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

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The Arctic is currently experiencing dramatic ecosystem changes with immediate effects on biodiversity. Sedimentary ancient DNA is a unique and valuable source of information on ecosystem changes over a long temporal scale. Understanding these past changes may help predict the relative impacts of climate change, herbivory, and anthropogenic effects on present ecosystems. In the BiodivERsA project "Future ArcTic Ecosystems" (FATE), we aim to assess changes in past herbivore abundance over large spatial (circumarctic) and temporal (Last Glacial Maximum until today) scales using three (semi-)quantitative methods on sedimentary ancient DNA of plants, herbivores, and herbivore proxies (i.e. coprophilous fungi and parasites) – metabarcoding, hybridization capture enrichment, and droplet digital PCR (ddPCR).

Metabarcoding was applied to DNA of plants and also of coprophilous fungi as proxies of herbivore abundance. This approach is an established and important tool for assessing biodiversity from recent environmental DNA; however, quantification of specific taxa may be complicated due to inherent methodological biases (e.g. amplification efficiency due to primer bias), and our current understanding of the factors affecting potential quantification by metabarcoding is still limited. Moreover, ancient DNA is highly fragmented, which may prevent PCR amplification altogether. As an alternative, target enrichment by hybridization capture is a method that does not depend on target PCR amplification and is typically not affected by DNA fragmentation. Furthermore, hybridization capture can be used to target numerous genetic markers of a vast range of highly diverse taxa. We are using hybridization capture to enrich DNA of a range of herbivore species and numerous proxy organisms. Metabarcoding and hybridization capture can be applied to a vast taxonomic range and may be used quantitatively based on relative sequencing read abundance; however, the respective read abundance may be confounded by random and systematic errors and other biases. We are therefore using an additional quantification method – ddPCR – on several selected taxa, which is taxon-specific but facilitates highly accurate quantification of template DNA molecules in a given sample. The

combined taxonomic and quantitative results of these three approaches are used to generate highly resolved datasets on past vegetation and herbivores, which allows us to reconstruct past vegetation changes over large spatial (circumarctic) and temporal (Last Glacial Maximum until today) scales.

Detailed inferences on herbivore abundance and reconstructing past ecological conditions may be important for ecosystem management and conservation in the face of accelerating changes in Arctic ecosystems due to global climate change.