A pan-Arctic model to assess faecal nutrient content of herbivores in the tundra using Near-Infrared Reflectance Spectroscopy (NIRS)

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Background

Herbivores are important drivers of nutrient dynamics in terrestrial ecosystems[1,2]. By consuming plants and releasing nutrient-rich resources to the soil, herbivores affect nutrient cycling[3]. Traditional chemical laboratory analyses to assess nutrient content of faeces are laborious and expensive, and often limit the number of samples that can be processed. Near Infrared Spectroscopy (NIRS) represents an effective, low-cost method to assess the nutrient contents of herbivore dung[4], but it requires calibration with samples of known chemical content[5]. Developing NIRS calibration curves for faecal nutrient content of Arctic herbivores will allow processing the large number of samples needed to address ecologically relevant questions at finer temporal and spatial scales. This project will provide a significant methodological advance in the field and will increase our understanding of how herbivores contribute to nutrient cycling in tundra ecosystems.

One limiting factor for understanding the nutrient contribution of herbivore faeces is the need to obtain fresh faecal matter from accurately identified herbivore species. This protocol describes an **opportunistic sample collection** for researchers that have access to **very fresh faecal matter that can be unequivocally ascribed to a species of Arctic herbivore** (e.g., when working with live trapping, culled animals, enclosure experiments, or visiting colonies and nests).

Equipment to bring with you to the field

- Ziplock bags: one per sample. Sandwich bag size (16.5 x 14.9 cm) or even smaller
- If possible, GPS for recording the geographic coordinates (otherwise site name will be enough)
- Permanent marker to label the plastic bags
- Small paper bags or coin envelopes for storing dry samples
- Plastic gloves to collect the samples

Sample collection

This project includes **wild and free ranging vertebrate herbivores**, both **mammals and birds**, small and large. Faecal samples from individual animals need to be collected **fresh, immediately after defecation or from the guts** (e.g., if the animal has been harvested). We aim at collecting ca. 2 mg of dried faecal material per sample. In the table below you can find estimates of how much this means in terms of pellets.

Herbivore size	***Examples of species***	***Quantities for adults***
Small	Lemmings, voles	Ideally min 50 pellets for adults, but as piles are usually smaller, less can be collected
	Arctic hare	6-8 pellets
Medium	Goose	1-3 droppings
	Ptarmigan	6-7 droppings
Large	Reindeer, sheep, muskox	Half a clump or 6-8 pellets

Each fresh sample will be placed in a separate Ziplock bag labelled with a **unique ID** following this pattern: last two digits for year of collection - site (initials) - species name (three first letter of the

genus and the species name) - unique number". For example, *23-CB-LAGMUT-1* corresponds to the sample number 1 collected in 2023, in Cambridge Bay for rock ptarmigan (*Lagopus muta*).

In addition, the following information will be recorded separately in the data template associated to the protocol:

- *GPS coordinates (LAT/LON coordinates) and/or location name*
- *Species of herbivore: recorded as scientific name*
- *if known, age and sex of the individual e.g., adult, calf*
- *Date of collection*
- *Method of collection*
- *Name of person collecting the sample*

Sample processing and storage

As soon as possible after collection, samples will be oven-dried at **40°C for about 48h**, until they are completely dried. If it is not possible to oven-dry the samples immediately after collection, you can freeze them at -20°C until you have the chance to dry them. Dried samples will be stored in a coin envelope or a paper bag with the information collected in the field and shipped to the Agricultural University of Iceland:

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In addition, you will need to report field information and a list of the samples in the data entry template and email it to: mathilde@lbhi.is

What will happen after you ship the samples

Samples will be further processed in the lab at the Agricultural University of Iceland, where they will be milled to fine powder. Part of each sample will be used for chemical analyses (C and N, and possibly more) and part will be compressed to a thin tablet that can be scanned with the NIRS instrument. With that information we will build NIRS calibration curves that, once validated for the different species, will be able to predict the chemical composition of faecal samples based on NIRS scans alone. In addition, the data obtained from these samples will contribute to an open database on nutrient content of herbivore dung. Data contributors will be invited as co-authors of relevant outcomes of this project.

If you have any questions, please contact Mathilde Defourneaux (mathilde@lbhi.is).

References: 1. Peller, T., Marleau, J. N. & Guichard, F. Traits affecting nutrient recycling by mobile consumers can explain coexistence and spatially heterogeneous trophic regulation across a meta-ecosystem. *Ecology Letters* ele.13941 (2021) doi:10.1111/ele.13941. **2**. McInturf, A. G., Pollack, L., Yang, L. H. & Spiegel, O. Vectors with autonomy: what distinguishes animal-mediated nutrient transport from abiotic vectors? *Biol Rev* 94, 1761–1773 (2019). **3**. van der Wal, R., Bardgett, R. D., Harrison, K. A. & Stien, A. Vertebrate herbivores and ecosystem control: cascading effects of faeces on tundra ecosystems. *Ecography* 27, 242–252 (2004). **4.** Villamuelas, M. *et al.* Predicting herbivore faecal nitrogen using a multispecies near-infrared reflectance spectroscopy calibration. *PLoS ONE* 12, e0176635 (2017). **5.** Dixon, R. & Coates, D. Review: Near Infrared Spectroscopy of Faeces to Evaluate the Nutrition and Physiology of Herbivores. *Journal of Near Infrared Spectroscopy* 17, 1–31 (2009).