Protocol for observational study on herbivore diversity

Isabel Barrio, Elina Kaarlejärvi

Herbivore diversity in tundra is changing[1]. Large-scale biogeographical studies report diversity of vertebrate herbivores in tundra to be positively related to plant productivity[2,3] and climate[1]. Similar positive associations of herbivore diversity with plant productivity have been reported at large spatial scales also for invertebrates, while at local scale invertebrate diversity is more strongly related to plant diversity[4,5]. For vertebrate herbivores, drivers of diversity at local scales remain poorly known and finer-scale data is needed to assess these relationships. Since different herbivores have different nutritional requirements[6], we can expect that at local scale herbivore diversity would be more strongly linked to productivity and nutritional composition of plant communities than to climate. Here we plan on collecting local-scale data on vertebrate herbivore diversity, plant biomass and plant nutrient content (C, N, P) to test the generality of these relationships both at local scale and across multiple sites in nutrient-limited and low-productive tundra.

In addition, we will collect faecal samples of different species of herbivores to explore species-specific nutritional stoichiometry (both related to nutrient requirements and output to ecosystem) and diet overlap as possible drivers of diversity in local herbivore assemblages. We will use chemical analyses for assessing C:N:P stoichiometry of different herbivore species, and DNA metabarcoding for identifying diets and diet overlap between different herbivores.

Study questions

Question 1: What is the relative importance of aboveground biomass, nutrient content of vegetation and climate as drivers of herbivore diversity at local site scale and across the tundra biome?

Question 2: Is herbivore diet overlap related to aboveground biomass, nutrient content of vegetation or plant community composition?

Question 3: Is nutrient content of herbivore faeces related to nutrient content of vegetation (C:N and C:P)?

You can participate in this observational study whether or not you are planning on establishing a long-term herbivore diversity experimental site (see *Protocol for the distributed tundra herbivory diversity experiment* for more details).

Timing of study and sampling

This observational study will be carried out once, during the peak of the growing season (late July or early August for most tundra sites in the northern hemisphere).

Spatial design across tundra: sites with two habitats

We welcome *sites* anywhere in tundra ecosystems, where some vertebrate herbivores are present. Sites will include **two *habitat* types**: the first representing the most fertile habitat in the landscape, used by different herbivores, and the second will be less fertile, but still used by some herbivores (**Figure 1**). Targeting two habitats will allow us test whether productivity and nutrient composition of vegetation are drivers of herbivore diversity both at site-scale and across sites. Optimally habitat fertility will be the main gradient separating habitats, so please minimize differences in other abiotic factors (altitude, slope, aspect …) between habitat types.

Within-site spatial design: three patches in both habitats

Within each habitat type in each site, we will select **3 relatively homogenous habitat *patches***, roughly 10 m x 10 m in size (since there are two habitats there will be 6 of these patches; **Figure 1**). Habitat patches of the same type should be located at minimum 400 m apart from each other to represent independent replicates from the point of view of small- and medium-sized herbivores. Note however, that the priority should be that patches of same habitat type are similar and distance between patches can be shorter than 400 m, if necessary to ensure similar vegetation, but always more than 100 m. Ideally a larger set of potential patches within both habitat types would first be identified (e.g., using field surveys or aerial photographs), and then three patches of each habitat type would be drawn randomly from this larger set.

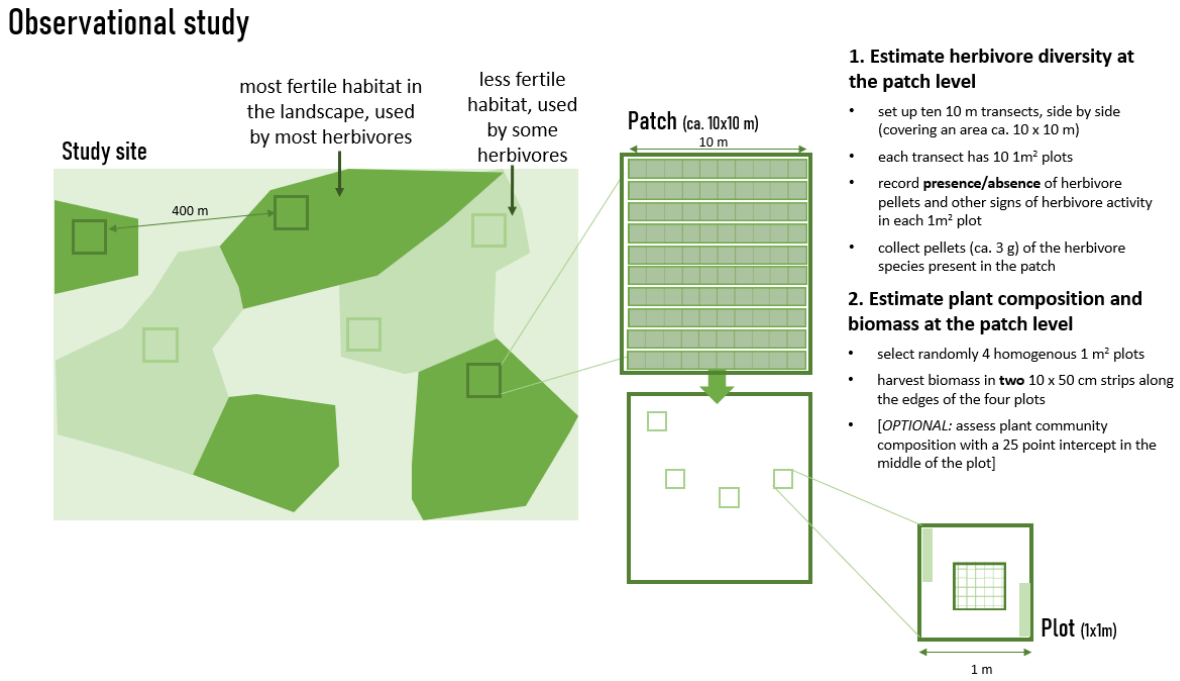

Figure 1. Sampling design. At your study site, you will select two habitat types used by herbivores. In each habitat you will select three homogenous habitat patches, roughly 10 x 10 m in size. At each patch you will assess herbivore diversity (in ten parallel 10 m transect, for a total of 100 1m^2 plots) and in four 1 x 1 m plots you will assess aboveground biomass (sampling two 10 cm x 50 cm strips per plot, 8 strips per patch) and plant community composition (optional).

Within-patch design: ten 10 m transects

We want to assess the diversity of the herbivore community that is using the area at the patch scale. The herbivore recordings proposed here serve two purposes: they characterize herbivore species richness at the patch level, and they allow us to compare prevalence-based abundances of herbivores within and between sites. By prevalence-based abundance we mean the fraction of all observation points within a patch, where a species was observed.

Assessment of herbivore diversity. We will set up ten parallel 10 m transects, side by side, to cover an area roughly 10 x 10 m in size (**Figure 2**). The shape of the area does not need to be perfectly squared, but the surface sampled has to be 100 1m^2 plots. This may sound like a lot, but we want to make sure we record enough signs of herbivory. You can temporarily mark the start and end points of the transects with flags. You can set up the transects sequentially: start setting up two transects, count pellets on them, and then move to the next pair of transects (**Figure 2**). Within each patch (where we are fitting the 10 transects) we will also randomly select 4 homogenous 1 x 1 m plots for additional

measurements (see below). It is good to mark the location of these plots first to avoid disturbing them while counting pellets along the transects), but you can also do this at the end, after recording data from the transects.

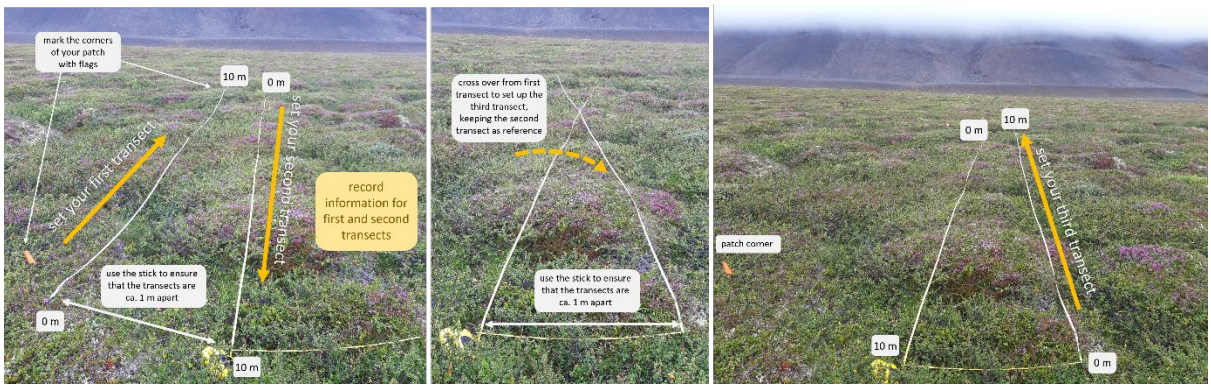

Figure 2. To set up your transects, start by setting up the first transect, marking the corners of the patch with flags for reference. Set up the second transect parallel to the first one, 1 m apart. Record pellets on these two transects, before setting up the next two transects. Cross over the first transect to the starting point of your third transect, keeping the second transect as reference, and then move the other end to set up the third transect parallel to the second one, and repeat the process to move the second transect to the fourth. Record pellets on transects 3 and 4, and repeat the process until you have 10 transects.

Walk down the transects inspecting 50 cm to each side of the transect, 1m^2 at a time (**Figure 3**). You may want to use a 1m stick or even a 1 x 1 m frame. In each 1 m^2 along the transect, record the **presence/absence of pellets, and other signs of vertebrate herbivore activities**, which you can identify at species, genus or functional group level. Examples of these signs of herbivore activity include lemming nests, browsing marks of reindeer or eating marks of grouse.

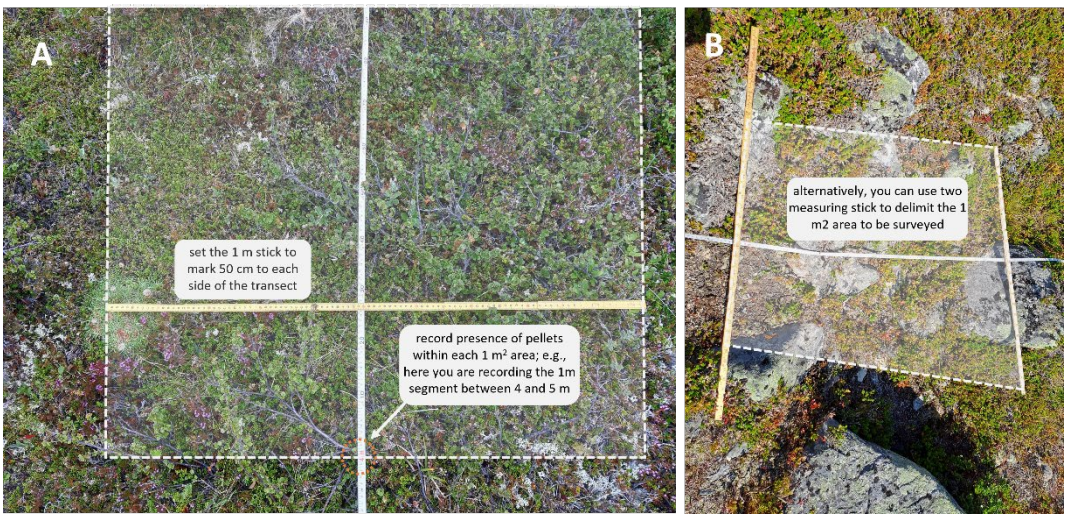

Figure 3. Use the 1 m stick as a reference to identify 1 m^2 areas along the transects where you will record the presence/absence of pellets and other herbivore signs. Using the transect lines as reference you can identify the 1 m2 area; for example in (A), you would be recording presence/absence of pellets in the segment between 4 and 5 m. Some teams found it easier to use two measuring sticks to delimit the 1m segments (B) – use the approach that works better for you! Use up to 10 seconds to detect large herbivore pellets, 10 seconds for small herbivore pellets and 10 seconds for other herbivore activity signs (if you detect them sooner, just move on).

We recommend starting with searching for pellets of large herbivores first, then taking 10 seconds in each 1m^2 plot to look for presence of pellets of small herbivores (this short time ensures that attention is focused on searching for pellets of small herbivores which might be more difficult to detect and gives some standardization of sampling effort across sites), and finally taking 10 seconds to search for other signs. Pellets in each plot will be identified to species or group level (e.g., reindeer vs. voles). In this way, each 1 m^2 plot should take you ca. 30 seconds, each transect 5 minutes, and the ten transects in the patch about 50 minutes.

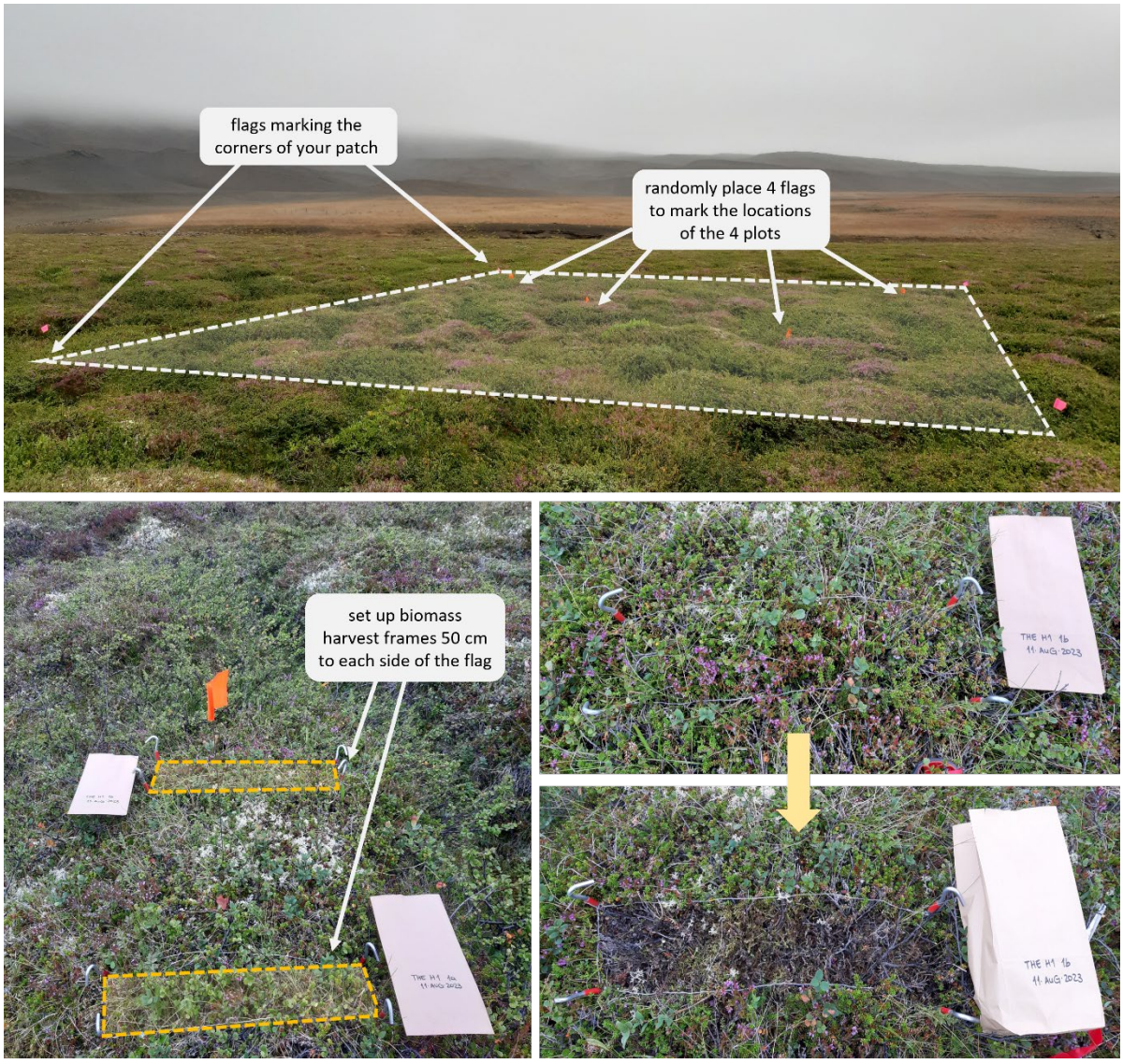

Figure 4. Randomly select 4 locations (homogeneous 1 m^2 areas) within your patch and mark them with flags. Set up your biomass harvest frames to each side of the flag. Harvest biomass and keep it in a paper bag (one paper bag per strip).

Collect a sample (ca. 3 grams) of pellets (preferably as a pooled sample of several pellet groups) of each herbivore species at each patch. Pellets should be as fresh as possible (although in many cases pellets will be relatively old). Please make notes when you are not certain about the species of herbivore producing the pellets[7], for examples when several species of herbivores present at your site produce morphologically similar pellets. Place pellets of different species in separate paper envelopes

(or similar; coffee filters or empty teabags work well too). Place each paper bag inside a separate ziploc bag, indicating the species (or species group, e.g. 'voles') of herbivore, patch ID, habitat, study area, and date. Place silica gel in ziploc bags to keep samples dry. Part of this sample (ca. 2 grams; to be used for nutrient analyses) will be dried in an oven at 60°C 48h when you are back in the lab, while the other part (ca. 1 gram[8]; to be used for DNA analyses) will be preserved dry with silica gel. Alternatively, keep pellet samples dry with silica gel for shipping (i.e., we will separate the parts for nutrient and DNA analyses in Iceland).

Assessment of aboveground biomass of vascular plants. Since we are interested in the local scale (patch), we need to collect biomass data at that scale. These biomass samples will also be used to measure nutrient content (see below). Biomass will be samples along the edges of the 4 randomly selected 1 m x 1 m plots described above (**Figure 4**). The aerial projection of aboveground vascular plant biomass, including woody species, is clipped on two 10 cm x 50 cm strips along the edges of the 1 m x 1 m plots, leaving the bottom layer (i.e. bryophytes and lichens) intact. Keep the samples from each biomass strip separate (i.e. in different paper bags; you will have a total of 8 biomass samples per patch). Biomass samples are oven-dried at 60 °C for 48 h when back in the lab and weighed to mg. If you have a bit of extra time, it would be great if you could sort out the biomass to vascular woody plants, vascular non-woody plants and non-vascular plants, and weigh these fractions separately.

Nutrient content of plant community After weighing separately the 8 biomass samples per patch, create a pooled subsample (ca 10 g in total), removing woody parts. In total, this yields 1 pooled nutrient sample per patch, three samples per habitat, six samples per site. These six samples are milled and shipped to Iceland for C, N and P analysis.

Plant community composition [optional]. To assess the implications of diet overlap between different herbivores (question 3) it would be very interesting to have information on availability of plant species. For example, diet overlap can indicate interspecific competition when the shared foods are scarce, but not necessarily when herbivores share an abundant resource. While an interesting question, collecting plant community composition data is *optional* as it requires some botanical skills; if you are running out of time, you can skip this part. We will assess plant species availability using the point intercept method. At each of the 4 homogenous 1 m x 1 m plots (as described above for biomass harvesting) place your 50 x 50 cm point frame with 25 pins in the middle of each plot. For each pin, we record two things:

1. Number of vascular species hits (1st hit and lower hits recorded separately for quantifying both cover and abundance, see Point_frequency_field_sheet). Please record vascular plants at species level (for nomenclature we follow The Plant List http://www.theplantlist.org). For sterile graminoids and other potentially challenging individuals, please look for flowering individuals for identification next to the plot. If not possible to species-level identification is not possible, use genus or functional group.
2. Species identity in the bottom layer (bryophytes, lichens, litter and bare soil). If species is not known, use for lichens: crustose, foliolose or fruticose; for bryophytes use the 12 functional groups defined in Lett et al. 2021 Arctic Science.

After recording all hits for all 25 pins, all species occurring in the 50 cm x 50 cm area, but not hit by any pins are recorded (mark 'X' in column 'first hit' on the Point_frequency_field_sheet) to get an estimate of species richness.

Materials needed

Herbivore diversity assessment:

- one (or several) 10 m tape measures to help set the 10 m transects
- 1 m stick or 1 x 1 m frame to count pellets in 1m^2 plots along the transects
- 36 flags (20 flags to mark the start and end of the 10 parallel 10-m transects, and 16 flags to mark the four randomly selected 1 m^2 plots)
- field sheet and pencil to write
- little shovel or spoon to collect pellets
- ziploc bags and permanent marker to collect pellets (the number of bags needed will depend on the number of herbivores in your site; say there are 5 species, then you will need 5*3 patches*2 habitats= 30 ziploc bags).
- small paper bags, envelopes or similar to store dry pellets (same number as for ziploc bags; coffee filters or empty teabags also work well)
- silica gel

Aboveground biomass sampling:

- 10 x 50 cm frame to harvest biomass (you can make this easily with large nails for the corners and metal wire for the sides)
- scissors and pruners to clip biomass to ground level
- paper bags to keep biomass (2 per plot, 4 plots in a patch, 3 patches in a habitat, 2 habitats → 48 per study site); choose paper bag size depending on how much biomass you expect
- permanent marker to label the bags

Nutrient samples:

- access to lab having a ball mill to mill samples
- back in the lab, you will need 6 pieces of ca 20 ml plastic vials to store and ship the milled samples, one for each patch.

Plant community composition:

- point frame (50x50 cm, 25 intercepts)
- field sheets (4 point frames per patch, 3 patches, 2 habitats → 24 sheets total)

Time estimate

In the field, if you have earlier selected your six patches, we estimate:

- mark five parallel 10 m transects: 15 min
- randomly select four 1 x 1 m plots along the transects, mark their corners with flags: 5 min
- record presence/absence of vertebrate herbivore signs in fifty 1 x 1 m grid cells: 50 min
- collect herbivore pellets: 30 min
- [optional: do point intercept assessments on the four randomly selected plots: 15 min per plot → 60 min (this depends on the vegetation at your site)]
- harvest biomass in 4 plots: 30 min per plot → 2 hrs (of course this depends on how much biomass there is at your site)

Time per patch: ca. 3.6 hrs (4.6 hrs if doing point intercepts) → 22 hours for 6 patches (28 hrs); walk between patches (>400 m): 10 min → 50 min between the six patches. **Total sampling time in the field: ca. 23 hours for one person** (29 hrs if doing point intercepts)

In the lab/office:

- preparation of field equipment
- planning of sampling sites (checking aerial photographs to select potential patches, etc)
- drying biomass samples
- milling 6 nutrient samples
- shipping pellet and nutrient samples to Iceland for analyses
- entering data herbivore and biomass data to database

Total work time in the lab and office: ca. 12 hours for one person

Authorship and collaboration

We anticipate two people will be participating per site (from the time estimates above, total time: 35 hrs / 2 persons per site → ca. 18 hours of work per person). Please be in touch if you foresee having a larger contributing team per site. Participants will be invited to contribute as co-authors in the resulting manuscripts, where they will be asked to help out with writing, data analyses and commenting on the manuscript.

Contact and additional information

If you have any questions, please get in touch with Isabel C Barrio (isabel@lbhi.is).

Please send your samples to:

Isabel C Barrio
Faculty of Environmental and Forest Sciences
Agricultural University of Iceland
Árleyni 22, 112 Reykjavík, Iceland

References

1. Speed, J. D. M. *et al.* Will borealization of Arctic tundra herbivore communities be driven by climate warming or vegetation change? *Glob. Chang. Biol.* **27**, 6568–6577 (2021).
2. Barrio, I. C. *et al.* Biotic interactions mediate patterns of herbivore diversity in the Arctic. *Glob. Ecol. Biogeogr.* **25**, 1108–1118 (2016).
3. Speed, J. D. M. *et al.* Trophic interactions and abiotic factors drive functional and phylogenetic structure of vertebrate herbivore communities across the Arctic tundra biome. *Ecography (Cop.).* **42**, 1–12 (2019).
4. Haddad, N. M., Tilman, D., Haarstad, J., Ritchie, M. & Knops, J. M. H. Contrasting effects of plant richness and composition on insect communities: A field experiment. *Am. Nat.* **158**, 17–35 (2001).
5. Ebeling, A. *et al.* Plant diversity impacts decomposition and herbivory via changes in aboveground arthropods. *PLoS One* **9**, (2014).
6. Sterner, R. & Elser, J. J. *Ecological Stoichiometry: the Biology of Elements from Molecules to the Biosphere*. (Princeton University Press, 2003).
7. Soininen, E. M. *et al.* Highly overlapping winter diet in two sympatric lemming species revealed by DNA metabarcoding. *PLoS One* **10**, e0115335 (2015).
8. Soininen, E. M. *et al.* Analysing diet of small herbivores: the efficiency of DNA barcoding coupled with high-throughput pyrosequencing for deciphering the composition of complex plant mixtures. *Front. Zool.* **6**, 16–25 (2009).