Distributed tundra herbivore diversity experiment (Tundra Exclosure Network, TExNet)

Version 2.1

Elina Kaarlejärvi, Isabel C Barrio, Eeva Soininen, James Speed, Dorothee Ehrich, Johan Olofsson, Ingibjörg Svala Jónsdóttir, David Hik

Goal

TExNet is a collaborative research network, which studies the impact of herbivore diversity on tundra ecosystem properties and functions by means of coordinated multi-site field experiment. We focus on vertebrate herbivores.

Background

Herbivores strongly shape diversity and functioning of tundra ecosystems (Bråthen et al., 2007; Cahoon et al., 2012; Gough et al., 2007; Jefferies et al., 1994; Manseau et al., 1996; Olofsson et al., 2004; Petit Bon, Gunnarsdotter Inga, et al., 2020; Zamin & Grogan, 2013). To understand drivers and predict consequences of biodiversity changes in rapidly warming tundra, we therefore need to consider herbivores: presence of different herbivore species across circumpolar tundra, and changes in their abundance over space and time. Cyclic dynamics and low diversity of herbivores is typical for tundra systems (Ims & Fuglei, 2005; Olofsson & Post, 2018), but also greater compositional changes of herbivore communities can happen, such as replacement of livestock with wild species in some areas (Speed et al., 2019).

Herbivore diversity in its multiple forms (e.g. species richness, evenness, identity of dominant species, functional diversity), regulates the impacts of herbivores on plant communities and functioning of ecosystems (Ritchie & Olff, 1999). Herbivore species differ in their habitat preferences, food choices and feeding styles leading to different impacts on ecosystem. Large herbivores are not able to feed very selectively due to their large mouths, but instead consume high quantities of plants, consequently with lower nutritious value on average (Müller et al., 2013). Smaller herbivores, in contrast, can be more selective, thanks to their smaller mouths, and target high-nutritious plants to fulfil their nutrient requirements (Clauss et al., 2013; Steuer et al., 2014). Consequently, removal of smallest herbivores should result in the greatest increase in nutrient-rich plants in the communities, while removal of large herbivores in the tundra, should benefit all types of plants regardless of their nutritional value.

These differences in diet and in ability to absorb nutrients (for example nitrogen (N) vs. phosphorus (P)) from food to body lead to variation in nutrients excreted by different herbivores (Roux et al., 2020; Sitters & Venterink, 2021). Thus, herbivores do not only alter plant community dynamics by consuming biomass, but they also modify plant available nutrients and nutrient cycling in ecosystems via their dung (Sitters et al., 2017). Plant growth in nutrient-poor tundra ecosystems is generally N-limited or co-limited by both N and P (Gough et al., 2000; Shaver & Chapin, 1980), but in the more productive habitats P can be the limiting nutrient (Giesler et al., 2012; Sundqvist et al., 2014). Theory predicts that dung of large herbivores should contain more N relative to P than dung of smaller herbivores, because large animals absorb relatively more P for sustaining their skeletons (Reynolds, 1977; Schmitz, 2020). If this pattern holds in tundra, it implies that relative more N-rich dung of large herbivores may have a greater positive effect on productivity and nutrient cycling in nutrient-poor N-limited habitats than in more productive P-limited habitats. In these P-limited habitats, relatively more P-rich dung of small mammals may have a greater impact on productivity and nutrient cycling than dung of large herbivores.

By excluding different sizes of herbivores from different tundra habitats, we will investigate their impact on tundra vegetation and ecosystem functions. By ecosystem functions we mean ecological processes that take place over time (Farnsworth et al., 2017; Garland et al., 2021; Manning et al., 2018). Some processes are fast and can thus respond rapidly to changes in herbivore pressure and concomitant shifts in abiotic conditions, for example flower production and enzymatic activities of soil microbes. Other processes take a growing season (e.g. decomposition, biomass accumulation) or from several years to decades (e.g. changes in carbon sequestration and soil nutrient pools (Stark et al., 2019). We will characterize herbivore diversity using multiple metrics: species richness, functional group composition, evenness and dominance.

Aims

Core study questions & resulting papers

These are only some core questions which this experiment aims to investigate. The standardized distributed design enables answering many other questions too, as well as designing add-on experiments to study specific questions.

Paper 1, data from year 0:

What is driving herbivore diversity at local site scale vs. across tundra biome? Is herbivore diversity associated with vegetation biomass, nutrient content of vegetation or climate both locally and across tundra?

> Hypothesis 1: We expect that climate explains variation in herbivore diversity at tundra biome scale, while vegetation biomass and nutrient content would be more tightly associated with herbivore diversity at local scale.
>
> Data 1: Total biomass, plant nutrients (C, N, P) and herbivore recordings. Climate data (temperature & precipitation from WorldClim). Also, data from external sources to account for hunting, management and possible other anthropogenic influences. This observational data can be collected anywhere without establishing an experiment. For more details, see Observational protocol for herbivore diversity.

Paper 2, data from years 2-3:

What are **short-term** effects of exclusion of different sizes of herbivores on fast ecosystem processes, such as flower cover, nutrient content of vegetation, decomposition and soil microbial enzyme activities? These are ecosystem processes, we expect to respond rapidly to changes in plant community composition, trampling and changes in external nutrient input via feces and urine (Garland *et al*. 2021).

> Hypothesis 2: Varies depending on function.
>
> Data 2: Measurements of flower cover, plant nutrients, decomposition (tea bags), potential enzymatic activities in soil in control and treatment plots.

Paper 3, data from years 0-5:

What are **medium-term** effects of exclusion of different sizes of herbivores on temporal stability of plant community composition?

> Hypothesis 3: Because different-sized herbivores feed primarily on different plant species, and because plant growth in tundra is resource limited (as opposite to light of water limited (Ritchie & Olff, 1999)), we expect that higher herbivore diversity may have compensatory effects on plant community composition by reducing competition between plant species (Holt, 1977; McNaughton, 1977). This means that We will investigate how different aspects of herbivore diversity (species richness, identity of dominant herbivores, richness of functional groups) influence the relationship between herbivore diversity and stability of plant community composition.

Data 3: Plant species abundance data using standardized point frequency method from year zero to year 5.

Paper 4, data from years 0-5:

What are the **medium-term** effects of exclusion of different sizes of herbivores on slow ecosystem processes, such as productivity and turnover rates of N and P in relation to their pools in soil?

Hypothesis 4: We expect that diverse herbivore assemblages will have greater impacts on ecosystem processes than less diverse herbivore assemblages.

Data 4: Plant biomass, soil samples. Any site, which provides data and samples on each these variables by November 2027 can participate, both with single and multiple habitats per site.

Timeline for papers

	Data from years	Data in database at latest by	Ms submitted
Paper 1	0	November 2023	December 2024
Paper 2	2-3	November 2025	December 2026
Paper 3	5	November 2027	December 2028
Paper 4	5	November 2027	June 2029

Rules

All participants agree on the experimental design and the core measurements (see below). All participants aim at maintaining the design and carrying out annual measurements for five years at their own cost. This entitles the authorship for max four scientists per study site. Note that annual sampling is required for paper 3. TUNDRAsalad project can contribute with a small amount towards the material costs in the first year. In future, the network can apply for funding for example for PhD students or postdocs to carry out more intensive sampling on participating sites.

Data management

All participants agree on delivering data and samples of the core measurements within three months after each field season to Agricultural University of Iceland to be stored in the database and analyzed in the lab. The data will be open for all participants. In addition to the agreed core measurements, the participants are encouraged to carry out additional non-destructive measurements on the study plots. The network would benefit from add-on experiments utilizing the standardized multi-site design.

Benefits for the participants

Value of this experiment increases with time. The distributed standardized design allows us to explore effects of herbivore diversity across tundra, but also tackle some unanswered ecological questions with a solid experimental setup. These kinds of results are likely to appear in high-quality scientific journals.

Max four researchers per experimental study site will become co-authors of the first paper given they deliver the baseline data (year zero) at latest by the end of November 2023. For the subsequent papers, we invite data providers to contribute to idea development, analysis or writing, i.e., data contribution alone is not enough. Also, for later publications, max four researchers per study site are entitled a co-authorship. Identity of these four researchers can vary between papers for example due to varying field teams.

EXPERIMENTAL PROTOCOL

Spatial design across tundra biome

We welcome sites anywhere in tundra, where some vertebrate herbivores are present. An optimal site would include two habitat types: the first representing the most fertile habitat in the landscape, used by different herbivores, whereas the second being less fertile, but still used by some herbivores. The second habitat could for example be the typical (zonal) habitat of the location as defined by the Arctic Vegetation Map (Walker et al., 2005). While we encourage researchers to use two habitat types, we realize the substantial cost and effort needed for sustaining such an experimental set up and carrying out measurements for five years. Therefore, we also welcome sites with just one habitat type (3 patches, 8 study plots in each patch, see below). This arrangement will allow us to compare variation in ecosystem responses to treatments across tundra by using habitat within each site as a replicate, but also across scales by calculating local-scale variation in responses by comparing responses between habitats in the subset of sites, which use two habitats.

Within-site spatial design

Within each site and habitat type, we select **3** homogenous habitat patches (minimum 10 m x 10 m; Fig. 1). It is utmost important that the three patches are as similar as possible (e.g. slope, aspect, soil moisture, fertility, vegetation). 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. However, if this distance would increase heterogeneity among patches, the distance can be shorter, but always larger than 50 m. The motive behind the distance between patches is to sample independent small and medium-sized herbivore assemblages. Ideally a larger set of potential patches would first be identified and selected (e.g. using aerial photographs and reconnaissance walks), and the three targeted patches drawn randomly from this larger pool of potential patches.

Within-patch design

In each habitat patch, we select and mark 8 homogenous 1 m x 1 m plots. Plots should be at least 2 m apart from each other. Try, however, to avoid the presence of a same plant ramet in several plots. The plots are randomly assigned to the treatments outlined below. In case a site lacks a given herbivore type, a treatment is not needed. *Note: before building the fences, read through the sampling plan for year zero below!*

- **Large:** Large-mesh sized fence (L-fence; regular livestock mesh size for example 30 cm x 10 cm) is built around six of those plots so that the lower edge of the fence is 30-40 cm above the soil surface allowing medium-sized herbivores like hares and ptarmigan to enter the plots (Fig. 1). This fence protects the smaller cages from disturbances, such as snow mobiles or large herbivores.
    - If this protection from disturbance is not needed, it is possible to build smaller L-fences around two separate L-plots.
    - A third alternative is to build the L-fence around the two L-plots, but having the other treatment plots outside this fence. Also in this case make sure you randomly assign plots to treatments, so do NOT select two closest plots to become L-plots.
    - Consult your local herders or large mammal specialists to decide whether it is a good idea (or potentially risky for example for reindeer calves) to lift the lower border of the fence up from the soil surface. If the mesh size is large enough, your medium-sized herbivores will go through the fence and then lifting is not needed.
- **Medium:** M cages prevent entrance of medium-sized herbivores (5 cm mesh size) but allow entrance of small mammals.
- **Small:** S cages exclude all mammalian herbivores (1 cm mesh size, + ca. 30cm skirt, Fig. 2).
- **Control:** unfenced plots. In case a large fence surrounds the small cages, place the control plots as close to the big fence, but not adjacent to it to avoid trampling.

These cages are at minimum 1.2 m x 1.2 m to allow some space around the 1 m x 1 m study plot. Their height should be high enough to protect even the tallest species over the years. The top of these cages is covered with the mesh used for their walls to exclude entrance of ptarmigan, geese and small mammals. The cages will be attached to the ground with metal pins. Each plot is marked with respective plot code on a metallic plate attached for example with a long nail in the south-eastern corner of the plot (Fig. 5).

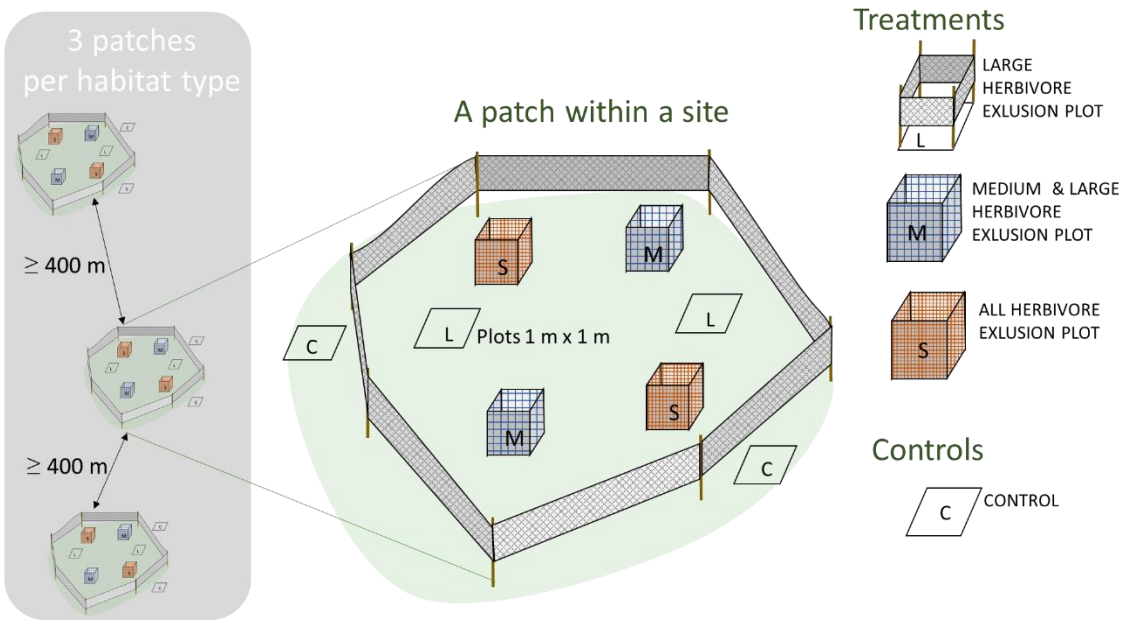


Figure 1. Experimental design within a site consists of three patches replicated within one habitat type. Each patch contains two replicates of each treatment and two control plots. This yields 8 plots per patch and 3*8=24 plots per habitat. The large fence protects smaller cages from potential damages from for example snowmobiles or large herbivores. However, it can take any shape, or if you expect no external disturbances, you are free to build two separate large-mesh fences around the L plots.

Figure 2. Example of a S cage with a skirt preventing small mammals' entrance. This cage has an openable side, it is attached to the ground with poles. See below drawings for another cage model, which you turn on its side, when working on the study plot (Figs 3-4). The skirt is hammered down so that it follows the ground as tightly as possible, and it is attached with screws, tent pegs or U-shaped solid metal wire pieces. Stones are here used as additional weight in the corners where the skirt of two sides overlaps. Too many stones may attract small mammals under the skirt. Photo by Eeva Soininen.

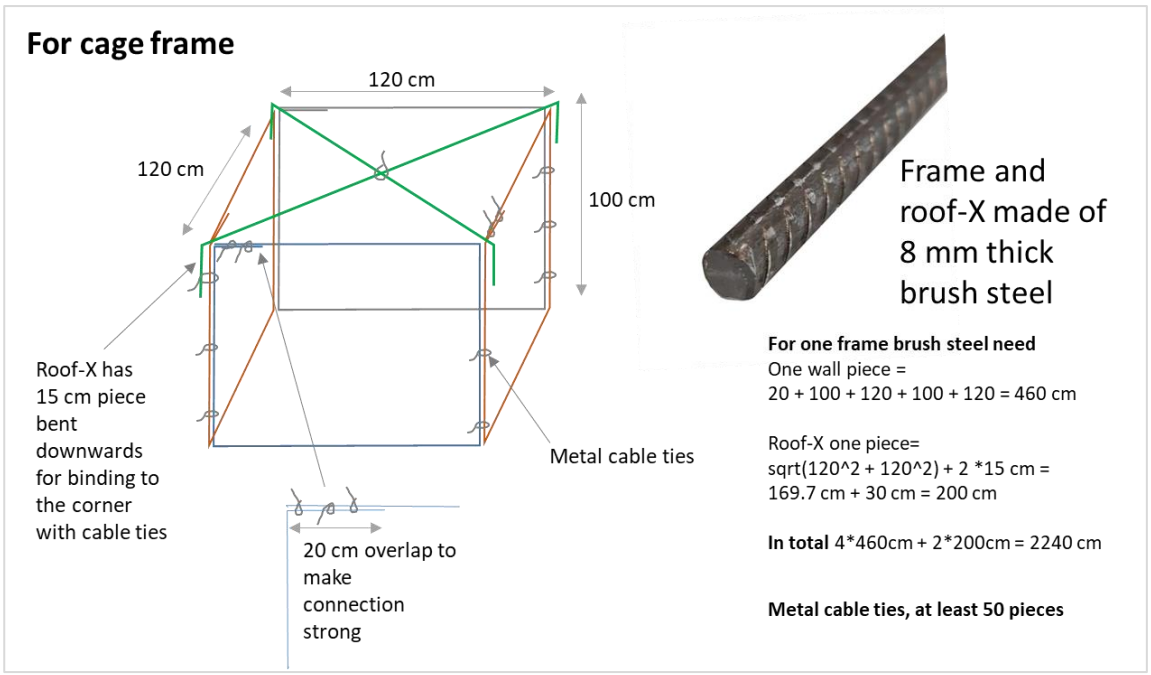


Figure 3. An example of a cage frame and material needed.

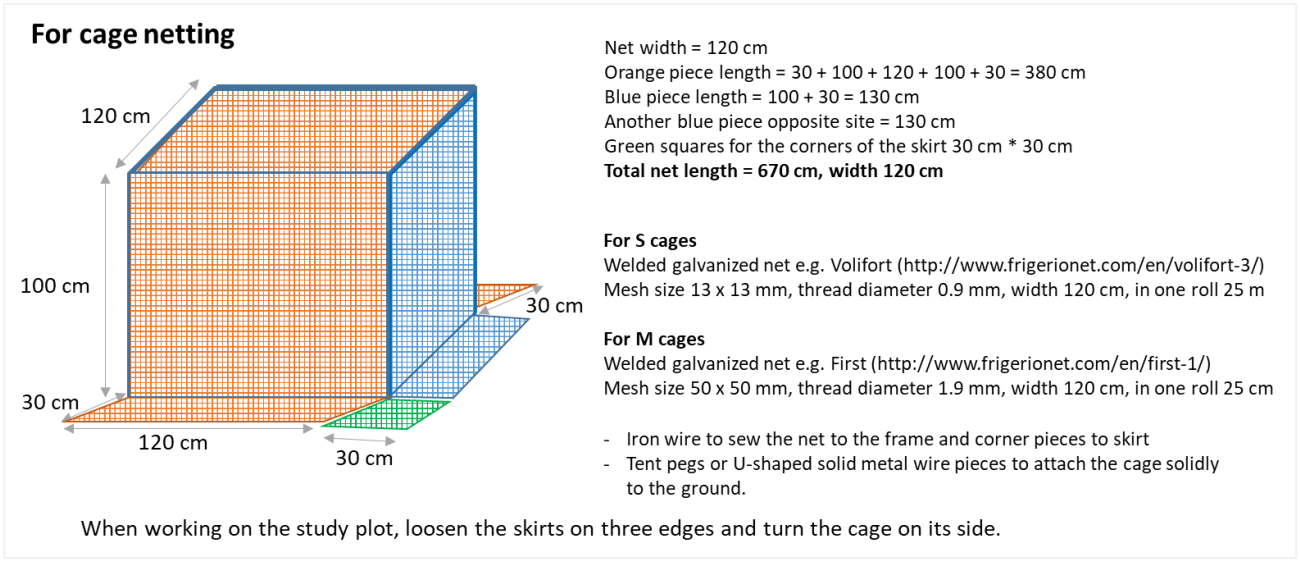


Figure 4. Specifications of S and M cage nets and suggestion for cutting.

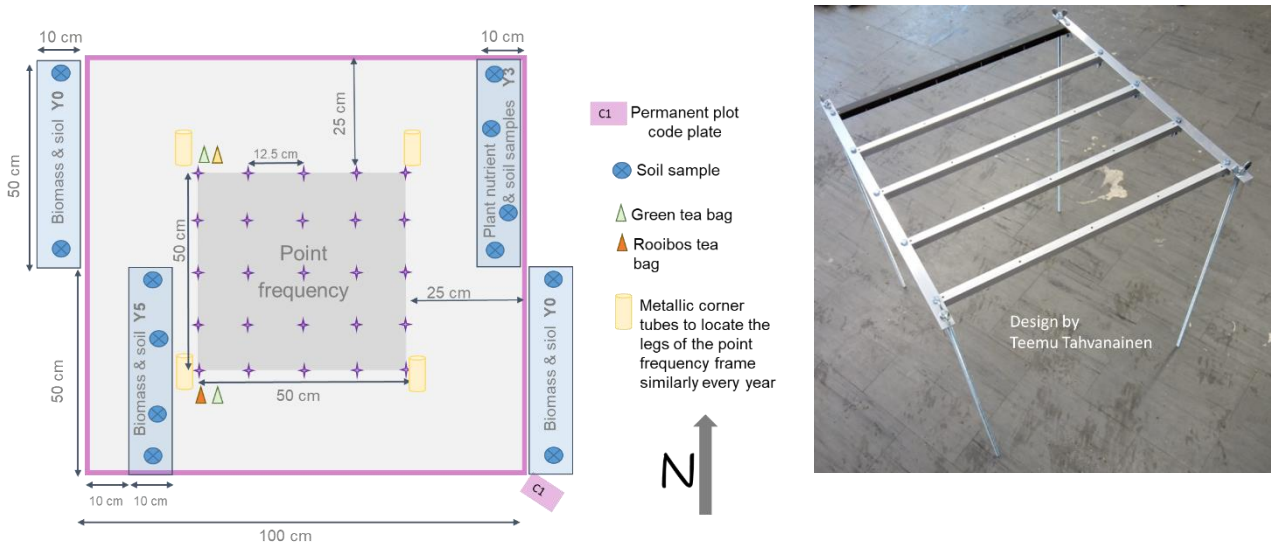


Figure 5.a) Schematic illustration of a 1 m x 1 m study plot. The central 50 cm x 50 cm area is dedicated to the annual plant abundance and richness survey and should be kept intact. The 25 cm edges can accommodate add-on measurements and destructive sampling (for biomass and soil) in different years. The teabags are placed in the illustrated locations so that they do not disturb the central point frequency area. If these locations are too stony to be used, any other position outside the central point frequency area can be used. **b)** An example of a point frequency frame. Each participating site will receive a tailor-made frame, pins, and metal corner tubes from TUNDRAsalad project.

Sampling plan for different years

Year zero

Year zero refers to the growing season, when the fences will be built. Alternatively, if the fences were built in the autumn, year zero is the following growing season, when the first measurements are taken. The sampling should take place at the peak of the growing season optimally just before building the fences. In year zero, you have the possibility to contribute to the observational study on herbivore diversity with very little extra effort, and we strongly encourage you to do so, as this study will provide valuable baseline data, comparable across a larger number of sites. The four steps outlined in Figure 6 describe how to accommodate the observational herbivore diversity survey into your experimental patches in year zero.

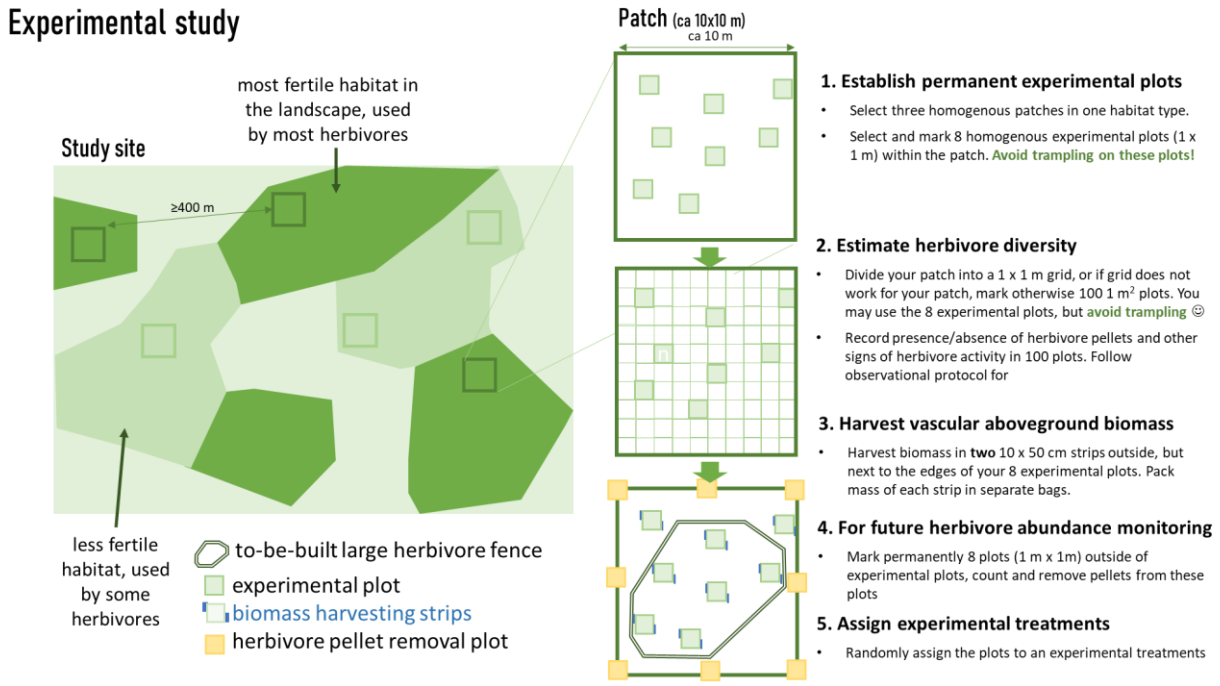


Figure 6. Illustration of a site and description of the first practical steps within a patch.

Basic information, only collected in year zero (needed as background information)

Take coordinates and photos of each plot in year zero. A photo should include the 50 cm x 50 cm central area, with the metallic corner tubes or the frame showing the edges of it, and as little extra area as possible. The photo should also include a plot label, written for example on a wipe-clean book. Also take a general photo of each patch. Record aspect (N, NE, E, SE, S, SW, W or NW) of each patch using a compass. Measure slope angle (0-90°C) of each patch using clinometer app on smartphone.

Recordings of herbivores (needed for year zero paper AND for long-term data)

Herbivore recordings serve three purposes: first, they characterize herbivore richness at patch level; second, they allow us to compare prevalence-based abundances of herbivores among sites; and they measure interannual variation in herbivore prevalence-based abundance at patch level. By prevalence-based abundance we mean a fraction of all observation plots within a patch, where a species was observed. Herbivore recordings will be done at two levels: an initial assessment of herbivore diversity (only in year zero, coordinated with the observational study) and long-term monitoring of herbivore abundance (from year zero on).

Initial assessment of herbivore diversity. This part is common to the observational study and will be only conducted in year zero. After marking your experimental plots (to make sure you do not trample on them), divide your ca 10 m x 10 m patch into 1 m x 1 m grid (Fig. 6). You can temporarily mark the grid with flags. If grid does not work for your patch, mark otherwise in total 100 plots including your 8 experimental plots. You may want to use transects, but please stay within the same habitat type. In each plot, record presence/absence of pellets, feeding and nesting signs of herbivores, which you can identify at species, genus or functional group level. For example, nest of lemming, browsing marks of reindeer or eating marks of grouse. We recommend starting with searching the pellets of the large herbivores first, and then taking 10 seconds in each 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 for searching feeding and nesting signs. Pellets in each plot will be identified to species or group level (e.g., reindeer vs. voles).

Long-term monitoring of herbivore abundance. In year zero, mark 8 1 m^2 plots permanently for long-term herbivore recording. Count and remove pellets from these 8 plots. In subsequent years, use these 8 plots and the above-described method (large pellets + 10 sec + 10 sec) for recording presence/absence of herbivore signs. Pellet counting and removal will take longer time in the first visit, but it will take shorter time in subsequent years, as not so many pellets will have accumulated.

If you have the possibility to equip your patches with a game camera, audiomoth or camera-trap, that would be very cool! Those devices would yield higher accuracy of data on herbivore species and also on their abundances.

Plant and lichen species abundance

Plant and lichen species abundance is recorded in the center of each plot using point frequency method. We use a standardized frame of 50 cm x 50 cm with five systematically arranged rows of five pins (Fig. 5b). 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). Whenever possible we encourage you to work at species level, if you can maintain the same accuracy over the years at your site, since this kind of long-term datasets are very valuable. For nomenclature we follow The Plant List (http://www.theplantlist.org). If species level identification is impossible, aim at genus level. If genus level is impossible, use the pre-defined functional groups specified in the 'Functional_groups_vascular_plants'. Please do not invent your own group taxa.
2. Species identity and depth in the bottom layer. We record possible hit by bryophyte and lichen species, litter, and bare soil. Note, that there might be more than one hit per pin in the bottom layer, please record them all, for example a bryophyte and a lichen. Always, when you know species name, record it. If you do not know species name, use following functional groups:
    - For lichens: crustose, foliolose or fruticose
    - For bryophytes: 12 functional groups defined in Lett *et al*. 2021 *Arctic Science* Note that you need to familiarize yourself with these groups **before** going to field.

    If no bryophyte or lichen hit the pin, record **either** bare soil **or** litter.

    As a proxy of bryophyte and lichen abundance, we measure depth of bryophyte-lichen layer. We use wooden grill stick with pre-marked centimeters. You will receive a set of these sticks with the frame. Wood creates more resistance than a metal pin, allowing you to "feel" the bryophyte/lichen-soil interface as an increased resistance. At each pin, we do one height measurement of bottom layer and record it in the bottom of the Point_frequency_field_sheet either for bryophyte or for lichen, whichever seems more abundant at the pin. Measure depth at 0.5cm accuracy.

Species richness (needed for long-term data)

After recording all hits for all 25 pins, the frame is removed and all species occurring in the central 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. Even if some species were registered at genus or functional group level for abundance data, aim at registering their presence at species level. Here a good trick is to search similar looking but more mature or flowering individuals outside but next to the plot. You can collect a herbarium sample of those individuals, record plot and genus or functional group you used when recording this 'species' and bring it to your institutes botanists for identification.

Litter layer depth (needed for long-term data)

Depth of the litter layer is measured in the four corner pins of point frequency grid to 0.5 cm accuracy. We define litter as dead plant material consisting only of separate plant parts, like leaves or branches, no soil. This is opposite to top soil, where partially decomposed plant parts can be found in between moder/soil. Note that living bryophyte or lichen cover is not considered as litter. Litter can lay on top of the bryophytes, or depth of litter can be zero. Record values on the Point_frequency_field_sheet.

Biomass sampling (needed for year zero paper AND for long-term data)

Aerial projection of aboveground vascular plant biomass is clipped on two 10 cm x 50 cm strips in Year zero and in one strip in Year 5 (Fig. 5). We focus on vascular plants and leave the bottom layer (i.e. bryophytes and lichens) intact. In Year 0, keep the samples from each biomass strip separate (*i.e.* in different paper bags; you will have a total of 8*2 = 16 biomass samples per patch). Biomass samples are oven-dried at 60 °C for 48 h, or longer until dry, and weighed to mg. Note here that we want dry weight separately for each of the strips in Year zero!

Community-level nutrient samples: After weighing the dry biomass samples, remove any woody stems, as we are interested in leaves, which are the main food of herbivores. Merge the two biomass samples originating from the same plot in a bigger bag and using scissors homogenize the sample. After homogenizing, collect a subsample of ca 100 ml of the biomass per plot, mill it with a ball mill (if possible), label with the plot code and ship to Iceland for C, N & P analysis.

Please note that this is different from observational protocol! We want to measure nutrients for each experimental plot separately to have plot-specific baseline data for temporal changes of nutrients.

Soil sampling (needed for long-term data)

Using a soil corer of 2-3 cm diameter, four soil samples are collected in specific strip(s) in each study plot as illustrated in Fig. 5. In Year 0, these are divided to two biomass sampling strips. In Years 3 and 5, we sample 4 cores within one 10 cm x 50 cm strip. We focus on top 0-5 cm of soil, because it contains most of the roots and microbes. This upper 5 cm can include both organic and mineral soil, it is not a problem. In the lab, we quantify the proportion of organic matter and report all results (e.g., total N content) standardized per organic matter.

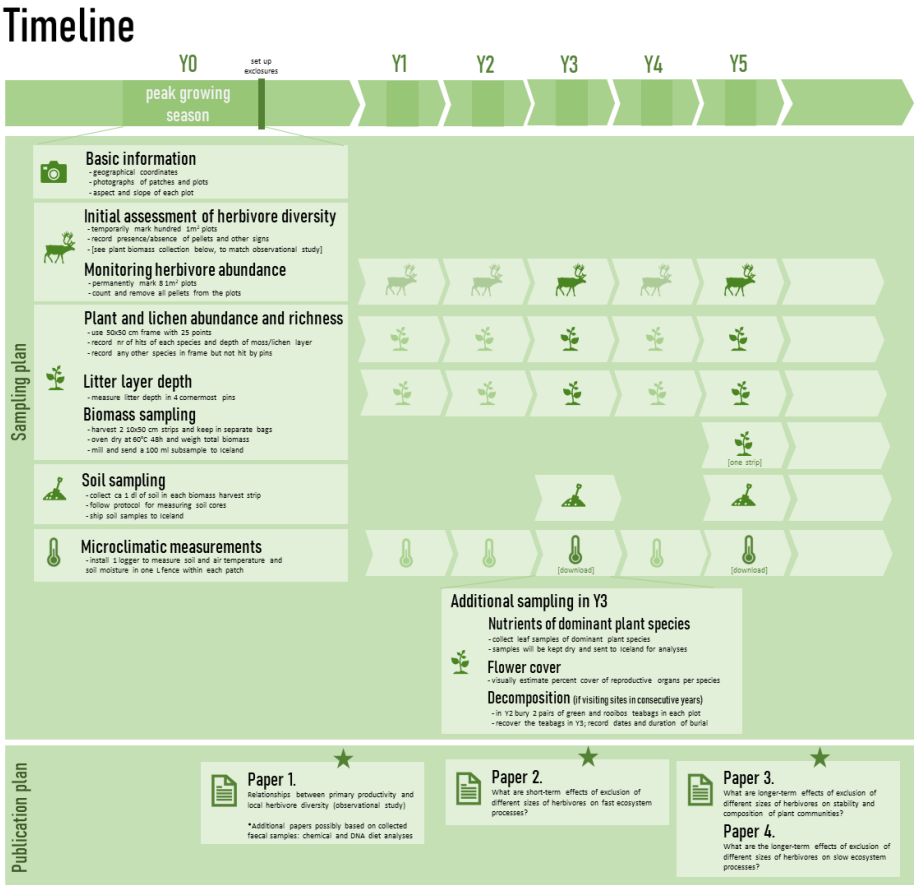
- Measure and record the diameter of your corer! We need this for calculating the sampling area.
- Remove vegetation and litter layer, core until ca 10 cm depth. Using ruler, collect top 0-5 cm of the core (Fig. 5). Record the collected depth with 0.5 cm accuracy on the Soil_sampling_sheet. In the optimal case the depth is 5 cm, but in stony ground it can be less. We need the depth for calculating sampled volume.
- Place the collected part of the core in a plastic bag. Take the next sample. Again, record the collected depth.
- After collecting 4 cores, evaluate total volume of your pooled sample the bag. If it is clearly less than 1 dl, collect more cores within current year's sampling strip(s) until you reach ca 1 dl total volume. Record the depth of each collected core! For example, if the soil is stony, you might be able to collect < 5 cm cores and need more than four cores to reach in total ca 1 dl.
- Label the bag with the plot code. Close well.

- If possible, store soil samples frozen. Otherwise, let soil dry in room temperature. Ship to Iceland.

Figure 7. A soil core showing the top 0-5 cm part we collect. Here the top 5 cm includes both organic (dark) and part of mineral (light brown) horizons. This 'soil sampling tube' is from nhbs.com in UK.

Microclimatic measurements (preferred for long-term data)

To quantify soil and near-surface air temperature and moisture at each patch, we use one Tomst TMS-4 or similar logger per each patch. TUNDRAsalad provides Tomst loggers for each experimental site. To protect loggers from potential large herbivore or other damage, we place the logger inside the L-fence, but out of the study plot area. Later, potentially in Year 2, we will compare effects of different fencing material on snow accumulation using soil temperature loggers in one S cage, one M cage and in a control plot within each patch.



Annual sampling

Annual sampling means long-term measurements, which will contribute to paper 3 'Long-term effects of treatments on stability of plant community composition'.

- ✓ *Recordings of herbivores*, as described for long-term monitoring of herbivore abundance in year zero.
- ✓ *Plant and lichen species abundance and richness,* as described for year zero.
- ✓ *Litter layer depth*, as described for year zero.

Sampling in year 3

- ✓ *Recordings of herbivores*, as described for long-term monitoring of herbivore abundance in year zero.
- ✓ *Plant and lichen species abundance and richness,* as described for year zero.
- ✓ *Litter layer depth*, as described for year zero.
- ✓ *Nutrients of dominant plant species*

  In year 3, we collect leaves from vascular plant species, which together make up at least 80% of aboveground biomass in the plot. For defining those species, we use either raw point intercept data from the same plot in year zero, or possible regression coefficients to convert them to biomass. We collect 5 fully developed undamaged leaves from each of those species per plot prioritizing the 10cm x 50cm strip devoted to this sampling (Fig. 5), and if not enough leaves found there, extending the sampling area to whole plot excluding the central point frequency area. We place the 5 leaves per species per plot in a small paper bag, press them dry in a plant press and within next 3 days dry them at +60°C for 48 hours. After drying the samples can be stored in their original paper bags at room temperature until NIRS analyses (Murguzur et al., 2019; Petit Bon, Böhner, et al., 2020)
- ✓ *Soil sampling*

  In year 3, sample soil as described for year zero, but only in one 10cm x 50cm strip, see Fig. 5. Please use the same strip as for nutrient sampling.
- ✓ *Flowering of insect-pollinated species*

  In year 3, we visually estimate areal percent cover of flower buds, flowers, senescing flowers or fruits per species in the central 50 cm x 50cm point frequency area. Here we are interested in the effect of treatments on flower production, so please estimate the cover of reproductive organs only, NOT the total cover of species (including leaves & stems). In total this estimate can exceed 100%, if reproductive organs of different species are overlapping. We ignore here wind-pollinated species, such as graminoids.

  Make use of the point frequency frame, which consists of 16 little squares: each square, defined by the pin holes, covers 6.25 % of total area. Quarter of a square is ca 1.5%, two squares make up 12.5% etc. If total cover of reproductive organs is smaller than a quarter of a square, we record it as 0.5%. We aim at 1% accuracy with help of the grid. This yields continuous data, which is easier to analyze than categorical data. Record values to Flower_cover_sheet.

- ✓ *Decomposition (if visiting the sites in consecutive years)*

  In year 2, bury 2 pairs of green and rooibos tea bags, 5 cm depth, in each plot (locations shown in Fig. 5). Mark the location with a colored pin /tag. Dig tea bags carefully up the ***following*** summer (year 3). Note the dates and duration of the burial. See the separate protocol for handling the teabags.

Sampling in year 5

- ✓ *Plant species abundance and richness*
- ✓ *Litter and bottom layer depth*

- ✓ *Biomass sampling*, as described for year zero, but only on one 10 cm x 50 cm strip, see Fig. 5.
- ✓ *Soil sampling*, as described for year zero, but only on one 10 cm x 50 cm strip, see Fig. 5.

Estimate time use

Attachments

- Functional_groups_vascular_plants
- Point_frequency_sheet
- Soil_sampling_sheet
- Tea_bag_sheet (to be done)
- Flower_cover_sheet (to be done)

References

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