arrival of invasive species and monitor subsequent ecosystem and population changes over time.

The invasions of *Dreissena polymorpha* (zebra mussel) and *Dreissena rostriformis bugensis* (quagga mussel) in the perialpine region show this phenomenon. In our study, sedDNA was used to detect these dreissenid mussels in sediment cores and surface sediments, revealing the timing, mode of colonization, and spatial distribution of these species. Using seven sediment cores from the perialpine region, we identify the initial colonization of mussels and track population development over time.

To assess spatial distribution within a water body, we quantified quagga and zebra mussel DNA in Lake Constance using a duplex droplet digital PCR (ddPCR) assay. Surface sediment samples from 54 sites, collected during the SeeWandel project's quagga monitoring campaign, were analyzed. Mussel density and biomass measurements provided by Eawag (CH) were used as references for genetic quantification. Our results demonstrate that sedDNA analysis describes mussel distribution, highlighting its potential as a valuable tool for invasive species monitoring and management.

Long-term genetic diversity patterns of ostracods and diatoms in Nam Co sediments

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Sedimentary ancient DNA (sedaDNA) metabarcoding has revolutionised the field of tracking biodiversity of target organisms from lake sediment cores, spanning thousands of years. Traditionally, past aquatic communities are studied using morphological identification of the microfossil remains. However, sedaDNA sequencing data has an incredible potential e.g. in cases where no fossil remains were preserved. Ostracods, as well as diatoms are sensitive to environmental factors, such as e.g. temperature, pH, salinity, conductivity and water depth, and are therefore, used as bioindicators and for reconstructions of past environments. However, no investigations on possible effects on phylogenetic changes and phylogenetic diversity (PD) have been conducted so far.

In this study we analysed the last 2000 years of a sediment archive from Nam Co (Tibetan Plateau) to investigate temporal assemblage changes of diatoms and ostracods.

Here we focus on the sedaDNA metabarcoding data which shows notable changes in species richness and diversity in both datasets. While diversity peaks differ throughout the analysed time period, both show a drastic increase for the longest time period since the 1970s. Overall, the observed temporal genetic diversity shifts can be linked to climatic changes such as in precipitation and temperature increase causing e.g. lake level changes. This study also highlights the PD changes associated to distinct events like the Little Ice Age and the Medieval Warm Period.

From the Early Anthropocene to the Great Acceleration: Comparing biodiversity dynamics in water bodies under differing human impact

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Aquatic biota are experiencing tremendous changes due to direct human impact on species and habitats, as well as anthropogenically induced changes in abiotic factors. However, we largely lack empirical data on the long-term trajectories of biodiversity change, and on the ecosystem baselines prior to the onset of current changes - on how stable they have been, which drivers have been historically important, what factors increase biodiversity and resilience, and how previous changes compare to the current ones. Sedimentary ancient DNA can offer such information, as well as elucidate the past role of humans - and potentially point to past sustainable practices that can guide the future. Lakes of the northern pre-alpine region have been under human impact for millennia, have experienced severe 20th century eutrophication and are currently under pressure from rising temperatures and invasive species. Records of sedimentary ancient DNA based on both metabarcoding and shotgun sequencing point to millennial old, significant and irreversible aquatic community shifts in relation to early human impact. Importantly, in contrast to the situation of the 20th century, the initial, putatively anthropogenic changes resulted in taxonomic biodiversity increases. Comparing these records with analyses of the past decades, we can connect scales to understand whether and how current biodiversity changes are specific and what factors can increase biodiversity and resilience of current ecosystems.

Environmental Controls on Recovery of Plant Environmental DNA in Lake Sediments from Wisconsin, USA

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Ancient environmental DNA (aeDNA) is a powerful tool for reconstructing past vegetation dynamics. Recent advances have shown that aeDNA from local plant communities is well preserved in small, deep lakes. Yet, disentangling the factors that affect the preservation and recovery of aeDNA from lake sediments remains an evolving area of research and understudied in temperate lowlands. Here, we study the relationship between plant aeDNA yields and various lake properties across 20 lakes in Wisconsin, USA, with three surface samples retrieved from each lake at center-lake and nearshore locations. These lakes, located in four distinct ecological landscapes, span a wide range of environmental gradients, making them an ideal setting for understanding the mechanisms governing aeDNA deposition and stability. We investigate how lake properties such as surface area, maximum depth, and sampling distance to shore affect plant aeDNA recovery using targeted enrichment capture. We also explore how pH, electrical conductivity, and dissolved oxygen impact plant aeDNA preservation. Additionally, we examine how variations in geological substrate, soil characteristics, and organic carbon content influence aeDNA yield. Furthermore, we ask whether aeDNA recovery varies more among lakes or within different areas of the same lake. By addressing the influences of environmental factors on aeDNA preservation, this work aims to enhance the accuracy and interpretive power of aeDNA as a paleoecological proxy.

Spatio-Temporal Dynamics and Drivers of Plant Diversity in the European Alps: Diversification and Homogenisation Over 12,000 Years

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Plant species richness in the European Alps has increased since the Last Glacial Maximum, but whether this rise has diversified or homogenised the alpine landscape remains uncertain.

Rapidly changing climate and the glacial retreat created new environments, which may have facilitated colonisation and increased landscape heterogeneity in the Alps. With advancing time and stabilising climatic conditions, species likely dispersed more widely, leading to the homogenisation of alpine habitats. In recent millennia, humans introduced livestock farming that may have created highly diverse local habitats while potentially spreading plants and thus homogenising local diversity across the Alps. We explore these spatio-temporal dynamics of diversity change in the European Alps during the past 12,000 years using sedimentary ancient DNA from 14 different lakes. Pairwise changes in dissimilarity between sites are temporarily correlated with the local introductions of domesticated cattle, sheep and goats, as well as climatic variables. Preliminary results indicate an overall diversification trend from past to present, with this trend being especially pronounced in the Western Alps. Yet, the spatio-temporal pattern of similarity change is complex and characterised by local features.

SediQuest: A user-friendly pipeline for the analysis of nuclear DNA capture data from sediment samples

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Sediments preserve DNA, including DNA from ancient humans. This allows for the study of past populations when skeletal material is unavailable, and enables the reconstruction of the occupational history, e.g. of Palaeolithic cave sites. To study sediment samples researchers have often focused on the mitochondrial genome due to its high copy number. Mitochondrial DNA (mtDNA) carries a high proportion of informative sequence variants, making it easy to identify species. However, mtDNA offers limited resolution for population genetic analysis as the genome is small, does not recombine, and is only maternally inherited. While nuclear DNA can be enriched for specific genomic regions of interest, this enrichment will still result in a mixture of sequences from hominins and other faunal species. Here we present SediQuest, a user-friendly implementation of a workflow previously developed for processing hominin sedimentary capture data¹. SediQuest uses a measure of the average sequence divergence between the human genome and those of other mammalian species at sites of interest, and the coverage distribution of the sequence data to filter undesired faunal sequences. We tested this pipeline using published sediment and bone-derived datasets and demonstrated its efficacy and accuracy in removing faunal sequences. We evaluated the amount of hominin data that could be recovered and its usefulness for reconstructing population relationships.

1.B. Vernot, et al., Science 372, eabf1667(2021)

Wild and domesticate mammals drove late-Holocene alpine plant diversity in the European Alps

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In the face of human land use and climate change, it is essential to know the key drivers of plant diversity in montane regions. However, the relative roles of climate and ungulates in shaping and maintaining alpine ecosystems is an open question. Neither observational data nor traditional palaeoecological data have the power to resolve this issue over decadal to centennial timescales. Sedimentary ancient DNA (sedaDNA) provides an alternative approach that provides both detailed information on former plant communities and the presence and abundance of ungulates in alpine landscapes. Here we record 603 plant taxa, as well as 5 wild and 6 domesticated mammals, from 14 lake sediment records over the last 14,000 years in the European Alps. Sheep were the first domesticated animals detected (at 5.8 ka), with cattle appearing at the onset of the Bronze Age (4.2 ka), and goats arriving later (3.5 ka). While sheep had an impact similar to wild ungulates, cattle became the primary driver of plant diversity over the last 2 ka. Modelling of the sedaDNA data revealed a significantly larger effect of cattle and wild ungulates than temperature on plant diversity. Our findings highlight the significant alteration of the Alpine vegetation, and entire ecosystem, by wild and domesticated herbivores. This study has immediate implications for how to maintain plant diversity in the face of ongoing human-induced changes in montane regions.

Environmental Dynamics of the Southern Baltic Region revealed by plant sedaDNA and diatom

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The study focuses on the environmental dynamics of the southern Baltic region following the last glaciation, utilizing multiproxy analyses of pollen, diatoms, lithological data, and high-resolution plant sedimentary DNA (sedaDNA) from sediment cores collected from Dūkštelis palaeolake in eastern Lithuania.

Observation highlights an intriguing scenario where two contrasting vegetation types coexisted in the same environment prior to 14000 cal yr BP, suggesting a transitional or “no modern analogue” ecosystem. This is supported by the presence of subalpine shrub taxa (e.g., *Arctostaphylos uva-ursi*, *Dryadoideae*, etc.) alongside a high representation of low-relief herbaceous plants (e.g. *Trifoliaceae*, *Mentheae*, *Ranunculaceae*, *Plantago*, various species of *Asteraceae*). The study indicated an early presence of *Picea* around 15000 cal yr BP, contrasting with pollen data that suggests its establishment at 12300 cal yr BP. This discrepancy highlights the sensitivity of sedaDNA methods in detecting isolated tree species.

Over time, a gradual transition to a forested landscape occurred, characterized by various riparian and deciduous species. By approximately 10000 cal yr BP, the lake's ecological state shifted from a shallow mesotrophic-eutrophic environment to a deeper eutrophic system. A decline in forest vegetation was observed when the lake evolved into a shallow wetland, with sedaDNA data suggesting that species growing around the lake may overrepresent local vegetation dynamics.

What can we learn by working with different sedaDNA fractions from plants?

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sedaDNA studies pay little attention to the differences and complementarity of different extraction protocols designed to target total, extracellular or intracellular DNA (i.e. tot, ex, inDNA). Tests are often carried out on selected samples to determine the best protocol to answer the scientific question. However, a more systematic use of different protocols could be beneficial, even beyond better estimates of community compositions.

We tested this hypothesis for plants recorded over the last 130 years in the sediments of Mare à Jonc (Réunion Island). Three phases were identified: one with more taxa detected in the in/tot DNA than in the exDNA (0-105 cm depth), another with very few taxa detected only in the exDNA (105-135 cm depth) and a last one below 135 cm depth, with more taxa mainly detected in the exDNA pool.

Old photographs show at least a dry lake in 1948 and the early 1970s. Although we do not yet know how many years this lasted, it may explain the better conservation of the DNA signal in the exDNA fraction below a depth of 105 cm. Indeed, exDNA is known to be protected from degradation (e.g. due to oxic conditions, UV light, etc.) by its adsorption on clays or other mineral/organic particles. On the contrary, the drying out of the sediments probably led to the physical rupture of the cells and then to the leaching and/or rapid degradation of the DNA contained in these cells. This result provides a new chronological constraint in a complex context for dating.

Reconstructing the past functioning of the biological carbon pump: a sedimentary ancient DNA approach

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Sedimentary ancient DNA (sedaDNA) is revolutionizing our understanding of marine ecosystems by offering a window into past biodiversity and its response to environmental and climate change. Recent findings have suggested that sedaDNA holds potential as a proxy for past particulate organic carbon (POC) flux, a key process for carbon sequestration in the oceans that is yet to be quantified from the geological record. We have reconstructed eukaryote biodiversity over the last ca. 150, 000 years from a 19.5-meter-long core from the Labrador Sea using DNA metabarcoding (18S-V9 region). Using a modern global scale surface-to-seafloor DNA dataset (>100 locations worldwide), we have separated sinking plankton DNA signatures from that of benthic taxa along the sediment core samples. In our sediment core, we identified diverse planktonic marine taxa including radiolarians, cercozoans, diatoms and dinoflagellates, alongside benthic organisms such as polychaetes. Our first results reveal shifts in the relative abundance of both plankton and benthic communities in response to past climate variations, possibly reflecting changes in POC export dynamics. We are developing a predictive model trained on a large collection of surface sediment samples, linking modern DNA signatures to current POC flux estimates. This calibrated model will be applied to our sediment core samples to reconstruct past POC fluxes, providing the first quantitative assessment of POC export over geological timescales.

Have global change and agricultural practices affected plant and soil microbial biodiversity? - a historical DNA approach

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The HerbaSoil project targets soil/plant-associated microbial communities that are largely excluded from the debate on the current biodiversity crisis, although essential to plant growth and agriculture. I evaluate the impact of ecosystem changes by unraveling, for the first time, “ancient” soil microbiomes in samples stored in herbaria associated to the roots of cereal plants and compare them with extant soil microbial communities associated with the same cultivated plants species.

This museomic study, performed on 60 historical and 60 modern soil samples, aims to quantify the ecological impacts of decades of intensive farming practices on soil microbes. It employs paleogenomics techniques to extract and sequence plant-soil-associated microbial DNA. Metagenomics sequence datasets from ancient and modern soils will be interpreted in terms of taxonomic, phylogenetic and functional diversity of the resident microbial communities to highlight potential long-lasting impacts on these communities of intensive management of agroecosystems.

Besides providing original information on the long-term evolution of microbial diversity in response to ecosystem disturbance, HerbaSoil may contribute to soil restoration in a context of agroecology.

Using sedaDNA for restoration and refunctionalization of degraded peatlands in Austria

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Raised bogs have been destroyed or damaged over the last centuries. In the light of the biodiversity and climatic crisis, restoration efforts are focused on such ecosystems as they have the capacity of storing large amounts of carbon in peat. Our research will focus on the reconstructing the chronology of late and post-glacial peatland development using sedimentary DNA (sedaDNA) from plants and pollen records in combination with geophysical methods (e.g., electric resistivity tomography). Core samples from various peatlands in Austria will be analysed for sedaDNA to reveal the chronology of historical vegetation changes over the past approximately 18,000 years. This study aims for reconstructing late and post glacial plant species composition, vegetation dynamics, assess changes in peat formation rates, and peatland responses to climatic and other environmental stressors. Sediment analysis together with radiocarbon dating (^{14}C) and pollen analysis will provide the stratigraphic background for the environmental reconstructions based on sedaDNA. This integrative approach, combining genetic, chronological, climate modelling data and geological methods offers a novel perspective on understanding peatland change. The study also fills a knowledge gap by investigating sediments underlying the peat to understand pre-peatland environments, such as post-glacial lake flora. Moreover, results should inform future management plans of peatland restoration in Central Europe.

Potential of SedaDNA as a Proxy to Distinguish between Nor'easter and Hurricane Overwash Deposits

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Sedimentary DNA (sedaDNA) offers a novel approach for identifying Nor'easter overwash deposits within sediment records, addressing the challenge of distinguishing these events from hurricanes. The short instrumental record limits our understanding of the relationship between Nor'easters and human-induced climate change, and traditional geological proxies cannot differentiate between these high-energy storm surge events. We hypothesize that marine eDNA from the North Atlantic Ocean, transported by Nor'easter-driven storm surges, is preserved in overwash deposits and reflects the distinct microbial communities associated with the seasonal occurrence of Nor'easters (November–March). These unique signatures could distinguish Nor'easter overwash from hurricane deposits (June–November). This study presents standard protocols for eDNA collection and extraction from salt marsh sediments and preliminary findings from the 2023/2024 and 2024/2025 Nor'easter seasons, using samples collected at the Rutgers Marine Field Station in Tuckerton, New Jersey. Additionally, we analyze a sediment core from Whale Beach, New Jersey, which contains confirmed overwash deposits from both a Nor'easter and a hurricane. Our results demonstrate the potential of sedaDNA to enhance the resolution of sedimentary records, providing new insights into the history, frequency, and impacts of Nor'easters in the context of climate change.

Hybridization Capture and Efficiency Enhancement for Ancient Environmental DNA

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Ancient environmental DNA (aeDNA) is increasingly utilized in paleoecology and evolutionary ecology, but insufficient DNA data per species from aeDNA metagenomes poses a significant challenge for genetic investigations. Hybridization capture enriches target DNA, thereby improving genome coverage for individual species and enabling population and evolutionary genetic analyses. However, no dedicated tool for aeDNA probe design exists to date. Here, we introduce eProbe, a flexible toolkit for capture probe design, assessment, and validation. Benchmarking a foxtail millet probe set generated by eProbe demonstrated an average 455-fold increase in target DNA recovery and high genome coverage, enabling comprehensive and accurate population and evolutionary inference. Subsequently, we conducted hybridization capture on aeDNA libraries simulated from ancient foxtail millet materials, performing a thorough evaluation of factors that may influence the efficiency of target species capture in aeDNA.

2000 Years of Ecological Community Dynamics in London- A Molecular Biography of an Urban Centre

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Understanding how increasing urbanism affects local ecologies is an important challenge for modern societies, one that would benefit from a palaeoecological perspective. London has been occupied almost continuously for the last two-thousand years and contains some of the richest urban archaeology of anywhere in the world. However, from a palaeoecological standpoint, environmental evidence relating to its urban ecosystem is scarce and heavily reliant on pockets of preserved organic remains that often only relate to short-occupational periods. Much of London is underlain by river alluvium, which does not commonly preserve traditional environmental proxies, but does contain a high enough minerogenic content to provide potential for sedaDNA research. Therefore, this project aims to use ancient sedimentary DNA from both urban archaeological deposits and alluvial sequences to examine how increasing urbanization, growth, and abandonment have impacted the ecologies of the city and its environs for the past 2000 years. By adopting shotgun sequencing as the primary methodology, we aim to demonstrate changes to both microbial and eukaryotic communities, exploring effects such as that of post-industrial heavy industry on soil quality. Furthermore, by providing a genetic record of localised plant and animal introductions into the city, we aim to showcase sedaDNA as useful tool for environmental archaeology, one that can benefit even a city as well historically documented as London.

SedaDNA & Environmental Archaeology- The Prehistoric Environment of the Stonehenge Landscape

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SedaDNA analysis of terrestrial archaeological sediments can be challenging. DNA preservation will be poorer and the depositional environment of the DNA is not as stable as lake sediment. However, the technique can provide a wealth of environmental data in these contexts. When applied alongside thorough dating frameworks, other palaeoenvironmental proxies (pollen, plant macrofossils, faunal assemblages) and a good understanding of the geoarchaeology, sedaDNA can be a powerful tool for environmental archaeology. Here we showcase one example of its application to archaeological research to demonstrate how sedaDNA can be used to examine prehistoric landscapes. Using sedaDNA data from multiple borehole and trench sequences from the Stonehenge environs we were able to illustrate the Mesolithic and Neolithic palaeoenvironment in combination with pollen analyses, providing both localised and extra-local records of changes to vegetation that help to elucidate why this area first attracted, then was modified by, human populations. Through such applications, we aim to highlight the ever-increasing potential for sedaDNA in environmental archaeology, without ignoring some of the challenges associated with complex sedimental sequences.

Central European Mountain refugia for the arctic-alpine flora: first insights from sedaDNA diversity in lakes of the Tatra Mts. and Karkonosze Mts

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The Quaternary was characterised by glacial and interglacial phases, driving significant changes in species distributions and community composition, especially in mid- and high-latitude regions. During glacial periods, extensive ice sheets in the North forced species to migrate southwards. The Central European (CE) mountains, including the Carpathians and Sudetes, which experienced moderate glaciation, potentially provided important stable habitats for cold-adapted species both during cold and warm periods.

In this project we investigate the CE mountains as refugia for the arctic-alpine flora and their potential role in northern recolonisation. By metabarcoding lake sediments, we aim to provide first sedaDNA data from these biogeographically important mountain ranges, to study the evolution of local species composition and the persistence of arctic-alpine communities since the Last Glaciation.

Sediment cores from high-altitude lakes in the Tatra Mountains (Western Carpathians) and the Karkonosze Mts. (Sudetes) provide sedaDNA records since the Late Glacial since the Late Glacial. Analyses of test samples from all sites confirmed the presence of conserved sedaDNA. Initial data from the Sudetes indicate richer past arctic-alpine communities than today. Ongoing analyses will clarify the resilience and genetic diversity of these communities. Preliminary findings will be presented at the conference.

High-latitude plant refugia during Quaternary glacial-interglacial cycles revealed by species distribution modeling and sedimentary ancient DNA

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Milankovitch climate oscillation is a significant cause for geographical patterns in species diversity and range sizes in the Quaternary. High-latitude plants are particularly sensitive to such periodical variations, but the presence of local refugia may contribute critically to their long-term survival and early postglacial/interglacial expansion. Here, we presented a modelling framework based on the species distribution model to predict the locations of refugia across 669 plant taxa (covering 2331 species) in Siberia and Alaska during Quaternary glacial-interglacial cycles. The modeling results were validated by our sedimentary ancient DNA (sedaDNA) dataset compiled from 10 lakes. Our preliminary results show that refugia plays an important role in harbouring and accumulating taxa, especially interglacials with preceding strong glacials. Areas with high climatic stability (e.g., Beringia) and high topographic heterogeneity (e.g., mountain ranges) could serve as potential refugia. Our model also provides a tool to plan conservation strategies for high-latitude plants. Further analyses are pending and will be finalized and presented at the sedaDNA conference 2025.

Population Genomic Insights of Woody Plant species from Ancient Arctic Sediments

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Ancient DNA (aDNA) recovered from sediments has revolutionized our understanding of species presence and ecosystem composition in past environments. While sedimentary aDNA is widely used to detect species across temporal and geographic scales, leveraging these data for population genomic inferences remains a significant challenge. In this study, we analyzed both published and unpublished sedimentary aDNA shotgun datasets, ranging from 2 million to 40,000 years old, from multiple sites across the Arctic. We focused on woody plant species with high DNA abundance and applied an optimized filtering pipeline to identify genome-wide genetic variants. This approach enabled us to investigate population structure and potentially reconstruct the demographic history of Arctic plant species across different periods. Our findings provide novel insights into the genetic diversity and historical dynamics of Arctic ecosystems, highlighting the applications of sedimentary aDNA to population genomic research.

A High-Coverage Brown Bear Mitochondrial Genome from Early Holocene Sediment in Northern Sweden

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During the early Holocene, plants and animals migrated into Scandinavia as the Fennoscandian ice sheet gradually retreated. Bi-directional colonization events have been suggested for several species, including humans, brown bears, toads, shrews, and field voles. In brown bears, two different populations, represented by different mitochondrial clades, were involved in the colonization: those carrying clade 1a took the southern route through Denmark and brown bears carrying clade 3a arrived via the northern route through Finland. These two groups formed a contact zone located around central Sweden. Based on mitochondrial DNA, it has been suggested that this structure was upheld by female philopatry throughout the Holocene, including a recent (~100 years ago) hunting-induced population bottleneck. Ancient DNA preserved in lake sediments across timescales of millennia can provide valuable insights to the colonization routes of both plants and animals as a complement to osteological and other paleolimnological studies. Here, we present a high-coverage ancient brown bear mitogenome extracted from a lake sediment layer from northern Sweden dated to 9.6 cal. ka BP, which is close in time to when the area became deglaciated. This complete mitogenome represents the oldest known brown bear DNA from northern Sweden. Our analyses suggest that this mitochondrial genome originates from a single individual belonging to an unexpected clade given the present-day distributions of brown bears.

Reconstructing mid Holocene lake and catchment changes: using diatoms, SedaDNA and XRF to investigate drivers of change at Rostherne Mere, UK

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Only a few UK lakes have yielded complete palaeolimnological records since the early Holocene, resulting in discontinuous and fragmented knowledge of long-term interactions. Rostherne Mere, a lake in the Shropshire-Cheshire Plain, UK, is of particular interest because of its 10,000-year post-glacial history covering a wide range of possible lake states and long-term record of landscape change within the region. This study undertakes a multi-proxy palaeolimnological approach using diatoms, sedimentary ancient DNA (SedaDNA) and geochemistry (organic content, XRF) across a continuous, long sediment record made up of two sediment cores. RM-A (11.2m) was sampled for SedaDNA (non-captured n=44 and captured n=42) and organic content (n=333), whereas RM-B (10.45m) was sampled for diatoms (n=236), XRF (n=1770) and organic content (n=1002). This sedimentary archive offers an exciting opportunity to elucidate the interactions between different drivers of the local aquatic and regional terrestrial systems. It provides a robust assessment of environmental change across a decadal, centennial to multi-millennial scale. A complete record of diatoms, SedaDNA, XRF and organic content covering the last 9'000 years provides a detailed insight into Rostherne Mere throughout the Holocene that aims to provide a picture of cultural and natural disturbances over key periods and guide the future sustainability of the UK landscape.

Human impact on biodiversity focusing on palms of the genus *Pritchardia*

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It is inevitable that humans had and continue to have a strong influence on both ecosystems and biodiversity. But how did early humans impact vegetation and associated evolutionary processes? The remote Hawaiian Islands offer a great possibility to answer this question as they were only recently (less than 3000 years ago) inhabited. Human colonization led to a drastic decline of the native flora as palm forests were cleared to i.e. cultivate non-native crop and weed species (Nogué et al. 2021). Within this PhD project, I want to unravel shifts in genetic diversity and identify potential connections between human-induced changes and palm population dynamics. We will therefore extract ancient DNA from fossilized *Pritchardia* plant parts and lake sediment samples and compare the sequences with the genetic information of extant *Pritchardia* populations. This comparison will not only help us understand the evolutionary history of these palms but may also reveal the presence of "ghost lineages"—ancient lineages that are no longer present but detectable through genetic evidence in the fossil record. Combining sedaDNA analysis with population genomics has the potential to allow a profound understanding of ecosystem responses to anthropogenic pressures and may further provide valuable insights into conservation strategies of an endangered plant genus.

Integration of Indigenous perspective with sedimentary ancient DNA analysis to address local conservation in Nevada, USA

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Recent aridification of the American West is having significant environmental effects at both local and regional scales. Paleoenvironmental reconstructions from the Great Basin document major hydrological changes during the Holocene and provide the necessary framework for interpreting contemporary hydroclimate trends. However, a detailed understanding of how past droughts affected local biotic communities in the Great Basin is less documented. Sediment cores collected from Summit Lake, NV, span the entire Holocene and show evidence of megadroughts, thus providing an opportunity to reconstruct community responses to changes in climate. In collaboration with the Summit Lake Paiute Tribe (SLPT), we pair geological and sedimentary ancient DNA analyses to reconstruct changes in the hydrology and biotic communities of the Summit Lake watershed through the Holocene. Using a metagenomic approach, we reconstruct changes in taxonomic community composition following major hydrological shifts within the watershed. We pay particular attention to species of interest for the SLPT including the Lahontan cutthroat trout (*Oncorhynchus clarkii henshawi*, LCT), which is both culturally significant and listed federally as threatened. These data will be used to inform SLPT-led management strategies for the contemporary LCT population in Summit Lake. This research demonstrates how collaboration with Indigenous Tribes can direct research design to maximize scientific equity and conservation outcomes.

Reconstructing subtropical landscapes with sedaDNA: Insights from two lakes in Nepal's Terai Arc Landscape

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Sedimentary ancient DNA (sedaDNA) offers unprecedented taxonomic resolution for reconstructing past plant- and animal- communities over time, presenting a valuable tool for conservation policy development. However, sedaDNA analysis has rarely been applied in (sub)tropical regions due to presumed preservation challenges. However, some successful applications in warm climates suggest potential possibilities. This study examines the feasibility and limitations of sedaDNA in the subtropical Terai Arc Landscape of Nepal using a multi-proxy approach. Sediment cores from two lakes with distinct morphologies and depositional environments, but identical sampling and processing protocols, were analyzed. Fixed-interval subsampling enabled the generation of traditional paleoecological proxy data (pollen, phytoliths, fungal spores) alongside shotgun sequencing and mammalian mitochondrial capture. Preliminary results reveal significant differences in DNA abundance and diversity between lakes, yet ancient DNA is present throughout the cores, spanning 1,000–1,500 years. Ongoing analyses aim to enhance taxonomic resolution for target families (e.g., Poaceae, Bovidae, Felidae) and to finalize traditional proxy quantification. Comparative assessment of sedaDNA and traditional methods will illuminate their respective strengths and faults, enabling a robust reconstruction of landscape history. These insights are then able to inform conservation strategies in the Terai Arc Landscape.

Changes in haplotype composition of Japanese anchovy for the last 2000 years

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Genetic diversity is essential for assessing the vulnerability of populations. However, the historical processes underlying the genetic diversity of many species remain unclear, hampering understanding of population origins and current status. This study focuses on Japanese anchovy (*Engraulis japonicus*), one of the most heavily harvested marine fish species to elucidate the current haplotype composition in the waters surrounding Japan using fish body samples, and to uncover historical changes in haplotype composition using sedaDNA extracted from sediment core samples in Beppu Bay, Japan.

Sequencing analysis of Cyt-b region using the DNA extracted from both fish body samples and sediment samples revealed numerous haplotypes. Phylogenetic analysis of the nucleotide sequences identified several haplotype groups (clades) with unique genetic sequences specific to the waters around Japan. Some of the detected clades were associated with subpopulations inferred to have the unique local life histories based on cohort analysis. Haplotype composition derived from sedaDNA revealed three major shifts associated with the Medieval Climate Anomaly/Little Ice Age, climate changes in the 1700s, and intensified human disturbance in the mid-20th century.

Haplotype analyses using sedaDNA play an important role in understanding how the current composition has been shaped and what factors have altered and sustained genetic diversity over time.

Stickleback sedaDNA time-series from isolated Norwegian lakes reveal patterns and dynamics of early stages of freshwater adaptation

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In the recent years, aDNA has provided new means for acquiring insight into the evolutionary processes in contemporary and extinct species. The sampling of various remains of ancient animals and plants has deepened our understanding of adaptation, demographic history and extinction. However, the view that singular ancient samples or several samples separated significantly in space and time only partly represent the past. Rapid advances in the methods of extracting and processing sedDNA provide a new avenue to overcome these limitations, as genome-level data from this source allows generation of continuous time-series datasets of aDNA. Recently, applying a novel sedaDNA approach, we were able to capture time-series of environmental genomes from Late Pleistocene sedaDNA allowing us to track adaptive haplotypes of threespine stickleback (*Gasterosteus aculeatus*) across ecological change from marine to freshwater environment. In our approach, we increased sequence coverage by processing two orders of magnitude more sediment per sample than previous studies. Here, we extend this approach to multiple lakes of different geological ages and histories that have shaped the contemporary stickleback populations occupying them. With this expanded approach we aim to provide a comprehensive look into how availability of genetic variation, demographic history and ecological restrictions can shape the tempo and dynamics of the threespine stickleback freshwater adaptation.

The last mammoth in the Polar Urals and its correlation to vegetation change

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The cause of the megafauna extinction is among the most debated questions in palaeobiology, with human hunting or climate change being the major competing hypotheses. Advances in ancient sedimentary DNA now allow us to analyse changes in mammal and plant populations from the same sample, and thus provide more insight into the ecological context of extinction. DNA was extracted from two lake sediment cores from the Polar Urals, dating back to 23,500 cal. yr BP. The trnL P6-loop (plant) and 16S (mammal) regions were amplified and sequenced. The identified plant taxa were linked to their present day trait data and used to reconstruct the palaeoenvironment. Across both cores, we detected a total of 239 plant taxa and 19 animal taxa. The flora developed from a tundra ecosystem dominated by forbs and graminoids during the Last Glacial Maximum (LGM) to one that was increasingly dominated by trees and shrubs during the Allerød and Younger Dryas periods. This transition to a more tree and shrub dominated landscape at the expense of forbs coincided with the disappearance of woolly mammoths, while smaller herbivores such as Mountain hare and Ptarmigans appeared in the record. SedaDNA allowed for the identification of the palaeoenvironmental changes in the Polar Urals since the LGM. The large climatic and floristic changes observed in the Allerød and Younger Dryas most likely contributed to the local extinction of mammoth in the region.

sedaDNA reveals a late Holocene shift from wet to dry floodplain ecosystems

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Thousands of years of human intervention have significantly altered river and stream landscapes, disrupting natural river-floodplain dynamics. In Europe, widespread river engineering combined with deforestation has been so effective that it has led to profound changes in hydro-geomorphic connectivity, with long-lasting consequences for riparian ecosystems, water quality, discharge, and water residence time across the continent. In contrast, in natural rivers, biota plays a fundamental role in shaping fluvial dynamics. However, in the absence of modern analogues, it is necessary to look to the past to gain insights into the natural functioning of river systems. Sedimentary ancient DNA, combined with chrono-stratigraphical analysis, offers a way to reconstruct fluvial dynamics.

In this study, we examine three river reaches in Northern Germany, finding a transition from a low-lying, wet floodplain forest to a high and dry floodplain ecosystem. We analyzed Holocene chrono-stratigraphy and ancient DNA from river-floodplain deposits, alongside hydro-geomorphological characteristics. Using single-stranded, Illumina high-throughput shotgun sequencing, we uncovered a surprisingly rich and complex sedaDNA record. Our results suggest that the sedaDNA found in Holocene river sediments primarily has a local origin, providing an unprecedentedly detailed view of the shift from natural to human-dominated river ecosystem dynamics.

Decoding Time: A Phylogenetic Framework for Molecular Dating of Sedimentary Ancient DNA

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Sedimentary ancient DNA (sedaDNA) from permafrost, lake and marine sediments provides a rich source of genetic data that captures broad perspectives of past biodiversity. However, accurate dating is crucial for discovering ecologically relevant patterns from sedaDNA, and increasingly samples are too old for C-14 dating. While molecular dating allows for sample ages to be estimated from the recovered genetic material itself, the fragmented and damaged nature of short-read ancient DNA poses significant challenges. We have developed ratePlacer, a phylogeny-based method for analyzing sedaDNA that can combine information from many short reads in a sample while accounting for DNA damage to provide maximum likelihood estimates of sample ages. By applying ratePlacer to a diverse set of sedaDNA samples from various time points, we establish a timeline that allows us to contextualize exceptionally old samples from sites like Kap København and Fyles Leaf Beds against younger C-14 dated samples. This comprehensive dating approach enhances our understanding of the age distribution of recoverable genetic material and expands our ability to study ancient ecosystems.

Potential of ice caves to disclose long-term genetic variability of mountain vegetation

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Ice caves act as natural traps for plant remains, providing evidence of past vegetation changes. Due to the excellent preservation of plant remains in frozen environments, ice deposits potentially host ideal conditions for the preservation of their endogenous ancient DNA (aDNA), and, thus, to conduct paleogenomic studies. Analysis of aDNA from macrofossils can provide insights into the evolutionary history and the factors that have shaped the current genetic diversity of plants. This study focusses on analyzing aDNA from macrofossils of four keystone species (*Pinus uncinata*, *Pinus mugo*, *Dryas octopetala* and *Picea abies*) dating back to the Holocene that are preserved in ice caves across the central and southern European mountain ranges. We aim to investigate whether significant changes have occurred in the genetic diversity of past populations and how this diversity is related to that of present populations of the target species. Extracted aDNA was incorporated into single-stranded DNA libraries and shotgun sequenced. While we successfully recovered endogenous aDNA with anticipated damage patterns, the endogenous content was low. To enable a more detailed analysis of genetic variation, we will apply a capture-based method to enrich the endogenous DNA content of the libraries. This study will rescue untapped ice cave information that may vanish in a warmer future. Analyzing these unique archives can improve assessments of future ecosystem dynamics under global warming.

Sedimentary ancient metagenomics infers plant-mammal community dynamics across northern Eurasia and North America during the Late Pleistocene-Holocene

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The Late Pleistocene-Holocene transition witnessed significant reorganization of plant-mammal communities. Using a comprehensive paleometagenomic-based dataset (shotgun sequencing data) from six lake sediments in Siberia and North America spanning 25-0 ka, we investigate the compositional dynamics of plant-mammal communities through this critical period. Our analyses reveal synchronized change points in terrestrial ecosystems, with a notable peak in community turnover around 14 ka that coincides with major temperature shifts. The temporal alignment of plant and mammal community changes suggests strong coupling in their responses to environmental change. This ongoing research employs network analysis to examine plant-mammal co-occurrence patterns, aiming to understand mechanisms driving community turnover. Our framework will provide insights into how species interactions, inferred from co-occurrence patterns, influenced community stability during periods of rapid environmental transformation.

Plant interactions associated with a directional shift in the richness range size relationship during the Glacial-Holocene transition in the Arctic

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A nearly ubiquitous negative relationship between taxonomic richness and mean range-size (average area of taxa) is observed across space. However, the complexity of the mechanism limits its applicability for conservation or range prediction. We explore whether the relationship holds over time, and whether plant speciation, environmental heterogeneity, or plant interactions are major factors of the relationship of plant richness and mean range size within northeast Siberia and Alaska. By analysing sedimentary ancient DNA from seven lakes, we reconstruct plant richness, biotic environmental heterogeneity, and mean range-size over the last 30,000 years. We find positive richness to range-size relationships during the glacial period, shifting to negative during the interglacial period. Our results indicate neither speciation nor environmental heterogeneity is the principal driver. Network analyses show more positive plant interactions during the glacial period, which may contribute to positive richness to range-size relationships. Conversely, in the interglacial environment, negative interactions may result in negative relationships. Our findings suggest potential susceptibility to invasion but conservation advantages in far northern tundra given their positive interactions, analogues to plant interaction during the glacial period.

Keywords: Sedimentary ancient DNA, metabarcoding, open-air archaeological sites

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Archaeological science has recently seen a boom in sedimentary ancient DNA (sedaDNA) studies from caves and lake deposits, but few open-air archaeological sites have been studied despite their great potential for sedaDNA preservation. This study uses metabarcoding to analyze archaeological sediments from the Iron Age courtyard site of Rødskjær in Harstad, Norway. The site includes a minimum of 155 cooking and fire pits, and 16 house structures. The preservation of these features and faunal remains make Rødskjær ideal for assessing how sedaDNA can be used in archaeology for understanding site use and past lifeways. The sedaDNA results will be compared to the zooarchaeological record from the site to assess the similarities and differences between the two methods and how sedaDNA can be applied at other open-air sites with poor preservation of organic materials. A total of 62 samples from nine cooking pit features have been analyzed for sedaDNA. Most of the sediments have good sedaDNA preservation with a high abundance of both plants and animals. The animal sedaDNA matches well with the faunal record, with additional species being detected that were not detected in the zooarchaeological analysis. The plant DNA results included domesticated foodstuffs including barley (*Hordeum Vulgare*). The mechanisms of DNA preservation in these features will be discussed as well as any patterns of species presence between the features.

Linking sedaDNA to Middle Holocene Hunter–Gatherer Population Dynamics at Lake Baikal

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Prehistoric Hunter-Gatherers at Lake Baikal demonstrate remarkable population continuity during the Middle Holocene, broadly the period from 8500–4000 cal. BP. Archaeological findings from the substantial excavation history of cemeteries in this area show distinct differences in mortuary practices across this time range, however, which broadly correspond to the genetically inferred demography of these populations. To study the hypothesis that ecosystem changes throughout the Holocene can be linked to hunter-gatherer population dynamics, we obtained an ancient environmental DNA record of ecosystem succession, with sediment cores from Lake Ochaul, northwest (Cis-Baikal), and Lake Kotokel, southeast (on the opposite Trans-Baikal side). We combined this with a large dataset of ~200 shotgun-sequenced ancient genomes from hunter-gatherer communities at Lake Baikal to then elucidate human-ecosystem interactions. This is further informed by a thorough pollen record for both cores at high temporal resolution. In the Early to Middle Holocene (10,600–6800 cal. BP), an expansion of boreal forest in this region is apparent, though this appears to vary across the microregions represented in Cis- and Trans-Baikal. Similarly, the appearance of domestic ungulates differs, corresponding to cultural differences at different times. By explicitly studying associations between demography and ecosystem succession, we show that human prehistory can be analysed in a community ecology perspective.

Assessing biota changes and ecological quality in Italian volcanic lakes: a comparison of sedimentary DNA hybridization capture and metabarcoding

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In recent years, lake sedimentary DNA (sedDNA) has proven valuable in paleoecological research for reconstructing past biodiversity and tracking changes in terrestrial and aquatic biota over time. Combining shotgun sequencing with hybridization offers an innovative approach to sedDNA analysis. Here, we compare these techniques to the widely used metabarcoding method, within the framework of a PRIN project aimed at reconstructing the pre-human impact reference conditions of three Italian volcanic lakes: Monticchio (Basilicata), Nemi, and Martignano (Lazio). Biodiversity and community composition was investigated on 100 cm sediment cores. Custom DNA probes were designed based on ancestral sequences inferred with phylogenetic tools to target molecular markers (matK and rbcL from cpDNA, COI from mtDNA) of taxa indicative of water quality, including plants (Alismatales, Lamiales, Poales), algae (Charales), diatoms, and Diptera (Chironomidae). By comparing these approaches, we aim to refine tools for reconstructing past and present lake ecosystems, enhancing our understanding of biodiversity dynamics and the current ecological status of these lakes.

eWHALE: Combining environmental DNA sampling, whale watching and citizen science for stakeholder-driven marine biodiversity protection

James, McKenna

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The eWHALE project pioneers a large-scale, participatory approach to marine biodiversity monitoring by integrating environmental DNA (eDNA) sampling with whale watching tours, research cruises, and fishing vessels across Europe. This innovative collaboration among researchers, industry partners, and citizen scientists aims to provide a proof of concept for scalable, non-invasive monitoring methods critical to the conservation of marine megafauna and their habitats.

eWHALE leverages genomic approaches to extract population-genetic data from eDNA, focusing on cetaceans, porbeagle sharks, and basking sharks—species of significant conservation concern. By optimizing eDNA sampling workflows and enhancing molecular methodologies, the project will enable the detection of species, population structures, and even individual identification. This effort will yield datasets with unprecedented spatial, temporal, and taxonomic resolution, validated through comparison with biopsy samples and visual observations.

Through a citizen science module aboard whale-watching platforms, eWHALE educates the public about emerging eDNA technologies, fostering awareness and engagement in marine conservation. By actively involving stakeholders, the project not only generates actionable insights but also paves the way for future eDNA monitoring campaigns and their integration into institutional biodiversity assessments, supporting marine management at the seascape level.

Reconstruction of variability in past sea-ice over 150 000 years using ancient DNA- a case study from the Greenland Sea.

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During the past several decades, a substantial reduction in the extent and volume of the Arctic sea-ice cover has been documented. Based on climatic models, the Arctic Ocean may become seasonally ice-free by 2040. Thus, it is crucial to create a reliable dataset of Arctic cryosphere conditions. Here, I present the results of a comprehensive, high-resolution study on past sea-ice variability in the Greenland Sea (72.08.527'N, 014.49.858'W) using sedimentary ancient DNA (sedaDNA). By applying metabarcoding targeting the V9 hypervariable region and the Diatom V4 subregion of the 18S ribosomal RNA gene metabarcoding, along with quantitative measurements of the sea-ice dinoflagellate *Polarella glacialis*, I obtained the record of sea-ice conditions in the Greenland Sea over the last 150,000 years. The results of the study indicate the potential of sedimentary DNA as past-sea ice indicators.

Unraveling Post-LGM Plant Community Dynamics in Southeast Alaska using SedaDNA approach

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The South-East Alaska (SE AK) has been proposed to have provided ice-free refugia to different species during the Last Glacial Maximum (LGM). But recent cosmogenic-nuclide exposure dating showed that the Cordilleran ice sheet retreated onto the modern coast of the SE Alaska 15.1 to 16.3 \pm 0.8 ka BP. However, parts of the continental shelf along SE AK may have been subaerially exposed during the substantially variable local LGM due to low sea level. However, the information regarding the development of ecosystems in SE AK after LGM is scarce and is based on the pollen records which are prone to preservation bias. Hence, to investigate how plant communities colonized and/or expanded and evolved in SE AK after LGM, we performed metabarcoding of P6 loop of trnL intron using sedaDNA derived from 4-m-long sediment core from Parrot Lake, Dall Island (54.82° N, -132.87° W) spanning last 15 ka. Using Obitools3, we analysed the presence and changes in abundance of different plant communities. We observed a clear shift in the plant communities inhabiting dry-cold habitat to humid-cold habitat around ~ 11.4 ka BP, coinciding with the Pleistocene-Holocene boundary in SE AK. We observed the first occurrence of water lilies (*Nuphar polysepala*) around the same time. Our data also indicates that the grey alder (*Alnus incana*) replaced green alder (*A. alnobetula*) after 11.4 ka BP. Our data provides the first sedaDNA record from SE AK, addressing gaps in pollen-based paleoecosystem reconstruction.

Well drained archaeological sediments and post deposition processes seen in archaeobotanical studies

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One of the common issues to be solved during plant macro-remains analysis of most of the archaeological sites and sediments is detection of recent, or at least younger, items called usually 'contamination'. It concerns well drained (air-opened/dry) sediments in contrast to most of the waterlogged ones, which are usually oxygen depleted and less susceptible to bioturbation. However, besides the possibilities of any kind of redeposition, archaeobotanical data from dry sediments are a very useful source of information about past subsistence, local environment and several other aspects, usually supported by archaeological context and other paleoenvironmental methods.

The aim of the presentation is to show some examples of macro-remain redeposition, spectacular or common. Additionally, the possible ways to tackle the issues of possible assemblage contamination will be presented, which should be also useful as a discussion point in the case of chemical analyses.

Ancient environmental DNA preserved in Yukon ground squirrel burrows records Pleistocene ecosystems over the last 700,000 years

Tyler, Murchie; Scott, Cocker*; Sina, Baleka; Nicola, Vogel; Libby, Natola; Emil, Karpinski; Diana, Tirlea; McIntyre, Barrera; Danielle, Grant; Evan, Morien; Linda, Rutledge; Duane, Froese; Hendrik, Poinar

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Coprolites (palaeofaeces) are a well-known, but challenging, source of ancient DNA (aDNA). In ideal circumstances, they can provide genetic data on the host organism, their diet, their gut microbiome, and their local environment. Enzymatic inhibition from incomplete DNA isolation remains a challenge for accessing these archives given their abundance of humics, Maillard products, phenolics, and other partially degraded organic compounds. Here, we report on the ancient environmental DNA (aeDNA) results obtained from permafrost preserved coprolites and sediments recovered from ground squirrel (*Uroditellus* spp.) burrows in Yukon, Canada that date between 15,000 and 700,000 years ago based on radiocarbon and tephrochronology. We recovered not only an abundance of *Uroditellus* spp. aDNA that reconceptualizes the evolutionary history of American ground squirrels, but also a rich metagenomic spectrum of plants and animals, far exceeding our initial expectations of the taxonomic breadth to be expected from squirrel middens. These squirrel faecal derived data have also allowed for the reassembly of the oldest woolly mammoth mitochondrial genome to date from the Americas. We discuss coprolite metagenomics and associated simulated validations for our classifications, methodological testing of extracts to overcome inhibition, and the ecological/evolutionary implications of these rich datasets that exceed the aeDNA concentrations of both local permafrost-preserved sediments and bone.

The missing stickleback: finding and not finding target sedaDNA in Canadian lakes

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Hakai Institute, British Columbia, Canada

Three-spined stickleback (*Gasterosteus aculeatus*) independently evolved into a pair of ecologically and genetically divergent limnetic and benthic populations in Enos Lake on Vancouver Island, BC, Canada following isostatic rebound of the island around 13,000 years ago. Today, the pair has collapsed into a hybrid swarm with the benthic and limnetic forms being threatened by species introductions, habitat changes, and human encroachment. We set out to study the Holocene evolutionary history of the Enos Lake stickleback pair through sedimentary ancient DNA (sedaDNA) analyses of stickleback remains and sediments recovered from the site using a Livingstone-type corer modified to protect against modern DNA contamination. We were unable to find any macrofossils of stickleback in the Enos Lake sediments, and surprisingly, we were unable to sequence any stickleback sedaDNA from the core, even with a stickleback targeted bait-set for capture enrichment. The absence of target DNA remains challenging to explain given the relative abundance of plant sedaDNA, the present and historical presence of stickleback in the lake, and the recovery of sedaDNA from comparative Norwegian lake sediment layers dated to the Late Pleistocene (Laine et al. 2024). Here, we discuss these unexpected challenges and contextualize those efforts with that of other, as yet unpublished, lake sedaDNA projects carried out across Canada where our targets are at times, elusive, and at others, abundant.

Holocene drought and vegetative responses revealed through integrated sedaDNA and sedimentary analyses in a high-desert environment, Great Basin, USA

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Paleoenvironmental reconstructions using sedimentary ancient DNA (sedaDNA) provide valuable insight into past ecological responses to environmental change, thereby informing responses to future drought and warming. To date, sedaDNA studies have focused on Arctic and alpine regions, where colder temperatures favor DNA preservation, leaving desert ecosystems understudied. Here, we report successful DNA recovery from a 12-m lake sediment record collected at Summit Lake (2.8 km²; 1780 m a.s.l), a high-desert terminal lake located within the Summit Lake Paiute Tribe Reservation in northwestern Nevada, USA. By integrating traditional sedimentary analyses and metagenomic sedaDNA data, we generate a ~12,000-year record of coupled hydroclimate changes and local vegetative shifts. Results show deep-water conditions in the early Holocene followed by a transition to drought conditions that generally persisted from ~ 8 ka to 0.6 ka. Local vegetation responded strongly to this shift, with a notable decrease in trees and shrubs and an increase in grasses through the Holocene. Additionally, we examine the relationship between sediment character and DNA taphonomy in this high-desert environment and find organic content may strongly predict DNA preservation. This work expands applications of sedaDNA to desert environments, providing valuable context for anticipating future ecosystem shifts in the drought-prone Great Basin and insights into DNA taphonomy in similar climates.

Marine Eukaryote Community Responses to the Climate Changes in Storfjordrenna Over the Past 13.65 Kyr BP: Insights from Sedimentary Ancient DNA

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"Sedimentary ancient DNA (sedaDNA) analysis is a crucial method for understanding paleo-biodiversity of marine eukaryotes and their responses to past climate change. Here, we present a marine sedaDNA metabarcoding record spanning the last 13.5 ka from Storfjordrenna. We identified a wide range of eukaryote groups and categorized them based on their ecological roles: phytoplankton, mixoplankton, microzooplankton, mesozooplankton, zoobenthos, parasites, marine fungoid protists, and gymnamoebae. Significant changes in biodiversity were observed during key periods, including the onset of the Younger Dryas and the late Holocene period. The main environmental drivers of changes in eukaryotic biodiversity were ice cover, surface water cooling, and the influx of Atlantic water. These environmental changes were reflected in the eukaryotic richness and the relative abundance of phytoplankton, mixoplankton, and zooplankton. Our study highlights the complex interactions within eukaryotic communities, revealing positive and negative impacts on ecosystem. This study demonstrates the potential of high-resolution marine sedaDNA metabarcoding for elucidating biodiversity responses to past climate change and understanding the intricate interactions within microbial communities in marine ecosystems.

The study was funded by the National Science Center grant no. 2022/47/B/ST10/03050."

Beyond Genera, the Next Frontiers in Ancient Environmental Genomics

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Ancient environmental DNA (eDNA) has become a reliable proxy for recording past taxonomic diversity, although often limited to the genus or family level. Ancient eDNA in environmental archives contains in principle the necessary information to assess species or even population-level variation but this requires a substantial amount of on target reads to access. At present, methods for recovering and sequencing aDNA are becoming cheaper and more advanced, allowing metagenomic approaches (shotgun and capture) to obtain genomic data (nuclear SNPs) from environmental samples at levels that are comparable to traditional skeletal and teeth samples, as seen in a small number of studies so far.

Improving the resolution from family and genus to species and population-level opens many new avenues of research, such as the potential to fill gaps in the fossil record, which is stochastic by nature. They also enable investigations of populations at locations where taxa occurred but did not die, or the study of taxa that do not leave skeletal tissue behind at all. Within paleo-ecology, intra-species variation has the potential to provide genetic links between study sites and across time. Here I present new tools which enable many of these assessments and will discuss best practices, pitfalls, and highlight the potential for a new field of Environmental Population Genomics.

The potential of sedaDNA to increase our understanding of island ecosystem dynamics

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Islands, though a small part of Earth's landmass, contain a significant portion of its biodiversity but have experienced substantial biodiversity loss due to factors like small population sizes, limited dispersal, and high human impact. Recent advancements in sedimentary ancient DNA (sedaDNA) are enhancing palaeoecological studies, providing more detailed insights into the historical impacts of human activity on island ecosystems. We will present a map of which islands have been studied using sedaDNA, in order to highlight the geographic and temporal gaps in the literature. Further, we compare island floras of 113 archipelagos or island groups with the availability of a DNA reference sequence of P6 loop of trnL (UAA) intron, to evaluate the potential to conduct metabarcoding studies at high taxonomic resolution. We additionally present a case study from Iceland to illustrate the potential for using sedaDNA to reconstruct past ecosystems and evaluate human impacts on islands. Overall, we find sedaDNA offers great potential for reconstructing the palaeoecology of islands, although there are geographical differences in the coverage of DNA reference libraries of local floras at the species level. Better flora inventories and reference databases need to be compiled for regions outside of the Arctic if sedaDNA is to be extensively used to study temperate and tropical areas, where most island biodiversity is concentrated.

Arctic Greening: using ancient DNA to determine responses of willows and birches to climate changes

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Shrub willows (*Salix* sp.) and dwarf birch (*Betula nana*) were described as the major constituents for the arctic greenness (Mekonnen et al., 2021). However, in the case of *Salix*, the attempts to systematize its taxonomy have been failing since the Linnean era (Linnaeus, 1799). Morphological classification is complicated by the extensive variation in phenotypes within species, frequent natural hybridization, sex differentiation in plants, and differences in developmental stages between flowers and leaves (Cronk et al., 2015). Meanwhile, due to very common hybridization and polyploidization, even worse failures were experienced by approach based on plastid and nuclear barcodes (Percy et al., 2014, Wu et al., 2015). Although recent genome-based approaches managed to reveal some major lineages, essential incongruence occurred among different studies (Marinček et al., 2024; Sanderson et al., 2023), due to limited species number and distribution.

Genome skimming has been considered a cost-effective, rapid, and reliable method for obtaining genomic sequence data through high-throughput (HTS) sequencing (Coissac et al., 2016). It sequences plastid and nuclear simultaneously, so both assembled plastome and nuclear metrics (e.g. SCOs, SNPs) can be used for phylogenetic analysis. We acquire genome skimming and whole genome data of arctic shrubs to design a pipeline for species distinguishing...

30,000 years of flora diversity in southern Italy: metabarcoding and pollen analyses at Lago Grande di Monticchio

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Plant metabarcoding on sedimentary ancient DNA (sedDNA) from Lago Grande di Monticchio in southern Italy reveals 30,000 years of vegetation history in a temperate Mediterranean context. Metabarcoding recovered 95 plant taxa from sediments spanning 1.4–31 cal ka BP, demonstrating the potential of sedDNA analyses in non-alpine and low-latitude lakes. The metabarcoding results complement pollen and macrofossil data, providing detailed insights into floral shifts, particularly in the first 8 ka. Challenges such as reduced DNA recovery from minerogenic sediments highlight the need for improved methodologies for low-latitude applications. These findings offer new perspectives on the reconstruction of past vegetation dynamics in temperate regions.

Challenges and Opportunities of environmental palaeoproteomics

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Ancient proteins ostensibly persist across deeper geological timescales than ancient DNA, while still retaining genetic sequence information that can permit taxonomic identifications and even phylogenetic inference. However, while this robust survival of ancient proteins is well-demonstrated in macrofossils, it is not yet clear if this remains true for environmental samples. Mechanisms underlying the deep-time survival of proteins are not well-understood, but so far, their deep-time survival is restricted to highly-biomineralised tissues that present a suitable scaffold for protein-mineral binding, and/or exhibit closed-system behaviour.

Here, we report on preliminary results from different ancient environmental samples (including sedimentary samples and coprolites), documenting only limited recovery of peptide sequences. In light of these results, we review previous findings of environmental proteomics, and metaproteomics more generally. Overall, optimization of methods is required for the extraction of proteins from diverse environmental samples. Improved peptide identification tools are similarly required. Currently, tools for peptide identification remain highly reliant on reference sequence availability, with no option of a 'bait and capture' approach to extend sequencing. To circumvent this issue, we develop a workflow integrating de novo sequencing tool InstaNovo, to more appropriately validate peptides and more meticulously explore their search space.

Exploring sedaDNA from early settlement sites in the East-fjords of Iceland

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Earlham College

Historical sources preserve stories of people from the past. In Fjörður, Seyðisfjörður in East-Iceland, Bjólfur, Helga and Ísólfur were said to have been living in the period of the settlement in Iceland (after 870 A.D.). A fisherman with a knowledge of sorcery and the ability to cause landslides, Brandur, was said to be living in Brandsstaðir at Skálanes outside of Seyðisfjörður. Test pits made at a farm mound in Skálanes have confirmed there was a settlement from the 10th to 15th century A.D. During 2022, in Seyðisfjörður, longhouses were discovered underneath an 11th century landslide, showing a lively, seafaring place where weaving was done, game pieces were used, and perhaps jewellery was made. People were buried there according to the Pagan Viking belief. Soil samples were collected from profiles of the farm mound at Skálanes during the summers of 2019 and 2022, and in floors, midden, and prehistoric soil from cultural layers excavated at Fjörður in 2022. DNA was extracted from the soil samples for 16S rRNA and shotgun sequencing, and then analyzed with QIIME2 and an adaptation of the PALEOMIX-BAM workflow. We are currently analyzing both data sets to identify signatures in the longhouse locations and settlement layers. We hope to learn more about the utilization of plants and animals during the settlement from 10th to 15th century A.D., highlighting the potential of sedaDNA to uncover aspects of daily life not visible through traditional archaeological methods.

Reawakening ancient DNA for biotechnology

Amedea Perfumo; Pablo Iván, Nickel

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Here, we aim to spotlight the untapped biotechnological potential of sedimentary ancient DNA (sedaDNA), an innovative area that has remained unexplored until now. Shotgun sequencing technologies, paired with significant analytical advancements, have moved us beyond taxonomy, making it possible today to annotate and analyse ancient functional genes.

We have advanced further by combining sedaDNA with synthetic biology, with the ambition to bring ancient functionalities back to life. As biodiversity loss caused by human activities reaches critical levels, with severe impacts on ecosystems and human health, this approach offers a promising path forward by tracing the evolution of functional genes and taxa, rediscovering lost molecules, and potentially leveraging them to address pressing societal challenges. We share examples from our research on improving crop resilience to climate change through the identification and application of eco-physiological adaptive traits from ancient plant rhizomicrobiomes that thrived during the warm Eemian period. We present our framework for expressing and testing ancient protein-coding genes in the laboratory, as well as evaluating the potential of these paleomolecules as soil additives to boost plant growth under changing climate conditions. Finally, we explore the vast potential of ancient genes in biotechnology, highlighting their wide-range applications across numerous fields.

Reconstructing the impact of rising anoxia with paleoecology - an Arctic case study

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University of Umeå, Umeå, Sweden

Due to global warming, seasonal and permanent anoxia of bottom waters are increasingly observed in many aquatic systems worldwide. This leads to loss of habitat for macroorganisms, such as molluscs, aquatic plants, and fish. Anoxia is also accompanied by the appearance of anaerobic prokaryotes in the water column, that can create harmful by-products such as methane, hydrogen sulfide, or methylmercury. Fjords are particularly affected by this phenomenon of rising anoxia, as their sills impede water movement and thus impair oxygen renewal in bottom waters. To better understand how rising anoxia affects these ecosystems and their biochemistry from a sedaDNA point of view, we studied a sediment core from Rossfjordvatnet, a system south of Tromsø with anoxic marine bottom waters and oxic freshwater at the top. The sedimentary sequence indicated that Rossfjordvatnet used to be fully marine and a habitat for sessile species, and that it was isolated from the main fjord relatively recently. In modern days, c. 80% of the water column is anoxic, reaching up to the photic zone. Metabarcoding and shotgun sequencing were used to reconstruct the prokaryotic communities and the photosynthetic organisms that lived over time in Rossfjordvatnet, and to investigate how they could have shaped this ecosystem.

Reconstructing past cultural practices using Picuris Pueblo oral tradition and sedimentary ancient DNA

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Indigenous peoples of the Americas have recorded their history through oral traditions, songs, music, painted and sculpted art, architecture, and modified landscapes, which embody the ritual and cultural dimensions of community life. However, centuries of disruption caused by European colonization and the suppression of Indigenous religions and beliefs have created significant gaps in this body of knowledge. This collaborative project, initiated by Picuris Pueblo, a Northern Rio Grande Pueblo in New Mexico, USA, aims to address these gaps by analyzing ancient sedimentary DNA (sedaDNA) from a Kiva – a circular, subterranean room that serves as ceremonial structure central to Puebloan religion, where rituals and political meetings are held. Sequencing sedaDNA from a 1610 CE Kiva floor enables the reconstruction of the plants and animals used in ancestral rituals, offering insights into current and past practices and the community environmental context. By combining scientific methods with Tribal knowledge, this work aims to deepen the understanding of ancestral Puebloan traditions and supports the revitalization of Indigenous histories and cultural heritage.

Long-term resilience of Pyrenean subalpine ecosystems: a high resolution sedaDNA approach to reconstruct ecological communities using Holocene paleoenvironmental records

Cristina Ramos Capón, Penelope González-Sampériz, Alessio Cardillo, Hugo Saiz, Irene Julián Posada, Michel Zech, Laura Epp, Roland Zech, Ana Moreno, Angela Ara

Pyrenean Institute of Ecology-CSIC, Zaragoza, Spain

Understanding past mountain communities' responses to disturbances is crucial for predicting the long-term effects that climate warming and human activities on subalpine systems. Given their vulnerability to disturbances, these changes may reshape subalpine biodiversity, altering community assemblies and stability. This research examines ecosystem resilience to natural and anthropogenic disturbances using the Holocene paleoenvironmental record of ecological communities from subalpine Pyrenean Basa de la Mora lake (BSM, 1913 m asl).

We recovered a new lacustrine sequence from BSM to reconstruct physical and biological variables employing a sedimentary ancient DNA analyses (sedaDNA) to infer communities' composition. Additionally, we used organic biomarkers and charcoal to reconstruct temperature, precipitation, and fire time series. We inferred community stability and resilience, applying diversity and structural metrics, then comparing the results with preliminary causal ecological network reconstructions based on pollen abundance from a previous record.

SedaDNA is an innovative tool for reconstructing past local subalpine communities providing animal and plants presence data, allowing the inference of potential interactions, as herbivory, pollination, or mutualism. Analyzing resilience to disturbances with causal ecological networks reveals significant structural changes, synchronized with various factors like fire, human activities, and climate shifts throughout the Holocene.

From poppies to mammoth: comparing postglacial ecosystems and depositional environments on either side of the Norwegian Channel

Aloïs, Revéret; Jo, Brendryen; Hans, Sejrup; Haflidi, Haflidason; Christian, Eide; Inger, Alsos
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Trajectories of postglacial succession are largely controlled by climate variations and colonisation dynamics. At the southwestern rim of the Fennoscandian Ice Sheet, the Norwegian Channel isolated the Jæren province from Doggerland (subaerial North Sea), the latter still connected to mainland Europe. How differently did terrestrial ecosystems settle and evolve?

We used sedaDNA metabarcoding of P6 loop and 16S to reconstruct plant and animal communities. The Jæren lacustrine core spans from local deglaciation ~17 ka BP to the Boreal, while the four Doggerland sites give a snapshot into Bølling–Allerød and Younger Dryas. Doggerland is a challenging sedimentary setting: laminated clays in channel infills revealing a mix of marine and terrestrial biota suggest shallow rias where marine sedimentation overlapped with detritic land influx.

We show that Jæren vegetation transitioned from arctic pioneers to boreal plants, driven by stadial-interstadial transitions, while Doggerland was a complex ecosystem mixing tundra and forested landscapes. Reindeer is found both in Doggerland and Jæren, at similar times. Detecting marine and terrestrial mammals in the same Doggerland samples suggests an asynchronous and perhaps allopatric origin. Mammoth presence in Doggerland is supported by the rich reconstructed flora, largely overlapping with that of Jæren. We emphasise Doggerland deposits as an untapped source of sedaDNA, remaining the blind spot of postglacial succession patterns in Europe.

What are the drivers of plant species diversity in the Alps and northern Fennoscandia?

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UiT The Arctic University Museum of Norway, Tromsø, Norway

Human activities may accelerate species exchange, altering species interactions and composition, and thus diversity. We use sedimentary ancient DNA data from 24 lakes to show how plant species exchange varies between regions with low and high anthropogenic activity. Taxonomic richness, and the number of locally immigrating and extirpating taxa were higher in the Alps than Northern Fennoscandia. The species exchange ratio (SER) or turnover was higher in the Alps, from 16-12 thousand years before present (ka BP), compared to Northern Fennoscandia. However, turnover declined and remained relatively lower in the Alps after 11 ka BP, indicating increased stability or retention of taxa in the species pool. We attribute the recent lower SER in the Alps to anthropochory, which promotes the dispersal of synanthropes. However, the lower anthropogenic pressure in northern Fennoscandia likely results in fewer widely distributed synanthropes. Human land use may drive high plant diversity and species exchange in the Alps.

Wildfires and insect outbreaks in boreal forests. A combined approach using Paleo-Ecology and sedimentary DNA

Damien Rius; Eve Afonso; Adam Ali; Yves Bergeron; Coralie Bertheau-Rossel; François Gillet; Martin Girardin ; Pierre Grondin ; Jonathan Lesven ; Laurent Millet ; Miguel Montoro-Girona ; Hubert Morin ; Cecile Remy.

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Boreal forests comprise 30% of the world's forested areas and contribute 20% of the global forest carbon sink, underscoring their critical role in climate regulation. However, these ecosystems are increasingly threatened by disturbances, primarily wildfires and insect outbreaks, which are exacerbated by climate warming. In eastern Canada, the Spruce Budworm (SBW), a major defoliating insect, causes cyclical outbreaks that affect millions of hectares of forest. While the frequency of these outbreaks seems to have remained stable over the past 450 years, global warming has driven a northward shift in their occurrence since the late 20th century. Concurrently, wildfires, also intensified by climate change, are reshaping forest composition and age structure, potentially impacting SBW host tree populations. The complex interactions between climate warming, insect outbreaks, and fire regimes (including their frequency and severity) remain poorly understood, largely due to the lack of long-term datasets (ie before the Industrial Era). The RETROPEST project seeks to address this gap by reconstructing SBW outbreaks and fire regimes across millennial time scales, using sedimentary DNA (Spruce Budworm outbreak), paleoecological data (fire & vegetation), and modeling. Preliminary results from the St. Lawrence Côte-Nord region reveal millennial-scale SBW outbreak patterns through qPCR analysis of sedimentary DNA from lacustrine cores.

7,000 Years of Change: How Environmental Shifts Shaped Phytoplankton Communities in the Baltic Sea

Juliane, Romahn; Alexandra, Schmidt; Jérôme, Kaiser; Damian, Baranski; Helge W., Arz; Anke, Kremp; Laura S. Epp; Miklós, Bálint

Few marine ecosystems have been as affected by human activity as the Baltic Sea, due to its semi-enclosed, shallow and brackish nature, surrounded by many industrialised countries. When investigating human impacts on ecosystems, many studies focus on specific species with characteristic traits, or at least traits that can be inferred from higher taxonomy. This is problematic because in the field of environmental DNA, many sequences cannot be assigned to species level and often only 50% of the sequences can be assigned to family level. As a result, much information is lost and environmental and human impacts can only be superficially analysed. Here we show how the dynamics of phytoplankton communities have changed over the last 7,000 years. We focus on the dynamics of community composition of diatoms, cyanobacteria and dinoflagellates based on metabarcoding data. This study investigates the influence of environmental parameters, climatic shifts, and human activities on regime changes in the phytoplankton community over time. Our findings indicate major community composition transitions linked to increasing multiple pressures caused by human impacts and changing climate conditions starting 2,000 years ago. Combined with the variation in response to different pressures among taxonomic groups, our study highlights the early and complex mixed effect of climatic and anthropogenic pressures on ecological dynamics.

Using ancient eDNA to assess VOLcanic LAkes REference condition, biodiversity and ecological response to climate change and anthropic pressure -VOLARE

Elisa Rondoni, Marilena Marconi, Cristiano Vernesi, Matteo Girardi, Andrea Lami, Simona Musazzi, Caterina Carabelli, Diego Fontaneto, Ester Maria Eckert, Aldo Marchetto, Renato Spicciarelli, Donatella Battaglia, Laura Parducci

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This poster presents the results of a research conducted as part of a PRIN project.

The Water Framework Directive requires states of the European Union to ensure that the ecological quality of water bodies is maintained close to those of an undisturbed body of water (reference condition). In the Italian context, no environments are free from anthropogenic pressure and the methods used to define the ecological quality of the lakes are expensive.

The project, funded in 2022, aims to provide a solution through three actions: traditional paleolimnological methods, ecological surveys, and analysis of eDNA extracted from the sediments of volcanic lakes in central and southern Italy:

- Analysis of eDNA from surface sediments, to generate a list of organisms present in the last 2-3 years and to use it as an indicator of ecological quality
- Use eDNA from 1-2 meter sediment cores to track changes in the biological communities and ecological conditions over the last centuries, estimating the reference condition
- Integrate eDNA with ecological surveys on a sediment core from Monticchio Lake (Basilicata) to study the natural variability of volcanic lake ecosystems and distinguish climate-driven changes. A 14-meter core from this lake is also available, allowing reconstructions back to the last glaciation, 33000 years ago.

The aim is to increase awareness of sustainable practices to protect volcanic lakes, mitigate climate change impacts, and support water quality management.

Sedimentary ancient DNA analysis at the Sakitari Cave Site, Okinawa

Rikai Sawafuji, Ryohei Sawaura, Shinji Yamasaki, Masaki Fujita, Mikkel W. Pedersen

Kyushu University, Fukuoka, Japan

sedaDNA in archaeological sites is a powerful tool for reconstructing past ecosystems, primarily studied in caves in Europe and Siberia. However, its applicability in subtropical regions, where ancient DNA degrades rapidly, is limited and remains uncertain. Okinawa, a subtropical region in Japan, has many cave sites dating to the Late Paleolithic period, but only limited pollen analysis and few botanical remains, except for charcoals, have been recovered due to poor preservation conditions. This study presents a sedaDNA analysis at the Sakitari Cave site, Okinawa, Japan, including layers dating back from 24,000–19,000 years ago to 11,000–3,000 years ago. We collected 21 sedimentary samples from each layer inside and outside the cave, and performed shotgun sequencing. We found the DNA exhibit high levels of fragmentation and degradation, consistent with ancient DNA characteristics. Plants such as the genera *Celtis*, *Morus*, *Crateva*, and *Ficus* were identified, many of which aligned with botanical remains recovered from the site. We also identified DNA from the genus *Eriocheir* (Japanese mitten crab), which had also been identified as macro remains at the site. These results demonstrate the feasibility of sedaDNA analysis in subtropical regions such as Okinawa, expanding the potential for paleogenomic research in challenging climatic conditions.

SedaDNA preservation at Satsurblia Cave, Georgia using a microstratigraphic approach

Susanna Sawyer, Thomas, Beard; Omaima, Zaki; Florian, Exler; Olivia, Cheronet; Joanna, Nicole, D'Souza; Stephan, Krämer; Ron, Pinhasi; Mareike, Stahlschmidt

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Satsurblia Cave, Georgia, is an Upper Paleolithic cave site with evidence of repeated human occupation before, during and after the Last Glacial Maximum. The site is notable for its rich DNA preservation including sediment DNA. Previous analyses of a sediment sample revealed excellent DNA preservation of human, wolf and bison, highlighting the site's great potential for further sedaDNA analyses. The process of DNA preservation and movement in sediments is still poorly understood, but is especially important in archaeology to link cultural material with molecular data.

Here, we developed a two-fold sampling strategy based on microstratigraphic (MS) analysis at the site: (1) loose sediment samples associated with the MS blocks were taken in the field and (2) samples were drilled directly from the MS blocks in a clean room environment.

Both loose sediment and block MS samples were extracted and pooled in sets of up to five prior to library preparation, enrichment for mammalian mitochondrial DNA (mtDNA), and sequencing. Taxonomic classification of sequenced reads was conducted using euka. Our results indicate low levels of mammalian DNA in half of the samples, with no observable correlation for microbone-dense areas. However, potential inhibitory effects may be biasing our findings and steps to quantify and mitigate inhibition are currently being implemented.

Genetic Resilience and Long-Term Monitoring of Phytoplankton Dynamics Using Sedimentary Ancient DNA

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The Baltic Sea, a unique and sensitive ecosystem, faces significant pressures from climate change and anthropogenic impacts. To understand its historical dynamics and future adaptability, we integrated findings from three studies using sedimentary ancient DNA (sedaDNA) and genomic tools to reconstruct phytoplankton population dynamics over millennia. We focus on the diatom *Skeletonema marinoi* as a model species, combining target enrichment of sedaDNA with resurrection ecology and genomic analysis of ancient and modern strains.

In the first study, we use sedaDNA to develop a molecular index to analyze historical shifts in diatom and dinoflagellate taxon composition, revealing changes linked to environmental stressors. The second study investigates the genetic diversity of *S. marinoi* over 8,000 years, showing stability despite periodic disturbances. Finally, we analysed whole genomes of resurrected *S. marinoi* from 6,800-year-old sediments.

Overall, we find that *S. marinoi* populations exhibit genetic resilience despite environmental disturbances. The integration of genomic data extends historical baselines for ecosystem health, providing insights into the impact of natural and anthropogenic influences. This approach highlights the utility of sedaDNA for identifying long-term trends and resilience mechanisms, aiding marine conservation.

Development of a bioinformatic toolkit for paleometagenomic analysis using the example of sedaDNA data from Saxon Switzerland

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Sedimentary ancient DNA (sedaDNA) analysis is valuable for environmental reconstruction but challenged by degradation and contamination, demanding rigorous authentication. We present GRAFT, an interactive bioinformatic toolkit for analyzing paleometagenomic shotgun data. GRAFT visualizes key sedaDNA metrics: fragment length, GC content, as well as position-specific damage patterns. Validated with simulated ancient *Hordeum vulgare* DNA, GRAFT was subsequently applied to sedaDNA from Saxon-Bohemian Switzerland, revealing length-dependent mapping biases and bacterial contamination. Authentic sedaDNA signatures were enriched in low mapping quality (MAPQ) reads, suggesting that conventional MAPQ filtering may be insufficient for filtering out microbial contamination. Future development will integrate DNA degradation models for environment-specific validation.

Unlocking the untapped potential of ancient DNA preserved in plant macrofossils

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Lake sediment archives have been used to reconstruct past vegetation composition using metabarcoding or metagenomic approaches. However, they also contain remains of single individuals in the form of plant macrofossils such as leaves, needles, wood or seeds. Ancient DNA from these remains would allow to examine genetic changes over time, providing direct insights into population genetic and microevolutionary processes. Here, we present the results from more than 80 samples from four different tree species collected in 8 lakes across the Swiss Alps that date back to the Holocene and the Lateglacial. We extracted aDNA from plant remains using optimized protocols and applied a shotgun-sequencing approach to single-stranded DNA libraries. Preliminary results from organelle aDNA indicate that good aDNA preservation is restricted to lakes with a pH >7, i.e. within calcareous bedrock. Plant remains from lakes with siliceous bedrock and low pH show only trace amounts of endogenous aDNA, if present at all. By comparing the genetic information of ancient individuals over a large area and with present day populations, we are able to track postglacial forest expansion in detail. Future research directions will include testing the adaptive potential of mountain tree species facing rapid climate change. Plant macrofossils are an underused source of genetic information that can be used to open a window into the past and study the response of plants to demographic and environmental changes.

Spatiotemporal Dynamics of Riverine Benthic Microbial Communities and their Biodegradation Potential

Joeselle Serrana; Run Tian; Michael S. McLachlan; Francisco J. A. Nascimento; Elias Broman; Benoît Dessirier; Malte Posselt

The natural variability in species composition, ecological interactions, and complex mixtures of chemical pollutants impede the development of a standardizable and systematic approach to monitoring and assessing the impacts of freshwater pollution in impacted habitats. Most research focuses on how biological communities respond to pollutants, e.g., wastewater discharge, by examining shifts in community composition and diversity within specific groups. Benthic communities, particularly microorganisms, are naturally subjected to spatial and temporal variability. Exploring spatial variations and seasonal community dynamics in affected habitats can enhance our understanding of the link between the biodiversity of key organisms and their role in ecosystem processes (e.g., contaminant biodegradation). Using environmental DNA analysis, we assess the spatiotemporal dynamics of benthic community diversity and composition, i.e., prokaryotic, algae, fungi, protists, and metazoans, and their association with biodegradation potential from two wastewater-impacted rivers in Sweden. We also quantified ecological processes, e.g., stochastic and deterministic, regulating the seasonal assembly of the benthic communities. We aimed to understand the spatial and seasonal organization and dynamics of multitrophic community assembly and assess their link to the biodegradation capacities of the microbial communities in wastewater-impacted rivers.

Exploring the evolutionary and adaptive mechanisms of *Fragilariopsis cylindrus* using sedaDNA

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The polar diatom *Fragilariopsis cylindrus* is a key indicator species for reconstructing Antarctic sea ice, productivity, and climate patterns. Its genome has revealed traits like polyploidy, suggesting adaptive genetic changes that enable the species to cope with drastic environmental fluctuations in its surroundings. Positive selection on genes such as the zinc-binding MYND domains, is thought to drive these responses. In this study, we used a targeted hybridization capture approach to analyze *F. cylindrus* sedaDNA sequences from the Scotia Sea (West Antarctica). Including sequences of the complete chloroplast genome and select nuclear genes in our baitset led to a 1000-fold increase in the *F. cylindrus* sedaDNA signal compared to metagenomic shotgun sequencing. Preliminary analysis showed 60% of the retrieved sequences belonged to the chloroplast genome, 32% to the SSU gene, and smaller contributions were found from LSU (3.2%), ITS (2.05%), ice-binding protein genes (0.4%), zinc-binding protein genes (0.22%), COI (0.011%), psbO (0.004%), genes within the LHCx clade (0.003%), and trace amounts of the highly divergent alleles of the *F. cylindrus* nuclear genome (~0.002%). The detection of these genes in the samples, previously shown to undergo positive selection, allows for the reconstruction of a genetic time series and the assessment of signatures of accelerated evolution in *F. cylindrus*, providing insights into its long-term survival in one of Earth's most extreme habitats.

Holocene Paleoclimate and Paleoecology reconstruction using SedaDNA and lipid biomarkers: Toli lake, Garhwal Himalaya, India

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Toli lake, from the monsoon-dominated Garhwal Himalaya reflects a continuous record of paleoclimate, paleoecology and history of fire events spanning the last ~18 ka. Lipid Biomarkers (n-alkanes), Polycyclic Aromatic Hydrocarbons (PAHs), stable isotopes, and sedimentary ancient DNA (SedaDNA) helped reconstructing the record. Based on variations in n-alkane indices, the entire time span was divided into distinct climatic phases. The basal part of the section (~18 to 13.6 ka) is identified as loessic deposits, formed during aridity of the Last Glacial Maximum (LGM). At ~14.8 ka, the lake exhibits sign of post LGM climatic amelioration and higher biodiversity with significant proportion of eukaryotes. Further from ~13.6 to 3.2 ka, warm and wet conditions prevailed which enhanced dissolved organic matter leading to proliferation of bacterial population. Drier condition is recorded between ~3.2 and ~1.8 ka, subsequently up to present remained warm. Pottery sherds found at ~3 ka suggest human presence during this time, corroborated by a sharp increase in PAH concentrations after ~2 ka, which is an indicative of burning events likely tied to increased anthropogenic activity in the lake catchment. In contrast, SedaDNA provides evidence of human, as early as ~14.8 ka. This study demonstrates the potential of SedaDNA in capturing both human history and ecosystem changes, offering a valuable tool for reconstructing past environmental and anthropogenic influences.

Long-term community, interaction and functional shifts in (sub)polar regions detected by marine sedimentary ancient DNA shotgun sequencing

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Ancient sedimentary DNA from marine cores offers valuable insights into community and functional changes over geological timescales, enhancing our understanding of ecosystem–environment relationships. The global carbon cycle is tightly linked to marine primary producers, their interactions with higher trophic levels, and environmental factors such as sea ice cover and temperature, which together influence whether an oceanic region functions as a carbon source or sink. To address the lack of long-term ecological data, we applied shotgun sequencing of sedimentary ancient DNA (sedaDNA) from (sub)polar cores, including those from the North Pacific, generating taxonomic and functional time-series data. These records reveal major ecosystem shifts from sea ice-adapted glacial communities to ice-free Holocene systems, marked by a turnover toward smaller plankton. Advanced network analyses across trophic levels further indicate a transition from glacial bottom-up to Holocene top-down food web regulation. Overall, our sedaDNA-based reconstructions demonstrate that sea-ice and sea-level-driven ecosystem shifts may, under continued warming, lead to greater food web fragility and diminished carbon export capacity.

Empirical comparisons of metabarcoding, capture probe and shotgun sequencing using surface sediments, vegetation surveys and PhyloNorway

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PCR metabarcoding is widely used for studying degraded environmental plant DNA, while shotgun sequencing and capture enrichment are newer approaches in palaeoecology. The effectiveness of these methods varies with DNA preservation, reference libraries, and local flora, complicating cross-comparisons. We compared these methods using surface lake sediments and vegetation surveys from 18 lakes in Northern Fennoscandia, supported by the PhyloNorway genome skim reference library. Filtering criteria were evaluated to maximize true positives (TP=present in vegetation records) and minimize false positives (FP=alien to regional flora). Pre-filtered data showed more FPs than TPs for shotgun and capture, while metabarcoding had fewer FPs. Optimal filtering for metabarcoding required presence in one of eight PCR replicates and at least three reads. For shotgun and capture probe, optimal thresholds were detections of $\geq 0.0000023\%$ and $\geq 0.00006\%$ of embryophyte reads, respectively. After filtering, TP detection at family, genus, and species levels were similar for metabarcoding and shotgun but lower for capture. However, species-level FPs exceeded TPs for shotgun and capture, making them unreliable at this resolution. Metabarcoding detected only two FPs at species level, making it preferred for species-level studies. At family and genus levels, all methods showed low FP counts and are reliable.

Applications of sedaDNA for sedimentological studies and the role of sedimentary processes in the interpretation of sedaDNA records

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The number of sedaDNA studies is rapidly increasing, especially in palaeoenvironmental studies. However, although DNA can be recovered from various sediments, we know little about the sediment properties that favour DNA adsorption and preservation. In this presentation, we would like to highlight the potential applications of sedaDNA in solving sedimentological problems, as well as the importance of a good understanding of sedimentary systems for interpreting the sedaDNA record. SedaDNA can serve as a sedimentary fingerprint and help to trace the provenance of sediments. Especially in cases where sediments have been transferred from different environments. We present examples of tsunami and storm deposits in coastal areas of Japan and Portugal that contain preserved sedaDNA of marine organisms, that can complement the micropaleontological records. We also present the study of deep-sea sediments from the Japan Trench, where the sedaDNA record reveals not only palaeoenvironmental changes, but also downslope transfer of sedaDNA with sediments in turbidity currents. Taken together, our results highlight the potential of incorporating sedaDNA into the multi-proxy toolkit of sedimentary geologists, and reveal some of the limitations of this emerging method. In addition, we discuss potential experiments assessing the impact of sediment characteristics on the performance of sedaDNA studies. This work was funded by the Polish National Science Centre (grant 2020/37/B/ST10/03677).

Changes in protists communities in Lurefjorden over the last three centuries assessed using sedimentary ancient DNA

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Positioned in the land-ocean interface, fjords are particularly vulnerable to human activities and climate change. In Lurefjorden, western Norway, declining oxygen levels and basin water darkening have led to ecological shifts including mass occurrences of the jellyfish *Periphylla periphylla* and subsequent reduction of certain fish species. While we may have some knowledge on how larger organisms are impacted by environmental changes in Lurefjorden, it is not clear how the ecosystem as a whole is affected. Microbial eukaryotes, protists, play central ecological roles in marine ecosystems, yet their sensitivity to environmental changes is unknown. It is essential to address this, as changes in their taxonomic and functional diversity can impact higher trophic levels. This study aims to address this gap by using sedimentary ancient DNA (sedaDNA) to assess the diversity of microbial communities in Lurefjorden for a time-series reaching back to pre-industrial times (~1700). Biodiversity data will be correlated with reconstructed time-series on environmental variables. Through this, we will gain insight into how protist communities have changed through time, how sensitive they are to environmental changes and whether there are any key environmental drivers of change. By assessing how protist communities have been impacted by climatic and anthropogenic stressors in the past, we can get a better understanding of how they may be impacted in the future.

Reconstructing Past Microbial Communities in Lake Ecosystems Using Sedimentary DNA

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Microbial communities are an important component of lake ecosystems, forming the foundation of aquatic food webs and driving essential biogeochemical processes. However, these ecosystems are rapidly changing due to the combined pressures of nutrient pollution and climate change. Understanding how microbial communities may respond requires insights from responses to historical environmental change, which can be revealed through the analysis of DNA preserved in lake sediments. Sedimentary DNA (sedDNA) offers a unique palaeolimnological tool to study community dynamics over centuries to millennia. However, questions remain about its reliability in capturing historical microbial community change. To assess the performance of sedDNA as a palaeolimnological tool, DNA was extracted from lake sediment cores collected from the English Lake District which has concurrent long-term monitoring data. Metabarcoding analyses identified distinct shifts in microbial community composition corresponding to historical variations in nutrient availability. Metagenomic approaches further uncovered functional changes within these communities and can be used to assess the extent of DNA preservation over time. These findings demonstrate the potential of sedDNA to reconstruct past microbial community dynamics and inform predictions of how microbes and the broader lake ecosystem may respond to future environmental change.

Bryophyte sedaDNA from a Norwegian Arctic Lake Reveals Past Ecosystem

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The unique adaptation of bryophytes makes them primary colonizers of new landscapes. While ecosystem development of vascular plants after deglaciation is well-studied, bryophytes remain understudied. We test bryophyte primers on sedaDNA to trace their community development post-deglaciation.

We metabarcoded sedaDNA from a Norwegian Arctic Lake using bryophyte primers targeting P6 loop in plastid DNA. Retrieved taxa were compared with the bycatch from vascular plant primer. Cluster analysis done with CONISS on Bray-Curtis distance and PERMANOVA assessed cluster significance.

We retrieved 3,569 reads and 309 ASVs assigned to 163 taxa, of which 95 were identified at the species level—significantly higher than the bycatch data. Taxonomic richness steadily increased until 7.5 thousand years before present (k BP), surged to 3.7 k BP, plateaued until 1.3 k BP, and then rose continuously. Taxa formed four clusters: 10.4–9.6 k BP (*Grimmia* most frequent), 9.6–5.9 k BP (e.g., *Hypnum sauteri*), 5.9–1.6 k BP (e.g., *Racomitrium ericoides*), and 1.6 k BP to present (e.g., *Hygrohypnella ochracea*). These groupings align with glacial activity, reflecting environmental shifts.

Bryophyte primer was effective, providing superior bryophyte taxonomic resolution for sedaDNA analysis, highlighting its potential for reconstructing past ecosystems.

Consistent patterns of vegetation composition and taxonomic diversity over 20 000 years in two adjacent arctic-alpine catchments in the Polar Urals

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Throughout the late Quaternary, the vegetation of Polar Urals (Russia) endured dramatic changes in climate, but still seems to have maintained a high, yet compositionally dynamic species richness. The region was an important refugium for arctic-alpine plant taxa during the early Holocene forest expansion, but whether the survival of taxa and the turnover of species and growth forms was consistent throughout the region or dependent on local conditions remains unknown. Here, we present reconstructed plant assemblage dynamics spanning the past 22,000 years based on metabarcoding of sedimentary ancient DNA (sedaDNA) cored from Maloe Shchuchye (287 m a.s.l.). The record contains detailed and continuous trends spanning from 22.9-1.5 cal ka BP distributed among 173 samples. These were compared to the neighboring lake Bolshoe Shchuchye in terms of how taxonomic diversity and composition of growth forms developed through time. Throughout the study period, several large-scale vegetation changes occurred in both cores. Similarly, the timing of which taxa arrived in the region highly aligns in the two records. Thermophilic plant taxa occurred slightly earlier in the lower altitude Bolshoe Schuchye lake record, as expected. Furthermore, the survival and persistence of arctic-alpine taxa is similar in the two catchments, confirming the importance of this region for long-term survival of arctic-alpine species.

A trial assessment of chemical burden on marine mammal dynamics over the past 100 years by using sedimentary DNA: an example of finless porpoise

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Marine mammal populations have been suspected to be declined in the past century, with the adverse effects of chemical pollutants suspected to be one of the causes. However, it is unresolved questions regarding the historical impacts of chemical exposure due to scarce in situ data. Here, we tried to reconstruct long-term population dynamics of finless porpoise (*Neophocaena asiaeorientalis*), a sedentary species inhabiting coastal areas, by analyzing environmental DNA (eDNA) preserved in sediments collected from Beppu Bay in the Seto Inland Sea, Japan. eDNA concentrations in the sediment layers were determined with quantitative PCR. Their temporal concentrations showed a distinct increase from the 1940s to the 1950s, followed by a rapid decline in the early 1960s, then recovered around 2000. Further analysis identified a significant negative correlation between their sedimentary eDNA (sedDNA) and concentrations of polychlorinated biphenyls (PCBs), cadmium (Cd). Consistent with historical PCB production trends, PCB concentrations in finless porpoises from the sea during the 1960-1970s were obviously higher than those observed in the 2000s. These findings suggest the finless porpoise population in the sea may have experienced a significant declined around 1960 due to elevated chemical exposure. This study highlights the potential of the sedDNA-based approach to enhance our understanding of anthropogenic disturbances on marine animals from a long-term perspective.

Recovering ancient DNA from warm-climate sediments (Southern Levant): integrated approaches with XRF and FTIR spectroscopy

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Ancient DNA (aDNA) extracted from archaeological sediments provides a transformative tool for understanding human evolution and past ecosystems, especially in contexts where skeletal remains are scarce. This study evaluates the potential of integrating portable X-ray fluorescence (XRF) and Fourier Transform Infrared (FTIR) spectroscopy to identify sedimentary contexts favorable for aDNA preservation in archaeological sites of the southern Levant, spanning from the Paleolithic to the Early Bronze Age.

A total of 66 sediment samples from 15 sites, including caves and open-air contexts, were analyzed. aDNA analyses employed three approaches: targeted enrichment for mammalian mitochondrial DNA (Slon et al., 2016), shotgun sequencing, and a newly designed enrichment probe set to target Levantine flora and fauna. Results from mtDNA probes confirmed aDNA preservation in 17 samples from 7 sites, including the recovery of aDNA from open-air contexts in the region. In addition, hydroxylapatite, a mineral associated with bone preservation, was detected in sediments from several sites, often co-occurring with aDNA.

These findings highlight the utility of XRF and FTIR as non-destructive, in situ tools to prioritize sediment sampling, advancing aDNA recovery from challenging preservation environments. This interdisciplinary approach facilitates more targeted and effective exploration of ancient genetic material, contributing to broader research on human and environmental history.

Environmental DNA as a tracer for past behavior

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Ancient Environmental DNA (eDNA) has been used to study past ecosystems, evolutionary change, ancient resource utilization and even human population dynamics. Ancient DNA from skeletal elements has dramatically reshaped our understanding of the past, but this DNA is limited to the location and time of death of an individual. In contrast, eDNA is deposited throughout the life of an organism, and under appropriate conditions can be preserved for hundreds of thousands of years. However, in many instances the source of this eDNA is unknown, clouding interpretation.

To determine the conditions under which DNA is released into the environment, and as part of a practical course at the University of Leipzig, we investigated the deposition of eDNA in a real life context: in the offices and hallways of the Max Planck Institute for Evolutionary Anthropology. We recovered primarily DNA from humans and dogs, and found that this DNA is tied almost exclusively to extended physical presence. By comparing eDNA samples with DNA from individual dogs sharing an office, we found that eDNA can be used to reconstruct behavioral characteristics. This investigation suggests that eDNA can be used to reconstruct past behavior, and demonstrates that ""experimental archaeology"" projects will be useful for the interpretation of eDNA archives from archaeological and possibly other contexts.

Automated sequencing data production from ancient environmental samples

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A rich record of genomic data from the past has been archived as ancient environmental DNA (eDNA) in sediments over millennia. However, a series of technical challenges exist for robust, efficient and trustworthy metagenomic data generation. These include varying sediment composition, sub-sampling procedures, efficient extraction protocols, removal of inhibitory substances, and robust generation of high-quality sequencing libraries. As a part of our research centres, we have established an accessible automated laboratory infrastructure for production of sequencing data from sediments. Here I report on the progress and the technical insights we have gained from applying semi-automated workflows. The effort has enabled analysis of eDNA from thousands of sediment samples allowing exploration of past changes in the composition and genetic function of species in natural ecosystems.

Keelime: Hybrid assembly for ancient environmental DNA using pangenome graphs

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Ancient DNA (aDNA) and ancient environmental DNA (aeDNA) projects have surpassed the simple statement of species presences or absences. The vast improvements in laboratory, sequencing and bioinformatics techniques allowed researchers to achieve specific genomic analysis, including phylogenetic analysis, genetics divergence through time and the discovery of extinct genomes. However, the first step of these analyses is the construction of a consensus genome from one's data. The mitochondrial or chloroplast genomes are often used due to their relatively small size. De-novo assembly is able to capture all genetic variation present in the data without bias; however, it requires coverage depths that are uncommon in aDNA or aeDNA samples. Reference-based assemblers excel with low coverage, but they will be biased towards the reference genome used if a reference genome is available. We present keelime, a hybrid assembler for aDNA and aeDNA that uses reference-based and de-novo assembly features to improve consensus assemblies. Our mapping approach against a pangenome graph structure allows us to build contigs from overlapping reads, displaying the genetic variance of the data even on low-coverage depth. keelime's pangenome graph approach allows consensus assembly even with distantly related references. We demonstrate keelime's improvements in three different benchmarking experiments and show consistency with empirical data sets for both mitochondrial and chloroplast genome assembly.

Is sea-ice cover reflected in sedimentary DNA? – a case study from the Weddell Sea, Southern Ocean

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Sedimentary ancient DNA (sedaDNA) is an emerging tool in marine paleoecology. It allows reconstructing entire biological communities, including lineages without a fossil record. In polar regions, sedaDNA has great potential to be used as a sea ice proxy by tracing sea ice associated biological communities across time, adding unprecedented detail to reconstructions with e.g. diatom-based proxies.

A crucial step before we can reconstruct past sea ice conditions with sedaDNA, is to calibrate recent sedimentary DNA with modern sea ice conditions. Here we present results from a metabarcoding study on surface sediment samples across the Weddell Sea and the Drake Passage in the Southern Ocean, spanning open ocean, seasonal as well as permanent sea ice coverage. We extracted DNA from the sediment at >40 stations. We then amplified and sequenced the V9 region of the SSU rDNA targeting eukaryotes. First results indicate that communities at the coastal area around the Antarctic Peninsula are dominated by diatoms. In the central Weddell Sea and the Drake Passage small flagellates and ciliates are more abundant.

We explore if and how the communities are related to modern surface ocean conditions, especially sea ice cover. In addition, we combine the DNA metabarcoding data with other sea ice proxies, like diatom and dinoflagellate cyst assemblages and biomarkers, as well as sedimentological information to get a more complete picture of the environmental conditions in the modern ocean.

Advancing ancient environmental DNA target genome capture to facilitate genomic and genetic analysis

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A major challenge in genomic and genetic analysis of ancient environmental DNA (aeDNA) is the insufficient coverage of informative sites across species genomes. Target genome capture has been proven as an effective approach to enhance coverage of specific genome regions, yet significant gaps remain in several aspects of aeDNA capture methods. In this presentation, I will summarize recent technical advances we made in aeDNA target genome capture techniques, including

- 1) developments in probe design aimed at improved biodiversity profiling, phylogenetic reconstructions for common domesticated animal and plant taxa, and genetic diversity and population genetic analysis for single species,
- 2) understanding the effects of variables involved in hybridization reactions and different types of probe,
- 3) subsequent analysis of the capture-sequencing data for different research goals.

The aim is to provide a comprehensive framework for improved aeDNA target genome capture, hence, to facilitate efficient and precise genomic and genetic analysis using aeDNA.

Using sedimentary ancient DNA to trace changes in past marine biodiversity in Norwegian fjords

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Fjords are strongly affected by climate change and anthropogenic impacts. Rapid changes, such as increasing water temperatures, changes in oxygen conditions and decreasing sea ice in the Arctic will inevitably have profound effects on the marine biodiversity in fjords. Yet, so far, our knowledge on the impact of these changes on marine communities remains limited, despite their important roles in food webs and nutrient cycling. By applying sedaDNA sequencing to fjord sediment cores, we can now unlock information on past changes in marine biodiversity in correlation with past environmental changes to better understand ongoing changes and the resilience of marine communities.

In the project PASTIME, we focus on sediment cores from Arctic and western Norwegian fjords and assess ecosystem changes over the last centuries. Our work thus extends the timescales far beyond traditional observational data and allows us to trace changes from a preindustrial state of the ecosystem to the modern state. We are assessing the impact of various environmental factors (e.g. temperature, freshwater inflow, sea ice, oxygen) on biodiversity to elucidate key drivers of change and study the resilience of organisms. Here, we will present preliminary data on one sediment core collected in Kongsfjorden, Svalbard, and one core from Masfjorden, Western Norway. Both cores cover the last three centuries with a high vertical resolution and show marine community responses to past environmental changes.

Shifts in Marine and Terrestrial Ecosystem Inputs to marine sediments: Insights from sedaDNA, automated habitat classification and Biomass Estimation.

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This study investigates the interplay between marine and terrestrial systems in the Northern Pacific region using metagenomic sedaDNA from five marine sediment cores spanning the glacial, deglacial, and Holocene periods. Employing innovative analytical approaches and automated taxonomic classifications, we distinguished taxa by their terrestrial, freshwater, or marine habitats and estimated habitat-specific buried biomass using the accumulation of DNA, cell weight per taxon and DNA weight per cell per taxon (derived from curated databases) in marine sediments. During the glacial period, the system was predominantly marine, characterized by a higher prevalence of marine taxa and biomass, with minimal terrestrial contributions. In contrast, the deglacial and Holocene periods exhibited increased terrestrial influence, derived by permafrost, riverine woodlands, and wetlands. Archaea provided evidence of permafrost input, while bacteria and eukaryotes revealed spatial patterns of terrestrial sources. The composition of terrestrial inputs transitioned from coastal plants during the glacial period to expanded contributions from wetlands and riverine systems during the deglacial and Holocene. Comparative analyses of taxonomic distributions in marine and terrestrial sediment cores uncovered parallels in eukaryotic and bacterial assemblages, underscoring the intricate pathways of terrestrial taxa transport to the ocean and their ultimate burial in marine sediments.

Arctic Ocean ecosystem reconstruction using sedaDNA: a comparison of shotgun metagenomics and metabarcoding

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Arctic environments are an excellent resource for sedimentary ancient DNA (sedaDNA) studies due to cold conditions facilitating DNA preservation. As the micropalaeontological record is often sparse in Arctic Ocean sediments, DNA-based methods may provide an alternative tool to better understand past environmental changes. Metabarcoding is currently the most common method for generating marine sedaDNA, but it is difficult to apply to poorly preserved or deep-time samples. Shotgun metagenomics is an upcoming method that provides opportunities inaccessible from metabarcoding data alone, however the bioinformatics analysis is more complex and only limited suitable curated genome databases are available. In this study, we analysed DNA fragments from 35cm-long surface sediment cores, taken with a boxcorer along a transect from the continental slope of the Barents Sea to the Nansen Basin. We tested two different DNA extraction protocols, a standard method based on silica columns, and a custom protocol, optimised for marine sedaDNA. We then aimed to reconstruct marine ecosystem responses to Holocene climate change in this area, using both shotgun metagenomics and metabarcoding approaches. In addition, we tested novel bioinformatics methods to screen the shotgun metagenomics data for a wide variety of marine organisms at a genome-wide level. We detected a broad range of taxa from across the ocean ecosystem, with a notable strong and consistent detection of baleen whale DNA.

Permafrost thaw: changing Arctic landscapes, their microbiomes and global health

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With the Arctic warming at least twice as fast as the global average, Arctic environments are particularly vulnerable to ecological disruption as a result of climate change. However, the microbial communities of Arctic environments remain under-sampled when compared to more accessible environments (e.g. agricultural soils). Consequently, our understanding of how accelerated permafrost thawing is impacting the microbiology of Arctic environments and pathogen emergence, is limited. The preservative properties of permafrost also provide a unique opportunity to study ancient microbial communities, including virulence genes, antimicrobial resistance genes and pathogens (known and unknown) dating back thousands of years. There are few examples of cross-sectional studies covering deteriorating permafrost environments and this undermines our ability to assess the ongoing collision between contemporary and ancient microbial genetic material, as well as the wider implications for human, animal and plant health. To address this, we are using 'omics' techniques to survey an actively degrading discontinuous permafrost system in Abisko, Sweden. Specifically, we collected core samples from intact permafrost, transition and active layers from three palsas. To assess the potential impact of permafrost thawing on the wider microbial ecology of the area, we also collected thaw-pond water and mosquitoes. The collection, processing and preliminary analysis of samples will be discussed.

First steps to integrating aeDNA with the Neotoma Paleoecology Database

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Empowering researchers to conduct macroscale and integrative biodiversity research based on networks of ancient environmental DNA (aeDNA) records requires the integration of aeDNA data and metadata into open, community-led data systems. In response to recommendations by members of the aeDNA community, a new effort has been launched to extend the services of the Neotoma Paleoecology Database to support aeDNA and to build a community of aeDNA data stewards and contributors. Here, we share the major goals of the next phase of building the aeDNA constituent database and first steps taken to achieve these goals. Goals include: 1) Extending community-curated paleoecoinformatic data systems to support dynamic aeDNA-based inferences about taxon identity and age, 2) Building a linked open infrastructure that connects and leverages existing resources and standards in bioinformatics and paleoecoinformatics, 3) Building a community of data governance and trust that creates, shares, and uses high-value aeDNA data, and 4) Empowering and inspiring the next generation of aeDNA and biodiversity scientists. Initial work is underway to extend the Neotoma data schema to support aeDNA data and metadata, to build draft data templates for data submissions, and to build an international data governance structure for an aeDNA Constituent Database within Neotoma.

Tracing abrupt climate changes: SedaDNA reveals impact of sudden climate change on plant communities in Wales since the Last Glacial Maximum

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The deglaciation period after the Last Glacial Maximum, 20 - 10 thousand years before present (ka BP), is dominated by abrupt changes. Whilst many archives chart the sudden warming at the onset of the Lateglacial period, ~14.7 ka BP, it is rare to have a continuous record from the preceding cold phase through to the onset of the present interglacial. Llangorse Lake, South Wales, has recently been discovered to chronicle these climatic oscillations between 19 and 10 ka BP. Using sedimentary ancient DNA (sedaDNA), we reveal a treeless landscape during the stadials, characterized by cold-adapted plant taxa such as purple mountain saxifrage (*Saxifraga oppositifolia*), Arctic root (*Rhodiola rosea*), and mountain avens (*Dryas octopetala*). The animal record from this period includes Arctic lemming (*Dicrostonyx torquatus*), mammoth (*Mammuthus primigenius*), and rock ptarmigan (*Lagopus muta*). The interstadials depict a markedly different environment, with evidence of trees including European spruce (*Picea abies*), elm (*Ulmus*), and pine (*Pinus*), alongside herbaceous plants such as bulrush (*Typha latifolia*), water-blinks (*Montia fontana*), and stinging nettle (*Urtica dioica*). This study allows us to peer into recently unknown past ecosystems and the significant differences that changing temperatures drive. This sequence also represents the oldest British sedaDNA analysis, offering valuable insights into the past ecological dynamics after the end of the last ice sheet in Southern Britain.

Sedimentary ancient DNA from the Middle Paleolithic Cave site Ararat 1, Armenia

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Marine Isotope Stage 3 (ca. 60-27 Ka) witnessed rapid global changes in population dynamics including the possible turnover of sub-populations of Neanderthals and Modern Humans. Southwestern Asia was a melting pot during this time with multiple hominin species present in the region possibly co-occurring with the cultural transition from the Middle to Upper Palaeolithic, and rapid environmental change and ecological fluctuations. Spatial effects of these climatic oscillations on hominin populations and terrestrial mammalian communities are however essentially unknown, especially in the Armenian highlands/Southern Caucasus region.

Ararat 1 is an Armenian Middle Palaeolithic cave site (35-52 Ka) with lithic remains and anthropogenic ashes. In this project, we harness sedaDNA to further investigate presence of early humans/ other mammals and to genetically validate microscopic observations from the resin impregnated blocks.

Samples included bulk sediment and resin-impregnated blocks to generate shotgun libraries and mammal mtDNA capture. Mammal DNA was more abundant in the resin block samples, and more mammalian signal was found in the block deeper in the cave. Identified taxa included Hominids, Hyaenids, Felids, Bovids and Ochotonids. Hyaenas shed DNA primarily at the back of the cave and lions throughout the site. Results suggest that hominin use was ephemeral and concentrated at the back of the cave, alternating with large carnivores.

Revealing Holocene ice-ocean-biota relationships with sedimentary ancient DNA metagenomics in Melville Bay, North-West Greenland

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Qimusseriarsuaq (Melville Bay) is a marine nature reserve and wildlife sanctuary located in North-West Greenland, which is particularly vulnerable to anthropogenic climate change. Due to high ice mass loss of the Greenland Ice Sheet, glacier retreat, increased freshwater input, and sea ice decline in this area, it is a critical yet understudied Arctic region, particularly regarding the impacts on the marine food webs. To fill this gap, we investigate the marine sedimentary record from the Holocene Thermal Maximum (HTM) as an analog for warming scenarios projected for the 21st century.

We present metagenomic data of sedimentary ancient DNA from marine sediment core LK21-IC-st26, which was collected during the ICAROS expedition 2021 onboard HDMS Lauge Koch. Laminations at the base of this 320 cm long core indicate its location close to an ancient ice shelf and thus highlights that our core captured the full Holocene since the deglaciation (past ~11.7 ka).

Focusing on eukaryotes and primary producers, we will show composition and diversity changes following the deglaciation into the HTM and the Late Holocene. We thereby provide novel insights into ecological transformations driven by ice dynamics, ocean circulation changes, and climate variability of North-West Greenland. By exploring the fingerprints of these past processes, this study will advance our understanding of Arctic marine ecosystem dynamics and ultimately will inform predictions for future changes in the region.