

Measuring background invertebrate herbivory in the tundra

Invertebrate herbivory in tundra ecosystems has received little attention¹ and effects of foliar losses to invertebrates on tundra plants have been generally neglected². Most attention has focused on extensive defoliation events during outbreaks in the forest-tundra ecotone³, but we know little about background (non-outbreak) herbivory levels in tundra⁴, despite the relevant effects it has in other systems⁵.

The goal of this document is to provide guidelines for assessment of the occurrence and intensity of invertebrate herbivory at different tundra sites. In this initial assessment (summer 2015), samples will be collected from the field from a large number of tundra sites, and leaf damage will be assessed in a common lab by the same observer. This information will allow a quantitative evaluation of invertebrate herbivory, providing information to address the following questions:

- ✓ What are the average levels of background invertebrate herbivory in tundra?
- ✓ Is invertebrate herbivory similarly prevalent across tundra sites?
- ✓ Which plant species suffer most from invertebrate herbivory?
- ✓ How variable is the occurrence of invertebrate herbivory in tundra at different spatial scales?

Most studies investigating invertebrate herbivory have focused on leaf damage⁶. Signs of other types of invertebrate herbivory are not as easily recognizable (e.g. sap feeders, root herbivores) or sampling may require more intensive protocols (e.g. floral herbivory and seed predation). Although the effects of these herbivores might be as relevant as those of folivores^{7,8}, for a preliminary assessment of invertebrate herbivory in tundra we will focus on the impacts of herbivores that cause leaf damage.

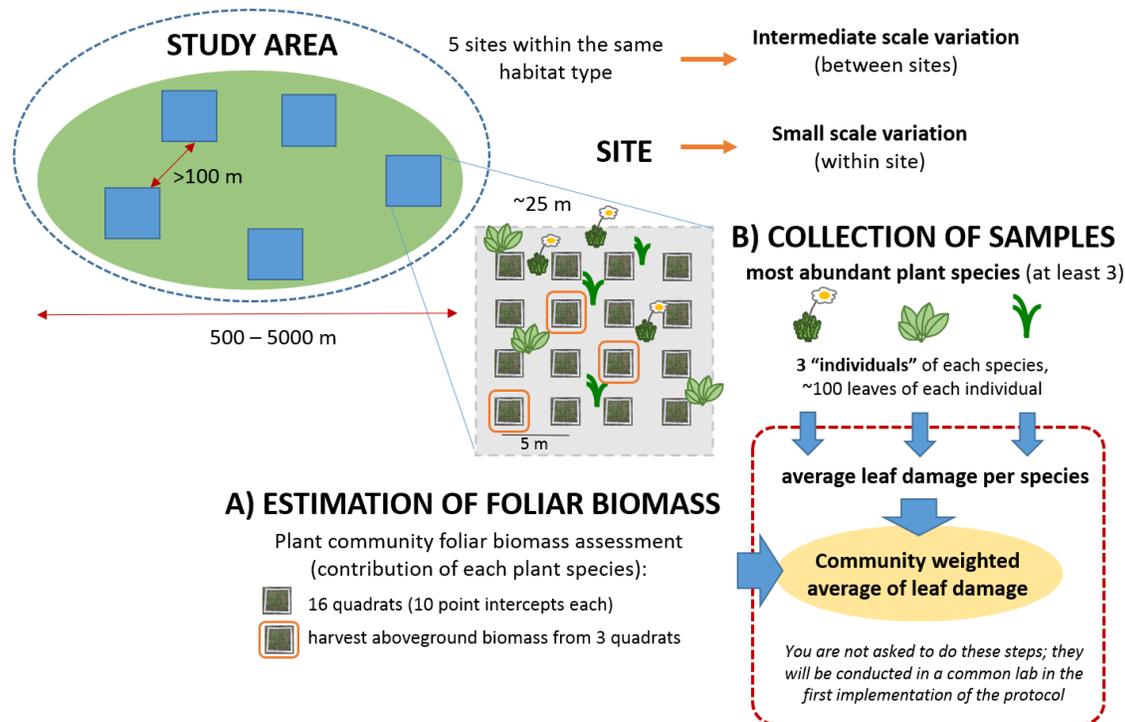


Figure 1. Study design for assessing variability in invertebrate herbivory at two spatial scales, between and within sites.

Information collected in this study will contribute to the development of a more general protocol to measure invertebrate herbivory in tundra, but also represents a standalone project. All contributors will be welcomed to co-author the resulting publication. This first assessment will focus on **spatial variability** of leaf damage; temporal variability, with large annual variation in herbivory (e.g. ^{8,9}), will be accounted for in future implementations, by conducting annual observations over several years. For the assessment of spatial variability and to calculate sample sizes needed to capture this variation more accurately in the future, sampling in the first implementation (summer 2015) will be structured within a study area, in a minimum of 5 sites (intermediate scale variation) within a common habitat type, sampling plants of the most abundant species within each site (small scale variation; **Figure 1**).

Study areas are loosely defined here at a scale of hundreds of meters to a few kilometers (i.e. areas separated more than 5 km apart would be considered different study areas); study area is, broadly, the place where you conduct your research. Within each study area, sampling will focus on the most dominant, typical habitat type (i.e. zonal habitat, *sensu* CAVM: areas where the vegetation develops under the prevailing climate, uninfluenced by extremes of soil moisture, snow, soil chemistry, or disturbance¹⁰). We suggest to sample in at least **5 sites** (corresponding to the same habitat) that will be at least 100 m apart. Sites will have a size of approximately 25x25 m – please note that this size is indicative and it is intended to give an idea of the spatial scale at which the variation will be accounted for. Please write down the habitat type and **take pictures** of all 5 sites! In this first assessment of invertebrate herbivory, sites should NOT be linked to existing monitoring plots, especially because sampling will be destructive (we are asking you to collect a lot of leaves! So probably you do not want to do that in your study plots). Sites should correspond to the same habitat type. Then, in each study area, the focal species (see below) will probably be the same in all 5 sites. You are more than welcome to include additional habitats, but each habitat type should then be represented by at least 5 additional sites.

In each site, you are asked to do two things:

- A) **Estimate foliar biomass at the community level.** Plant community assessments accounting for foliar biomass of each species need to be conducted for each site to allow calculation of the community-wide losses of foliage to invertebrate herbivores from the species-specific data. Plant cover will be estimated using the point intercept method¹¹, using 10 pins in a fixed position within a 50x50 cm frame, where all plant species that hit the pins will be recorded. For the three focal species it will be recorded if each hit corresponds to leaves or woody parts. 16 frames will be placed in a regular grid at each site, 5 m apart, to cover a 20x20 m area (**Figure 1 and overview**). From 3 of these frames aboveground biomass will be collected. These frames should ideally represent a range in abundance of the focal species (i.e., minimum, maximum and average hits) because we will then need to calibrate the number of hits to the foliar biomass of each species, so the broader the gradient we can get, the better – but if this proves to be too complicated to assess in the field, a random selection of 3 of the 16 frames will do. Live green biomass samples from each frame (it is extremely important to label uniquely the point intercept recording and the corresponding biomass collection!) will be sorted by species (at least for the three focal species) and dried in an oven (~70°C, 72 h), and packed together with the rest of the samples for shipping.
- B) **Collect leaf samples of the most abundant species.** A number of focal **plant species** (minimum of 3) will be identified based on their relative contribution to foliar biomass in each site. The most abundant plant species (the one contributing the most foliar biomass at each site) should be selected first, followed by the second and third most abundant. The more species per plot the better community estimates we will have, but the minimum is **three**; importantly, selection of focal species should not be biased towards plants receiving higher-than-average levels of foliar damage¹². For example, some species of arctic plants (e.g. *Salix* spp.) carry a disproportionate number of herbivorous species¹³. From each plant species in each site, samples from 3 individuals will be collected. Defining “individuals” in the tundra can be tricky, because many plants are clonal; however, plant genotypes vary in their susceptibility to insect herbivory

and therefore it is important to sample whenever possible several genotypes per site, at least at this test stage. For our purposes, individual plants (genets) can be defined as plants separated at least 10 m. For plants with smaller stems (e.g. *Vaccinium vitis-idaea* and *Salix rotundifolia*) or with less leaves per individual, several stems living close together (e.g. within 1-2 m) can be aggregated to collect ~100 leaves from 3 different “aggregates”. Individual plants (or aggregates) should be “blindly” selected from a distance that prevents recognising foliar damage by insects (e.g. 5-10 m)¹²; a number of individuals of each species in each site might be temporarily located using flags and then, from a distance, randomly select 3 flags and sample the corresponding individuals. Please try not to bias your sample to a specific height of the plant above the ground when you are collecting leaves (i.e. sample across all heights). Leaf damage levels in tundra are low (<1% on woody species⁴), so a relatively large sample (i.e. sufficient nr of leaves) needs to be collected for each individual. Based on a previous study reporting background herbivory levels in tundra⁴, we expect that less than one leaf out of 100 might be completely eaten (although most likely we will observe minor damage in a few leaves), so we will need to collect ~100 leaves from each individual to estimate damage accurately. Leaf samples can be temporarily stored in labelled plastic bags (labels should include information on study site, patch, plant species and plant individual), and be processed for preservation back in the lab. In this way, field sampling is not as time consuming, as entire plants or branches are collected in the field.

Leaf samples should be preserved as herbarium specimens (pressed and dried, to avoid growth of mould). Newspaper used for pressing plants needs to be changed daily in the first couple of days to ensure samples are kept dry. For plants with relatively large leaves (leaf size of *Betula nana* or greater), leaves should be detached from the stems before pressing, so that leaf damage can be easily identified on flat, unfolded leaves. Species with needle-like leaves (e.g. *Empetrum nigrum*, *Loiseleuria procumbens*) tend to have early abscission of leaves after damage, and this can be assessed by scars left on twigs after leaves are dropped; for these species it is therefore important to keep leaves and stems together when pressing them.

Pressed samples will be shipped to the University of Iceland and processed in the lab by the same observer (ICB). So, up to here, it is what you are asked to do; but if you are curious on what will happen to the samples after you ship them (in the red box in **Figure 1**), please continue reading 😊

In the lab, leaves will be carefully assessed for damage on both sides and through a source of light (some damage types are not so obvious to the naked eye). Each leaf will then be attributed to a damage class according to the proportion of area of leaf lamina that is consumed or damaged by insects: intact leaves, 0.01-1%, 1.01-5%, 5.01-25%, 25.01-50%, 50.01-75%, and 75.01-100%. Most foliar damage in tundra is imposed by leaf chewers, followed by gallers (the incidence of leaf miners, if any⁴, is very low), and evidence from leaf damage by each group will also be recorded separately when possible. The numbers of leaves in each damage class will be recorded, and for each individual plant the following measurements will be calculated:

1. **Proportion of damaged leaves:** ratio between the number of damaged leaves and the total number of surveyed leaves (separate measurements can be calculated for defoliators, miners, gallers and all herbivores combined)
2. **Average proportion of leaf area lost to insects:** the number of leaves in each damage class is multiplied by the respective median of the damaged leaf area (0 for intact leaves, 0.5% for the damage class 0.01-1%, 3% for damage class 1.01-5%,...); the obtained values are summed across all damage classes and divided by the total number of leaves (including undamaged ones) within a sample.

From the dry weights of the biomass samples collected, we build a calibration between the number of pins recorded in the field with the point intercept method and foliar biomass, to estimate the relative contribution of

each plant species to community foliar biomass. Community estimates of biomass loss can be then obtained by doing a weighted average for the foliar biomass removed by invertebrates on the dominant species in the community.

Additional information that can be collected opportunistically...

Additional information on unusual situations or ecological surprises related to invertebrate herbivory is also highly needed. Although the focus here is set on describing “typical situations” of invertebrate herbivory, opportunistic information on extremes is also highly valuable. If you happen to observe an unusually high level of plant damage, then please (a) take a photograph of the damage type and the damaging invertebrate, whenever possible; (b) preserve the damaging invertebrate in alcohol for future identification; (c) estimate the spatial extent of the excessive damage; and (d) collect leaf samples from at least 5 plant individuals both within and outside the ‘exceptionally damaged’ area.

Our knowledge of Arctic entomology is still relatively limited¹⁴, so it is also very interesting to know who is actually consuming the leaves. Whenever possible, please take pictures, collect and preserve insects seen on plants, along with a damaged part of the plant, and record if the insect was actually feeding on the plant or just sitting on it. When sufficient amounts of specimens are accumulated, we will transfer them to relevant taxonomists. In particular, aphid samples from tundra are especially needed and will be processed in the first line. Knowing the feeding habits of the invertebrates that are most commonly observed to damage plants in your study areas will help develop the protocols further.

Timing and time commitment

Invertebrate herbivory data should be collected once at the end of the growing season (cumulative signs of herbivory might be better assessed later in the season, before plant senescence). No big differences between mid and late summer in insect herbivory are expected^{4,9}. We expect sampling to take **one day of work in the field** per study area for one person over the whole season (max up to two days). A rough estimate of time dedicated to each activity (needs to be adjusted to each site):

- Estimates of community foliar biomass: 16 point frames and collection of biomass in 3 50x50 cm frame ~60 minutes in the field per site: ~5 hr in the field
- Collection of leaves (search of individual plants of each focal species in a patch and collection of 100 leaves per plant): 1 hr per plot, ~5 hr
- Processing and preserving samples (pressing samples): ~ 3 hr for all patches, but a bit of attention in the following days

Collected samples and data

Data collected from the plant community composition assessments, using the proposed field sheets (see appendix) or your own templates, can be entered following the data entry procedure described in the appendix, or scanned copies of the field sheets can be sent to us (herbivory.network@gmail.com) at the end of the field season.

If you have any questions, please contact: icbarrio@gmail.com

Leaf samples and biomass samples should be sent to:

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University of Iceland
Sturlugata 7, IS-101 Reykjavik, Iceland

Materials

- Frame (50x50 cm) and pins – for assessing foliar biomass of plant community
- Field sheets and pencil, to record data from point intercepts
- Large paper bags – for collecting biomass samples (15 per site, 75 total)
- Plastic bags for temporary storage of leaf samples (one per individual plant (3 individuals of 3 species at each site), minimum of 45 bags total).
- Permanent marker – for labelling bags indicating site, date, plant species and collector name.
- Clippers or pruning scissors – to collect aboveground biomass, pruning scissors might be needed to collect branches of woody species
- Tape measure 25m long (for delimiting sites)
- Camera – to take pictures of the sites

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Overview of the sampling protocol

