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[1] & hybrid capture[2,3]
- – *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[4,5]
- ✓ curated database for arctic-boreal plants
- ✓ database currently being expanded for complete chloroplast genomes (Tromsø Museum)

a) Metabarcoding of **plants** (trnL P6 loop[6]) and **coprophilous fungi** (ITS1-5.8S-ITS2)[7,8]

☆ **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, CO1, 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

quantification: HTS read abundance

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

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

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