



# Quantifying past arctic herbivore populations from ancient sedimentary DNA by metabarcoding, hybridization capture enrichment, and droplet digital PCR



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## Summary

The Arctic is currently experiencing dramatic ecosystem changes with immediate effects on biodiversity. **Palaeorecords** (e.g. sedimentary **ancient DNA** [aDNA]) are a unique and valuable source of data on long-term ecosystem development, which may help disentangle the relative impacts of climate, herbivory, and anthropogenic effects on ecosystems. **Herbivores** are keystone taxa of many ecosystems due to their impact on vegetation by mechanisms such as nutrient cycling, promoting food web diversity, modifying vegetation structure, and altering hydrology and fire regimes. In the project "Future Arctic Ecosystems" (FATE), we aim to assess changes in past herbivore abundance over a wide spatial range (*circumarctic*) and over long temporal scales (*Last Glacial Maximum – present*). The outcome will produce insights on past ecosystems which may help understand the effects of current changes in contemporary, ecosystems and for predicting future developments.

## Methods

### aDNA from Siberian lake sediment cores

#### Herbivorous mammals

- ✓ identification of terrestrial mammals from sedimentary aDNA by metabarcoding<sup>1</sup> & hybrid capture<sup>2,3</sup>
- but: detection unreliable (patchy), not quantitative

#### Herbivore proxies

- ✓ coprophilous fungi (e.g. *Podospora*, *Sporormiella*): spore abundance established as herbivore proxy
- ✓ endo- and ectoparasites of herbivores, e.g. **nematodes, mites, insects**

#### Plants

- ✓ robust metabarcoding assay is available<sup>4,5</sup>
- ✓ curated database for arctic-boreal plants
- ✓ database currently being expanded for complete chloroplast genomes (Tromsø Museum)

### a) Metabarcoding of plants (trnL P6 loop<sup>6</sup>) and coprophilous fungi (ITS1-5.8S-ITS2)<sup>7,8</sup>



☆ **high taxonomic resolution**

potential issues:

- length of conventional barcode regions (up to 700 bp)
- primer and length amplification bias

currently available PCR primers for fungal metabarcoding may not amplify taxa of interest and produce long fragments; -> design of novel barcoding primers for short fragments (100-200 bp) to include coprophilous taxa.

quantification: HTS read abundance

### b) Hybridization capture enrichment



enrichment of aDNA of **mammals** (complete mitogenome) & **proxy organisms** (barcode regions: ITS1/2, COI, 12S, 28S)

- ☆ complex bait set to target **multiple markers of multiple taxa**
- ☆ DNA fragmentation less problematic (PCR-free method)
- ☆ high bait-target divergence is unproblematic (up to 40% bait-target mismatch)
- enrichment success may be affected by amount and divergence of non-target DNA in the genomic library

bait set:

	marker	extent	# of sequences
Fungi	ITS1, ITS2	4 genera of obligate coprophilous fungi + representative sequences of 65 genera of facultative coproph. fungi	1199
	12S	representative sequences of obligate/facultative cp. fungi	7
Oomycota	ITS1, ITS2	3 selected species	12
Nematoda	COI	27 selected genera	98
	ITS2	27 selected genera	119
Acari	COI	4 selected genera	16
	28S	4 selected genera	16
Insecta	Diptera COI	6 selected genera	25
	Phthiraptera COI	5 selected genera	34
Mammals	complete mitogenome: 11 species		11

quantification: HTS read abundance

### c) Droplet digital PCR (ddPCR) - absolute quantification of aDNA of selected target taxa.



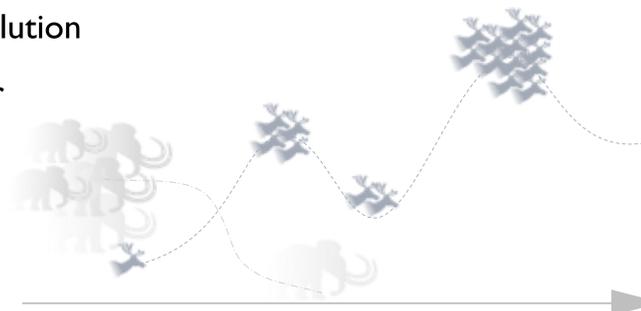
quantifiable PCR amplification within 20.000 nano-droplets

evaluation by preliminary experiments on contemporary eDNA of ungulate intestinal parasites & coprophilous fungi (e.g. *Trichostrongylidae* / *Podospora* sp.)

absolute quantification of template molecules

## Objectives & Data Output

- Quantitative data of herbivores, proxy organisms, and vegetation with high taxonomic resolution
- Assessment of correlation patterns of plant community structures with climatic changes or with abundance of herbivores
- Changes in diversity and relative abundance of plants, mammals, and fungi over time at different sites throughout the Arctic?



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Future Arctic Ecosystems



<sup>1</sup>Graham RW et al. (2016) Timing and causes of mid-Holocene mammoth extinction on St. Paul Island, Alaska. PNAS

<sup>2</sup>Seeber PA et al. (2019) Terrestrial mammal surveillance using hybridization capture of environmental DNA from African waterholes. Mol Ecol Resour 19:1486-1496

<sup>3</sup>Murchie T et al. (2019) PalaeoChip Arctic1.0: An optimised eDNA targeted enrichment approach to reconstructing past environments. 1-45. bioRxiv 730440

<sup>4</sup>Taberlet P et al. (2007) Power and limitations of the chloroplast trnL (UAA) intron for plant DNA barcoding. Nuc Acids Res 35, e14

<sup>5</sup>Sønstebo JH et al. (2010) A minimalist DNA barcoding approach for reconstructing past Arctic vegetation and climate. Mol Ecol Resour 10:1009-1018

<sup>6</sup>Willerslev E et al. (2014) Fifty thousand years of Arctic vegetation and megafaunal diet. Nature 506:47-51.

<sup>7</sup>Epp LS et al. (2012) New environmental metabarcodes for analysing soil DNA: potential for studying past and present ecosystems. Mol Ecol 21:1821-1833.

<sup>8</sup>Bellemain E et al. (2013) Fungal palaeodiversity revealed using high-throughput metabarcoding of ancient DNA from arctic permafrost. Environ Microbiol 15:1176-1189.



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