Letting the soil speak: High-throughput metagenomic sequencing of sedaDNA from two early Norse settlements in East Iceland

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The first settlers migrated to Iceland from Scandinavia and the British Isles during the 9th century.[1,2] Some settled in East Iceland around present-day Seyðisfjörður, including near the research station Skálanes. In 2022, a longhouse was discovered in Fjörður, Seyðisfjörður (hereafter Fjörður) underneath an 11th century landslide. The excavation revealed a lively, seafaring place where wool-processing and weaving were practiced, game pieces were created, and metalworking took place. Four excavated graves revealed burials according to Pagan Viking beliefs. Our present study investigates sedaDNA collected from the settlement farm at Fjörður (10th-12th century), and from a farm mound at nearby Skálanes (10th-18th century).

At both sites samples were taken in pre-settlement layers (6th-8th century) and in cultural layers (9th-19th century); at Fjörður a midden was also sampled. Our aim is to investigate whether sedaDNA from archaeological contexts can provide additional and novel evidence to what has previously been published on the utilisation of plants and animals before and after the settlement of the island than.[3-6] To "let the soil speak" we created a software package in Python to manage a multi-step workflow to identify and profile damaged sedaDNA. The findings of this analysis are expected to represent the living conditions and resources used at Fjörður and the Skálanes farm during settlement.

Sample Collection

At each sampling horizon, 5 cm of the outer soil layer was scraped away to reveal uncontaminated soil. Sterile 50 mL tubes were pushed into the soil profile to a depth of ~5 cm. Three biological replicates were collected per horizon. Tubes were immediately capped and placed into a double layer of ziplock bags, sealed and stored on ice and then at -20°C until DNA extraction. Technicians wore PPE including hair covering, eye goggles, a face mask, and double layers of gloves during sampling.

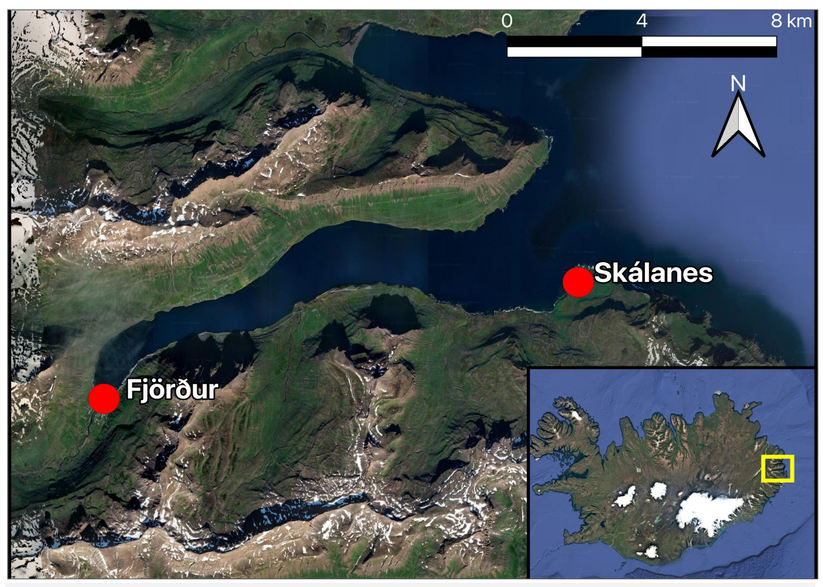

Figure 1. Location of the research sites near Seyðisfjörður, East-Iceland. Image credit: Open Street Maps, Google Maps, and the authors.

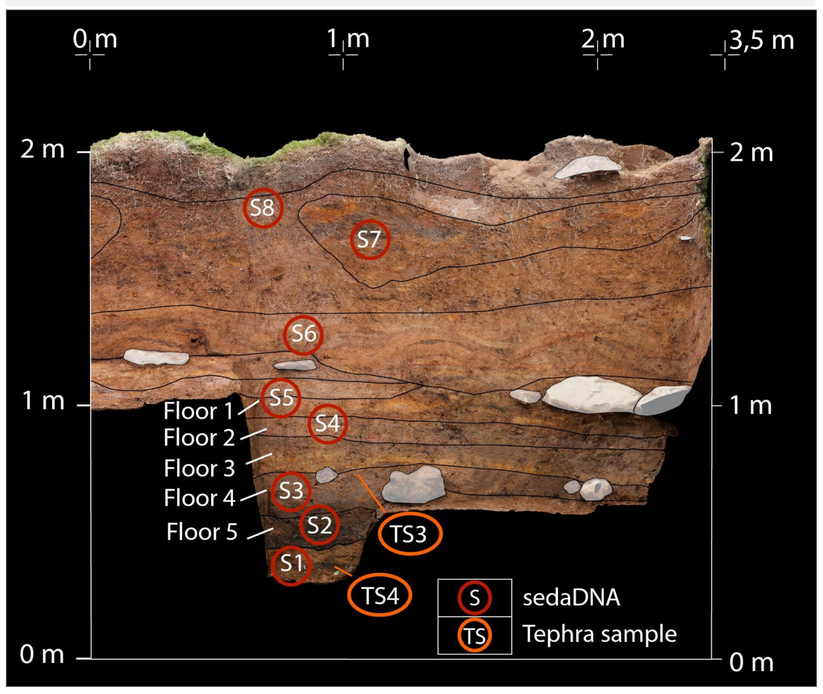

Figure 2. Skálanes farm mound profile. Samples were taken from profiles in the following archaeological contexts: **S1)** Undisturbed soil - before the settlement (700-900), **S2)** Floor layer 5 (950-1100), **S3)** Floor layer 4 (950-1100), **S4)** Floor layer 2 (1500-1700), **S5)** Floor layer 1 (1500-1700), **S6)** Turf structure (1500-1700), **S7)** Collapsed turf layer, around 1800, **S8)** Surface underneath 1875 tephra.

Figure 3. Fjörður Viking longhouse. Samples were taken from profiles at Fjörður from the following archaeological contexts: **S1)** Undisturbed soil from before settlement (700-900), **S2)** Top of peat ash midden (over 1100 landslide), **S3)** Bottom of peat ash midden (under 1100 landslide), **S4)** Upper floor of "weaving room" where landslide fell in 1100, **S5)** Lower floor of the "weaving room" that landslide fell in 1100, **S6)** Surface profile, for comparison.

sedaDNA Extraction and Sequencing

Soil samples were transported (USDA permit #201905-0022) to Earlham College's aDNA facilities for extraction and purification. Samples were extracted following sterile aDNA guidelines using Qiagen's PowerSoil Pro Kit with modifications.[5,6,7] After extraction, samples were purified with Zymo's DNA Clean and Concentrator MagBead Kit following manufacturer's instructions. Samples were sent to the sequencing core at UC Berkeley and size selected (<500 bp) before high-throughput sequencing (HTS) on a NovaSeq 6000 with a 100PE kit.

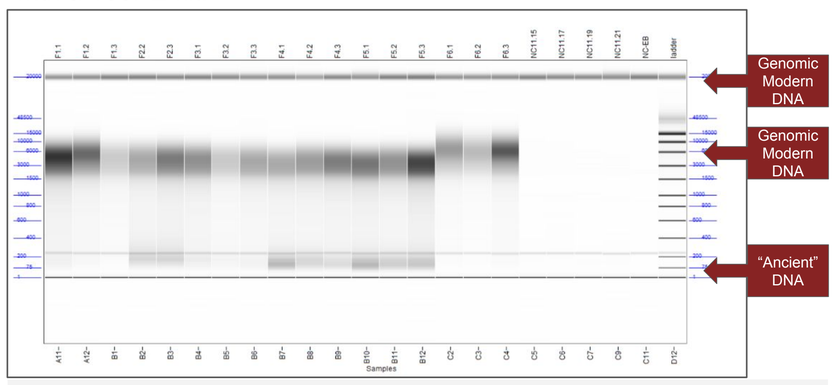

Figure 4. Fragment Analysis of Fjörður Longhouse Samples. Ancient DNA should appear as small fragment smears below 500 bp. aDNA appears in the samples collected within the longhouse (F2.2-5.3) and not in the modern samples (F6.1-6.3) or the samples taken from below the settlement layer (F1.1-1.3). All negative controls (NC) show no DNA, indicating we did not have contamination during DNA extraction.

Results

We began our workflow on the HTS data from the Skálanes Farm Mound. Thus far we have used two databases for alignment and damage analysis, the NCBI mitochondrial database and the PhyloNorway plant database.

Total Sequenced Reads	**1,027,957,930**
NCBI Aligned Reads	136,584
PhyloNorway Aligned Reads	297,367
Overlapping Aligned Reads	**3,938**

Table 1. Results of aligning to the NCBI Mitochondrial and the PhyloNorway Plant Databases. To compare overlapping aligned joined reads between both databases, 3 - 4 biological replicates from all sampling horizons (S1 through S8, Fig. 2) were combined.

To-date we have assessed the damage of all the aligned reads from the NCBI Mitochondrial database with mapDamage 2.0 and DamageProfiler 1.1. While the documentation for each tool indicates that they calculate the same summary information about deanimations at each base position, we had difficulty replicating this (see Figure 5 below).

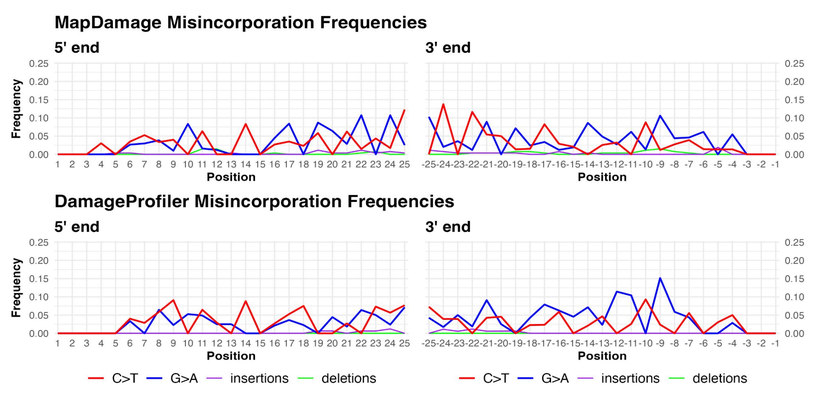

Figure 5. Comparison of mapDamage and DamageProfiler output when run on the *Vaccinium macrocarpon* reads and the NCBI mitochondrial database. The graphs also illustrate the general damage pattern we encountered in the oldest sampling horizons.

Many individual species that were present only in the oldest sampling horizons, e.g. Cranberry (*Vaccinium macrocarpon)*, were damaged, but not at the 5' and 3' ends as is typically found. Rather, most of the deanimations took place further along the strands, starting roughly at the fifth position from the 5' end and the third position from the 3' end. Our efforts to find broad damage patterns at each sampling horizon were confounded by differing results from tools and the overwhelming presence of bacterial species.

Bioinformatics

We developed a software package to support our "let the soil speak" approach. This package makes it practical to scale-up the analysis and manage thousands of files, ultimately identifying the comparatively few species with sufficient reads to be considered "present" and DNA damage consistent with the timescale of the sampling horizon provided by tephrochronology. The tool enables us to change input FASTQ, parameters, databases, thresholds, damage analysis tool, etc. and re-run the workflow from raw FASTQ through post-damage filtering with one command.

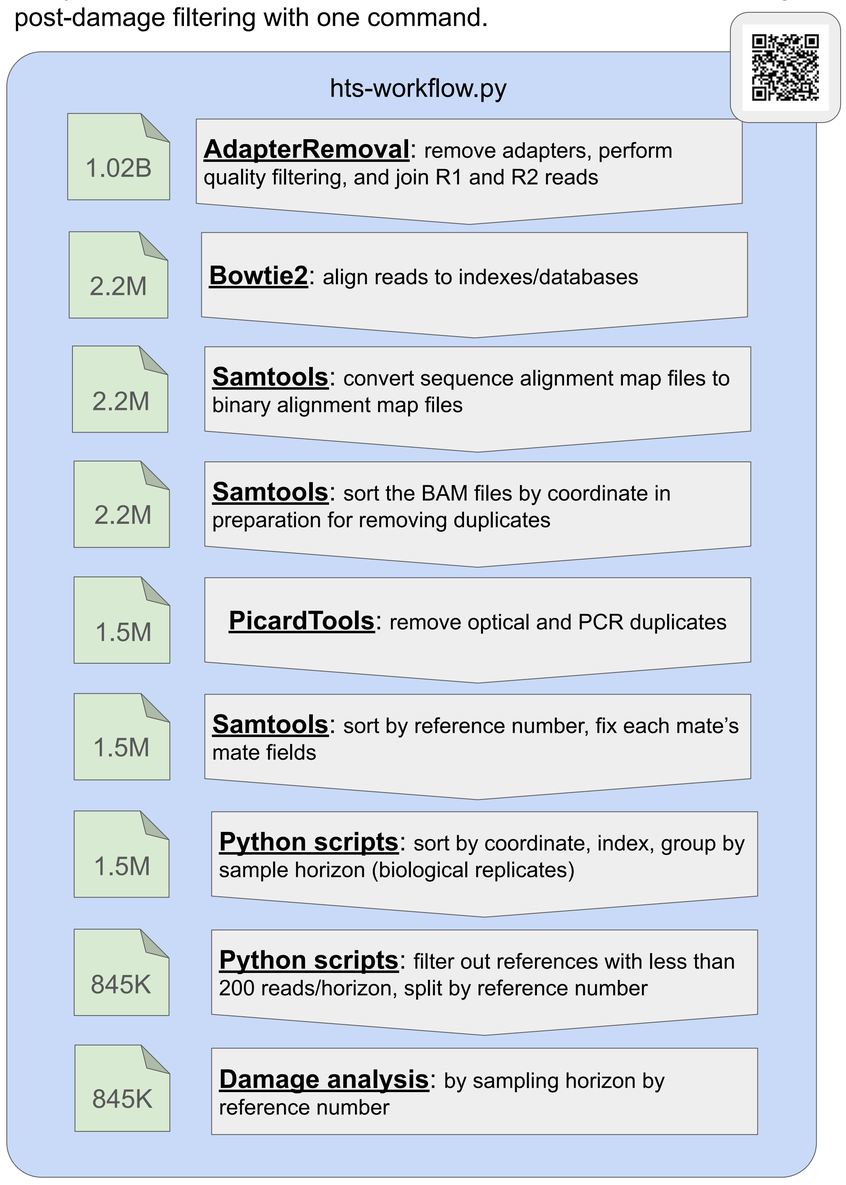

Figure 6. HTS and damage analysis workflow. Numbers in the green call-outs represent number of reads at each stage when run with the NCBI mitochondrial database.

Future Directions

- Compare HTS results with Kraken/Bracken results.
- Filter bacterial species and establish mean damage scores per sampling horizon.
- Interpret damaged DNA in context of Icelandic soil and archaeology sites.
- Characterize DNA leaching through soil layers.
- Reconcile mapDamage and DamageProfiler, consider metaDMG.
- Use the results to understand the ecosystem before and during the settlement of Iceland, highlighting the potential of sedaDNA to uncover aspects of daily life not visible through traditional archaeological methods.

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Acknowledgements

We thank the Skálanes Nature and Heritage Center for hosting us while in Iceland. Funding sources include the Skálanes Nature and Heritage Center; Earlham College's Stephenson Fund, Ford-Earlham Research Fund, and Cluster Computing Group Fund; the University of Iceland Research Centre in East Iceland; PhD funding [nr. 2410163-051] from the Icelandic Centre for Research (Rannís); SIE Scholarship grant from Soroptimists in East Iceland; and travel grants from Hagþenkir and the University of Iceland.