ARTICLE

Developing common protocols to measure tundra herbivory across spatial scales[1]

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Abstract: Understanding and predicting large-scale ecological responses to global environmental change requires comparative studies across geographic scales with coordinated efforts and standardized methodologies. We designed, applied, and assessed standardized protocols to measure tundra herbivory at three spatial scales: plot, site (habitat), and study area (landscape). The plot- and site-level protocols were tested in the field during summers 2014–2015 at 11 sites, nine of them consisting of warming experimental plots included in the International Tundra Experiment (ITEX). The study area protocols were assessed during

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2014–2018 at 24 study areas across the Arctic. Our protocols provide comparable and easy to implement methods for assessing the intensity of invertebrate herbivory within ITEX plots and for characterizing vertebrate herbivore communities at larger spatial scales. We discuss methodological constraints and make recommendations for how these protocols can be used and how sampling effort can be optimized to obtain comparable estimates of herbivory, both at ITEX sites and at large landscape scales. The application of these protocols across the tundra biome will allow characterizing and comparing herbivore communities across tundra sites and at ecologically relevant spatial scales, providing an important step towards a better understanding of tundra ecosystem responses to large-scale environmental change.

Key words: ecological monitoring, Herbivory Network, International Tundra Experiment (ITEX), Interactions Working Group (IWG), standardized protocol.

Résumé : Pour comprendre et prévoir les réactions écologiques à grande échelle face au changement environnemental à travers le monde, il est nécessaire de mener des études comparatives à travers les échelles géographiques avec des efforts coordonnés et des méthodologies normalisées. Nous avons conçu, appliqué et évalué des protocoles normalisés pour mesurer l'herbivorie de la toundra à trois échelles spatiales : la parcelle, le site (habitat) et la zone d'étude (paysage). Les protocoles au niveau des parcelles et des sites ont été vérifiés sur le terrain durant les étés 2014–2015 à 11 sites, dont neuf comprenant des parcelles expérimentales de réchauffement incluses dans l'« International Tundra Experiment » (ITEX). Les protocoles des zones d'étude ont été évalués en 2014–2018 dans 24 zones d'étude de l'Arctique. Nos protocoles fournissent des méthodes comparables et faciles à mettre en œuvre pour évaluer l'intensité des herbivores invertébrés dans les parcelles ITEX et pour caractériser les communautés d'herbivores vertébrés à des échelles spatiales plus grandes. Nous discutons des contraintes méthodologiques et formulons des

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recommandations sur la façon d'utiliser ces protocoles et d'optimiser l'effort d'échantillonnage pour obtenir des estimations comparables de l'herbivorie, tant aux sites ITEX qu'à grande échelle du paysage. L'application de ces protocoles dans l'ensemble du biome de la toundra permettra de caractériser et de comparer les communautés d'herbivores entre les sites de la toundra et à des échelles spatiales pertinentes sur le plan écologique, ce qui constituera une étape importante vers une meilleure compréhension des réactions de l'écosystème de la toundra au changement environnemental à grande échelle. [Traduit par la Rédaction]

Mots-clés : surveillance écologique, « Herbivory Network », « International Tundra Experiment » (ITEX), « Interactions Working Group » (IWG), protocole normalisé.

Introduction

Herbivores strongly influence the structure and composition of tundra plant communities (Barrio and Hik 2020), by consuming plant biomass, altering nutrient cycling, and disturbing soils by trampling (Mosbacher et al. 2019; Tuomi et al. 2019). Importantly, the activities of vertebrate and invertebrate herbivores can also mediate the responses of plants to warming (Post and Pedersen 2008; Olofsson et al. 2009; Barrio et al. 2016*a*). However, there remains much uncertainty as to how plant–herbivore interactions shape community responses to global warming and how local dynamics may scale up to affect regional patterns. To further complicate matters, the different methodologies used to assess herbivory across studies increase the variability in the observed patterns of herbivory, preventing meaningful comparisons, data synthesis, and upscaling (Halbritter et al. 2020).

Coordinated experiments that use standardized methodologies across broad geographical ranges have been proposed as a tool to advance our understanding of general mechanisms of ecological change (Fraser et al. 2013; Borer et al. 2014). One such coordinated distributed experiment is the International Tundra Experiment (ITEX; https://www.gvsu.edu/itex/), which investigates the responses of tundra plant communities to warming by using a simple, standardized experimental design applied at many sites across the tundra biome. Syntheses across ITEX sites suggest that many responses of tundra plant communities to warming depend on local site characteristics (Elmendorf et al. 2012; Prevéy et al. 2017; Bjorkman et al. 2018). Herbivory is an important process that could contribute to the site-specific responses of plants to warming but has not been systematically quantified at ITEX sites.

With an extensive network of tundra sites, the ITEX experiment provides a framework where methods for assessing herbivory within a coordinated distributed experiment can be tested. However, measuring the impacts of herbivory and characterizing herbivore communities is challenging, as herbivores and their impacts occur at different spatio-temporal scales. Methods such as capture–mark–recapture of individuals or transect surveys allow estimating population sizes or densities of herbivores at the landscape scale (e.g., Krebs 1999; Fauteux et al. 2018; Le Moullec et al. 2019), but are time consuming, expensive, or difficult to implement at a large scale in remote tundra sites. Observational methods to assess herbivory either quantify signs of herbivory on plants (e.g., Barrio et al. 2017) or infer the intensity of herbivory from direct or indirect estimates of herbivore abundance. For example, indices like fecal pellet counts have long been used in wildlife ecology to indirectly assess the abundance and habitat use of mammal and bird herbivores (e.g., Neff 1968; Putman 1984), including several northern herbivores (Krebs et al. 2001; Bråthen et al. 2007; Evans et al. 2007). Recommendations on how to conduct pellet counts have been proposed for other systems (e.g., Camargo-Sanabria and Mandujano 2011) but a unified protocol for pellet counts in tundra is still lacking.

Fig. 1. Diagram of the questions addressed in this study (indicated by numbers 1–4), at three different spatial scales (plot, site, and study area). The common protocols addressed ecological and methodological aspects. Assessments at the smaller spatial scale (plot) were based on herbivory signs, whereas at larger spatial scales (site and study area) assessments were based on pellet counts.
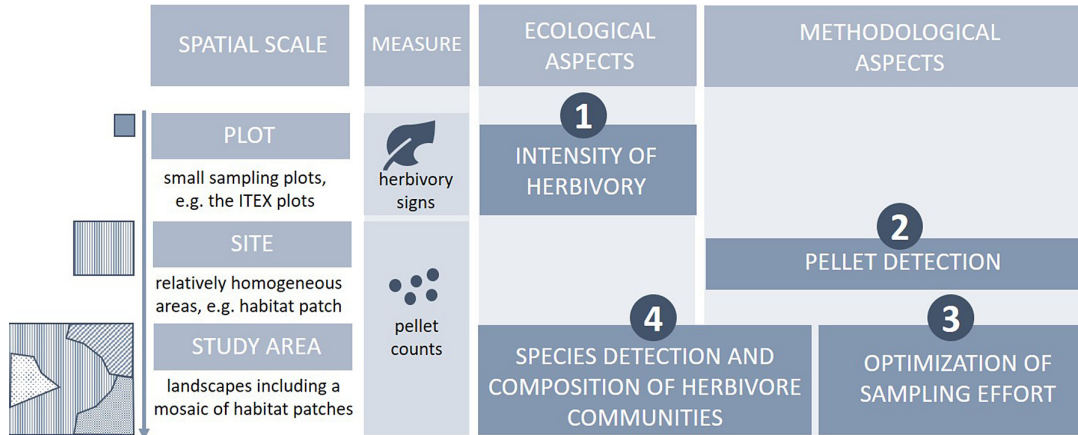
To address these issues, we designed and implemented a set of common protocols to measure herbivory using non-invasive, low cost methods following a spatially hierarchical approach (Fig. 1). The protocol specific to ITEX sites focused on the plot and site levels, whereas a pellet-count-based protocol targeted the study area level at other tundra sites. We were particularly interested in evaluating the efficiency of these methods, as well as optimizing sampling effort to propose easy to implement protocols that can be applied at different spatial scales and allow multi-site comparisons.

The overall aim of the study was to evaluate the ITEX protocol and to develop a recommended standardised protocol for studies specifically addressing herbivory related questions in tundra studies. At the plot level we used signs of herbivory by vertebrate and invertebrate herbivores to assess the ability of the ITEX protocol (1) to detect differences in the intensity of herbivory between plots. At the site and study area levels, we used protocols based on pellet counts to assess, (2) the detectability of pellets using different sampling units and across vegetation types, (3) how sampling effort affected the precision of pellet count estimates and how to optimize the allocation of sampling effort across study areas, and (4) whether the protocols could detect herbivore presence and capture differences in herbivore communities across the tundra (Fig. 1). Finally, based on these insights, we provide an update for the ITEX protocol and recommend a protocol for conducting pellet counts in tundra studies.

Methods

Standardized herbivory protocols

Following an expert workshop in 2013 at the International ITEX meeting in Davos, Switzerland, we designed a pilot protocol to measure herbivory at ITEX sites (Barrio et al. 2014; updated in 2016 after initial field trials, Appendix A). In this study we follow the hierarchical approach proposed in the updated ITEX herbivory protocol, including assessments at three spatial scales: plot, site, and study area. We also develop quantitative assessments at the study area level by building on standardized protocols implemented by other studies, like the Interactions Working Group (IWG) network (Meyer et al. 2020). The protocols we

Table 1. List of study areas and sites included in the plot-level assessment.

Study area	Region	Habitat (site)	Year	Design	Number of plots	OTC	Controls
Qikiqtaruk	Canada	Herschel vegetation	2014	Not paired	6	NA	6
Qikiqtaruk	Canada	Komakuk vegetation	2014	Not paired	6	NA	6
Kluane*	Canada	*Dryas* heath	2014	Paired	8	4	4
Auðkúluheiði*	Iceland	*Betula nana* heath	2014	Paired	20	10	10
Val Bercla*	Switzerland	Alpine tundra	2014	Paired	18	9	9
Endalen*	Svalbard	Snowbed	2015	Not paired	10	5	5
Endalen*	Svalbard	*Cassiope* heath	2015	Not paired	10	5	5
Endalen*	Svalbard	*Dryas* heath	2015	Not paired	10	5	5
Latnjajaure*	Sweden	*Dryas* heath	2014	Paired	10	5	5
Latnjajaure*	Sweden	*Salix herbacea* tundra	2014	Paired	8	4	4
Latnjajaure*	Sweden	Tussock tundra	2014	Paired	10	5	5

Note: A study area can include several International Tundra Experiment (ITEX) sites. Sites including ITEX experimental manipulations are indicated with asterisks. At some ITEX sites, open-top chambers (OTCs) and control plots are located adjacent to each other (i.e., paired), whereas at others, OTCs and controls are randomly distributed across the site (i.e., not paired). Study areas are indicated by triangles in Fig. 2.

evaluated here are based on signs of herbivory at the plot scale and on fecal pellet counts at the scale of sites and study areas.

Typically, ITEX experimental plots are 1 m × 1 m and are either randomly assigned to a passive warming manipulation using open-top chambers (OTCs) or remain as unmanipulated controls. We defined a site as a relatively homogeneous area with broadly similar environmental conditions (e.g., within the same habitat type or topographically homogeneous area), roughly one hectare or less in size, where the ITEX experimental plots (or other plots) are located. A study area can include several neighbouring sites (Table 1) and spans from several hundred square metres up to tens of square kilometres, thereby covering a more heterogeneous landscape (Fig. 1).

Plot-level assessments

In the summers of 2014 and 2015, the plot-level assessment was implemented at 11 sites (Table 1). The plot-level assessment was aimed at determining the intensity of herbivory within the ITEX plots (OTCs and controls) by both vertebrate and invertebrate herbivores. A modified point-intercept method was used where signs of herbivory were recorded at 100 regularly spaced point intercepts with a 1 cm buffer, in 1 m × 1 m or 75 cm × 75 cm quadrat frames as used in the regular ITEX vegetation assessments. The presence of herbivory signs and whether herbivory was due to vertebrate or invertebrate herbivores were recorded at each point intercept. Herbivory was expressed as the percentage of points intercepting leaves with signs of herbivory. Points only intercepting bare ground or cryptogams (mosses and lichens) were subtracted from the total number of point intercepts. In the plot-level assessment, most herbivory signs (97.5%) were attributed to invertebrate herbivores, so at this scale, we focus on invertebrate herbivory only.

Site-level assessments: a single habitat in a homogeneous landscape

In summer 2014, we conducted the site-level assessment of herbivory at 13 sites, some of them including ITEX manipulations (Table 2). The site-level assessment was confined to a single, relatively homogeneous habitat and targeted vertebrate herbivores, whose presence and abundance were assessed using fecal pellet counts along linear transects (Bråthen et al. 2007; Skarin 2007). Following the site-level assessment of the ITEX herbivory protocol, herbivore pellets were counted along one 100 m linear transect, 2 m wide, with pellets recorded within every 1 m segment (Barrio et al. 2014). In some cases, identifying pellets to species level was not possible, so broader categories (hereafter herbivore taxa) were used:

Table 2. List of study areas included in the site and study area assessments.

Study area name	Region	Geographic area	Northing	Easting	Year	Transect length (m)	Transect width (m)	Number of transects	Area surveyed (m²)	Herbivore taxa	Research leader(s)
Utqiaġvik[#]	Alaska, USA	North American Arctic	71.23	−156.75	2017	30	1	12	360	2; 2.2	Richard Lanctot, Sarah Saalfeld
Canning River[#]	Alaska, USA	North American Arctic	70.11	−145.80	2018	10	1	30	300	3; 3.2	Christopher Latty
Qikiqtaruk[+]	Canada	North American Arctic	69.58	−138.87	2014	100	2	2	400	3; 3	Isla Myers-Smith
Kluane[*+]	Canada	Subarctic/alpine	61.35	−138.47	2014	100	2	1	200	3; 3.2	David Hik
Kugluktuk	Canada	North American Arctic	67.82	−115.23	2014	100	2	21	4200	6; 6.1[R]	Noémie Boulanger-Lapointe
Cambridge Bay[#]	Canada	North American Arctic	69.12	−105.05	2018	10	1	35	350	5; 3.2	Jean-François Lamarre
Polar Bear Pass[#]	Canada	North American Arctic	75.72	−98.67	2017	30	1	20	600	3; 3.2	Paul Woodard
Arviat	Canada	North American Arctic	61.13	−94.14	2014	100	2	35	7000	4; 5.1[R]	Noémie Boulanger-Lapointe
Churchill[#]	Canada	North American Arctic	58.70	−94.08	2018	10	1	30	300	3; 4	James Roth, Laura McKinnon
Burntpoint Creek[#]	Canada	North American Arctic	55.24	−84.31	2018	10	1	30	300	3; 3.1	Glen Brown
East Bay[#]	Canada	North American Arctic	63.98	−81.67	2018	10	1	30	300	3; 2.1	Paul Smith
Igloolik[#]	Canada	North American Arctic	69.40	−81.60	2017	30	1	20	600	1; 1.2	Nicolas Lecomte, Marie-Andrée Giroux
Bylot Island[#]	Canada	North American Arctic	73.15	−80.00	2017	30	1	10	300	3; 3	Joël Bêty
Karupelv[#]	Greenland	Greenland	72.50	−24.00	2016	30	1	20	600	4; 2.2	Johannes Lang
Zackenberg[#]	Greenland	Greenland	74.47	−20.57	2017	30	1	10	300	4; 4	Niels M. Schmidt
Hochstetter[#]	Greenland	Greenland	75.15	−19.70	2016	30	1	15	450	4; 2.2	Olivier Gilg, Loïc Bollache
Auðkúluheiði[*+]	Iceland	Subarctic/alpine	65.20	−19.70	2014	50	2	3	300	3; 3	Ingibjörg Svala Jónsdóttir
Val Bercla[*+]	Switzerland	Alpine	46.48	9.58	2014	100	2	1	200	2; 2	Janet Prevéy
Forlandsundet	Svalbard	Svalbard/Scandinavia	78.45	11.44	2015	30	1	9	270	3; 3	Virve Ravolainen
Kapp Linné	Svalbard	Svalbard/Scandinavia	78.06	13.69	2018	10	1	29	290	3; 3	Øystein Varpe
Endalen[*+]	Svalbard	Svalbard/Scandinavia	78.19	15.76	2014	50	2	5	500	3; 3	Ingibjörg Svala Jónsdóttir
Adventdalen[#]	Svalbard	Svalbard/Scandinavia	78.20	15.80	2018	10	1	53	530	3; 3	Christian Stolz, Øystein Varpe
Isfjorden	Svalbard	Svalbard/Scandinavia	78.10	16.13	2015	30	1	47	1410	3; 3	Virve Ravolainen
Austfjorden	Svalbard	Svalbard/Scandinavia	78.93	16.26	2015	30	1	10	300	3; 3	Petr Macek
Billefjorden	Svalbard	Svalbard/Scandinavia	78.64	16.52	2015	30	1	40	1200	3; 3	Petr Macek
Ammarnäs[#]	Sweden	Svalbard/Scandinavia	65.96	16.29	2017	30	1	10	300	1; 2.1	Rob van Bemmelen
Latnjajaure[*+]	Sweden	Svalbard/Scandinavia	68.21	18.31	2014	100	2	1	200	4; 4[R]	Juha Alatalo
Erkuta[#]	Russia	Russian Arctic	68.22	69.15	2015	30	1	30	862	4; 4.1[R]	Aleksandr Sokolov
Belyi Island[#]	Russia	Russian Arctic	73.32	70.09	2015	30	1	9	270	3; 3.1[R]	Dorothee Ehrich
Sabetta[#]	Russia	Russian Arctic	71.24	71.80	2015	30	1	30	872	5; 4.1[R]	Natalia Sokolova

Note: [*] indicates International Tundra Experiment (ITEX) sites included in this study, and [#] indicates Interactions Working Group (IWG) sites; [+] indicates study areas where the site-level assessment was conducted, where each transect corresponds to a site. Herbivore taxa shows the number of medium and large vertebrate herbivores (species or groups of species), and small rodents if included in the survey (superscript R), observed in the pellet counts and the number of species known to be present in each study area; decimal points in the number of species indicate additional species known to be present in the study area but reported as rare.

Fig. 2. Map of study areas across the Arctic. International Tundra Experiment (ITEX) and Interactions Working Group (IWG) sites included in this study are indicated by triangles and pentagons, respectively. The biogeographic sub-zones (High Arctic, Low Arctic and subarctic) are drawn following the Arctic Biodiversity Assessment (CAFF 2013) using ArcGIS Desktop 10.5 (ESRI™, Environmental Research Institute, Redlands, California, USA).
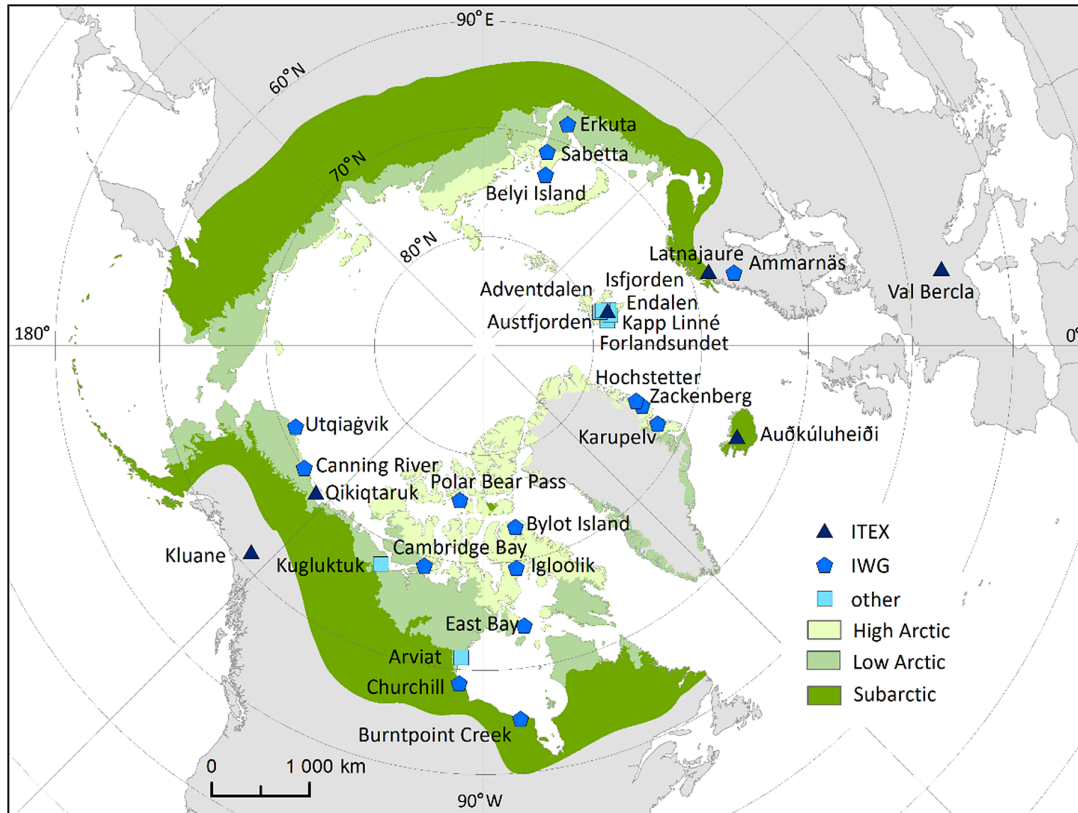
geese and swans, grouse species, ground squirrels, marmots, hares, muskoxen, reindeer/caribou, sheep, and small rodents.

Study area assessments: multiple habitats in heterogeneous landscapes

In 2014–2018, a standardized protocol using pellet counts along transects was implemented once at 24 study areas across the Arctic (Table 2; Fig. 2). Seventeen of these study areas were part of the IWG network (Meyer et al. 2020), where the aim of the surveys was to assess the composition of the herbivore community in tundra landscapes; the remaining surveys were conducted by members of the Herbivory Network (Barrio et al. 2016*b*) to evaluate protocols for pellet-based herbivore community assessments.

To account for landscape heterogeneity and obtain comparable estimates independent of subjective definitions of habitat patches within the study area, pellet count transects (usually 1 m × 30 m) were located at random within each study area by choosing random coordinates for the starting point and orienting the transect in a fixed direction. The observer walked along a measuring tape (transect width 1 m), recording pellet counts in each 1 m segment along the transect. The number of transects and their length varied slightly across study areas, depending on study area size, resulting in a surveyed study area

ranging between 270 and 7000 m^2 (Table 2). Pellets of small rodents were included in some study areas (Table 2).

Data analysis — evaluation of the protocols

We addressed four questions on the effectiveness of the standardized protocols at the different spatial scales (Fig. 1). At the plot level we used signs of herbivory to assess the ability of the protocols to detect differences in the intensity of herbivory. At the site and study area level we evaluated how simple field protocols based on pellet counts can be optimized to characterize herbivore communities.

Can the protocols detect differences in the intensity of herbivory between sites and treatments?

We compared the intensity of herbivory across ITEX sites with data from control plots only, including site as a predictor variable in a binomial generalized linear model (GLM) for proportional data. Similarly, we compared the intensity of herbivory in plots with and without experimental warming manipulations at different ITEX sites including an interaction term between warming and site as predictor variable in a binomial GLM for proportional data. In both cases, the intensity of herbivory (i.e., percentage of points intercepting leaves with signs of herbivory) was included as response variable.

Does pellet detectability differ between types of sampling units and between tundra vegetation types?

We assessed if the detectability of pellets differs when using different types of sampling units (linear transects or smaller sampling plots). We used data from three study areas (Erkuta and Sabetta on Yamal Peninsula, and Isfjorden in Svalbard) where fecal pellets were counted both along 30 m linear transects (30, 30, and 23 transects respectively) and in small plots (50 cm × 50 cm) located every 2 m along the transects. For each study area, we compared the density of pellets (pellets/m^2) estimated in the transects and in the smaller plots for different herbivore taxa. Deviations from a 1:1 correspondence between the two methods are interpreted as over- or under-estimation by one of the methods.

To assess the detectability of fecal pellets in different vegetation types, we conducted a field trial in Erkuta in 2017, targeting the six most common habitat and vegetation types in the area: (1) wet tundra, with a continuous *Sphagnum* moss layer (>10 cm thick) and abundant graminoids; (2) mesic tundra characterized by tussocks of *Eriophorum vaginatum* L., a deep moss layer (>10 cm) and abundant dwarf shrubs (<25 cm); (3) dry tundra, with a thin (<10 cm) or discontinuous moss layer, and abundant graminoids and dwarf shrubs (<25 cm); (4) ridges with limited plant cover, >25% cover by biological soil crust, and abundant prostate dwarf shrubs (<5 cm); (5) birch shrubs dominated by *Betula nana* L. (>25 cm); and (6) willow thickets dominated by erect *Salix* spp. shrubs (>90 cm). We established 9–14 plots (50 cm × 50 cm) in each vegetation type and all pellets were cleared from them. We then added a known number of reindeer pellets (5–11) to each plot, and a second researcher, unaware of the number of pellets in the plots, counted the number of pellets. We calculated the percentage of omissions in pellet detection as: % omission = $(T - O)/T \times 100$, where T is the true (known) number of pellets, and O is the observed (detected) number of pellets. Differences in the mean percentage omission of pellets between vegetation types were assessed using a Kruskal–Wallis test.

How does sampling effort affect pellet count estimates and how should sampling effort be distributed across a study area?

To evaluate how transect length at the site level and transect length and the number of transects at the study area level influenced the precision of pellet density estimates we used simulations based on observed data.

Site level

We assessed the influence of transect length on the precision of pellet density estimates within sites (homogeneous habitat) simulating small habitat patches (250 m × 250 m) with homogeneous pellet distribution. We used the observed data for one of the ITEX sites in Endalen located in a moss tundra habitat on a concave landform. We selected this site because it had the largest contrast in mean pellet density between the three herbivore taxa (reindeer, geese, and ptarmigan). For each herbivore taxon we simulated 500 random placements of one 2 m wide transect, varying between 10 and 150 m in length (20–300 m^2 surveyed area). Means and 95% confidence intervals (CIs) of the pellet densities estimated from the 500 simulations were plotted against transect area.

Study area level

We simulated landscapes (6 km × 6 km) where pellets were heterogeneously distributed, using data for the most abundant herbivore taxa in three study areas (Erkuta, Sabetta, and Isfjorden), located respectively in the Low Arctic, between the Low and High Arctic, and in the High Arctic. We selected these study areas for analyses because they contained a large number of sampled transects (Table 2). The simulated landscape was divided into patches of 1 km^2 each with different densities of pellets, to simulate habitat patches that herbivores use differently. First, we assessed the effect of transect length on the precision of pellet density estimates. We chose 20 random locations within the simulated landscape as the starting points for 1 m wide transects of different lengths (between 5 and 100 m). We repeated this procedure 500 times for each transect length. Second, we assessed the effect of the number of transects on the precision of pellet density estimates. We located a varying number of 1 m × 20 m transects (between 5 and 100 transects) at random locations within the simulated landscape 500 times. For both assessments, means and 95% CIs of the pellet densities estimated from the 500 replicates were plotted against transect length and against the number of transects.

To address how to best allocate sampling effort for herbivore pellet counts with respect to the number and length of transects in a landscape we performed simulations in homogeneous and heterogeneous study areas. First, we simulated a homogeneous landscape (6 km × 6 km), where the distribution of pellets was based on the empirical value of reindeer pellet counts from Erkuta (Table 2). Keeping the sampling effort (i.e., area surveyed) constant at 300 m^2, we simulated sampling of different numbers of transects of different lengths (between 2 and 60 transects of a length varying between 6 and 150 m). For each simulated sampling event, we calculated the mean estimates of pellet counts and compared the distribution of these means with the true density, which was the mean density calculated for the whole landscape. Second, we followed the same procedure on a simulated heterogeneous landscape. As above, the heterogeneous landscape (6 km × 6 km) consisted of patches of 1 km^2 each with different densities of pellets. In doing so, we assessed how the number and length of transects influenced bias and precision of pellet density estimates in homogeneous and heterogeneous areas. Third, because effort during fieldwork is usually measured in person days, we also simulated how the number and length of transects could be best allocated to reduce bias and maximize the precision of pellet count estimates. As an example we considered a total sampling time of three person days (24 working hours in total) and assumed it takes 20 min to conduct a transect of 30 m, and 25 min to pack, unpack, and walk between the random starting points.

Can the protocols detect the occurrence of all herbivores and capture differences in herbivore communities within sites and across study areas?

Site level

To assess the ability of the protocol to detect all herbivore taxa present within a relatively homogeneous area, as in the case of the ITEX sites, we used the data from Endalen consisting of five ITEX sites located on five distinct landscape units: a snowbed community dominated by bryophytes (*Sanionia uncinata* (Hedw.) Loeske, *Tomentypnum nitens* (Hedw.) Loeske) and *Bistorta vivipara* (L.), a moist *Cassiope tetragona* (L.) heath, a moist moss tundra dominated by bryophytes (*Sanionia uncinata*) occurring in two contrasting parts of the landscape (a concave and a convex landform), and an exposed *Dryas octopetala* L. heath. For each site we built species accumulation curves using the specaccum function in the vegan package in R (Oksanen et al. 2019). As only one transect was conducted at each site, we randomized the order of segments within each transect to assess how many species of herbivores were detected with increasing transect length (100 permutations). We also plotted the observed species accumulation curve along the transects (i.e., same order of metre segments) in each habitat because we found some spatial autocorrelation between metre segments (Supplementary Fig. S1[2]).

Study area level

To assess how sampling effort (i.e., transect length and the number of transects) affects our ability to detect herbivore taxa over heterogeneous study areas, we used data from the three study areas (Erkuta, Sabetta, and Isfjorden). For each study area, we built species accumulation curves estimated from 100 random permutations of transects and for transect lengths between 5 and 30 m.

To assess whether the sampling reflected the local herbivore community, we compared the number of herbivore taxa detected in the transects at each of the 30 study areas with the number of herbivores known to be present. This information was reported by the study area research leaders based on their general knowledge, independent of the pellet counts (Table 2). Research leaders at each site completed an online survey where they were asked which herbivore taxa were present in their study area, and if they were abundant or rare.

Finally, to assess whether the protocol captured differences in the herbivore community among the 30 study areas (Table 2), we used non-metric multidimensional scaling (NMDS) as implemented in the vegan package in R (Oksanen et al. 2019). The total number of pellets for each herbivore species was summed across transects for each study area, and pellet densities were calculated based on the total surveyed area. For some herbivores (e.g., reindeer/caribou and muskoxen), pellets were counted both as groups (clumps) and as single pellets, and conversion factors were used to estimate the number of single pellets. For reindeer/caribou one group equalled 30 individual pellets, and for muskoxen one group equalled 20 single pellets. For standardization, single clumps of reindeer summer feces were also converted into single pellet equivalents using the same conversion factor. The NMDS analyses exclude small rodents because pellets for this herbivore group were only recoded in some study areas. The NMDS analysis also included ITEX study areas (pooling the transects conducted at several ITEX sites within each area) for comparison but excluded one alpine ITEX site (Val Bercla) that clearly had a non-Arctic herbivore community (Table 2).

All statistical analyses were conducted in R 3.6.3 (R Development Core Team 2020).

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[2]Supplementary material is available with the article at https://doi.org/10.1139/as-2020-0020.

Fig. 3. Plot-level assessment of invertebrate herbivory (percentage of points intercepting leaves presenting damage by invertebrate herbivores) in experimentally warmed plots (open-top chambers, OTC) and control plots at different sites. Sites are arranged by longitude. Error bars show 95% confidence intervals. Significant differences between OTC and control are marked with asterisks (***$p < 0.001$).
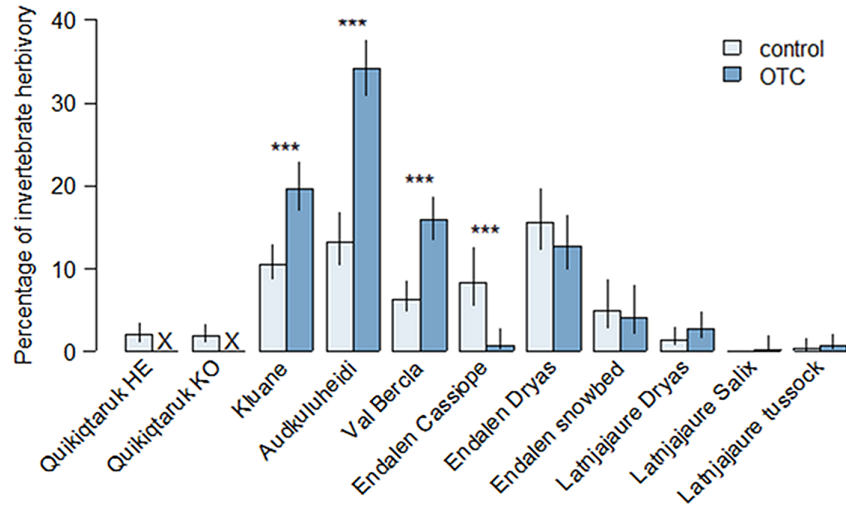
Results

Can the protocol detect differences in the intensity of herbivory between sites and treatments?

Invertebrate herbivory was widespread, with signs of herbivory being present in 81.3% of all plots and in the control plots at all 11 ITEX sites except one (Latnjajaure *Salix*). However, the overall intensity of invertebrate herbivory was low (mean ± SE = 6.36% ± 0.94% of the points intercepted leaves with signs of herbivory in control plots) and there was substantial variation across sites (binomial GLM, deviance = 266.41, $p < 0.001$; Fig. 3). The plot level assessments also detected differences between experimental and control plots at each site. We found a significant interaction between the experimental warming treatment and site (binomial GLM, deviance = 75.03, $p < 0.001$), indicating that at some sites the intensity of herbivory was higher in warmed plots, whereas in other sites no significant differences were detected (Fig. 3). At one site (Endalen *Cassiope* heath), this pattern was reversed, with a higher percentage of invertebrate herbivory in control plots (binomial GLM, $z = 3.380$, $p < 0.001$).

Does pellet detectability differ between small plots and transects and between tundra vegetation types?

When comparing pellet density estimates based on counts of pellets along a 30 m transect or using 50 cm × 50 cm sampling plots every 2 m (Fig. 4), we found that transects tended to underestimate the densities of smaller vertebrate herbivore feces (hares and small rodents), especially in the Low Arctic (Erkuta). In the High Arctic (Isfjorden), transects were able to detect goose and reindeer pellets and yielded similar estimates to the smaller sampling plots. For ptarmigan, the results were similar between transects and plots (not shown).

The mean percentage omission associated with the detectability of reindeer pellets per vegetation type ranged between 11% and 39% (Fig. 5). The detectability of reindeer pellets was higher in mesic tundra and ridges, compared with birch shrubs and willow thickets.

Fig. 4. Comparison of pellet densities (pellets/m^2) estimated using 30 m transects and smaller square plots (50 cm × 50 cm) every 2 m along the transect, for three study areas (Isfjorden, $n = 23$; Sabetta, $n = 30$; and Erkuta, $n = 30$) and four species of herbivores (reindeer, goose, hare, and small rodents). Solid lines indicate 1:1 correspondence between the two methods.
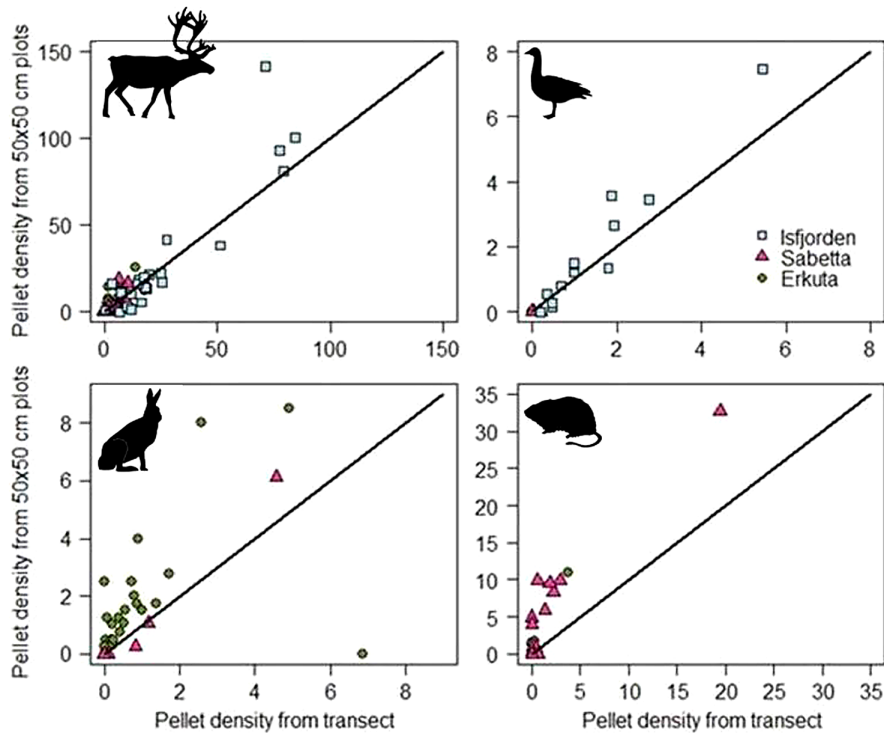
The mean percentage omission was highest in the habitats with taller vegetation ($p < 0.001$; Fig. 5).

How does sampling effort affect pellet count estimates and how should sampling effort be distributed across a study area?

At the site level, where transects were conducted within homogeneous habitat patches, transect length (and thus the total transect area surveyed) affected the precision of pellet density estimates. This is exemplified with the concave moss tundra ITEX site in Endalen, where increasing the sampled area decreased the CIs for pellet estimates (Fig. 6).

At the study area level, assuming a landscape with a heterogeneous distribution of pellets, average pellet count estimates were unbiased for transect length or the number of transects (Figs. 7 and 8). However, the distribution of means estimated from the different replicates varied considerably (indicated by the 95% CIs), especially for shorter (Fig. 7) and fewer transects (Fig. 8). Longer transects increased precision but increasing transect length beyond 20–30 m resulted only in little improvement in the precision of pellet density estimates (Fig. 7). Increasing sampling effort by increasing the number of transects improved the precision of estimates of pellet densities and narrowed CIs more than increasing transect length (Figs. 7 and 8).

Given a fixed sampling effort (here assumed to be 300 m^2), the simulations of different lengths and different numbers of pellet count transects showed that the optimal sampling depends on whether pellets were assumed to be distributed homogeneously over the

Fig. 5. Boxplots for the percentage of reindeer pellets not detected by observers (percentage omission) in a field trial conducted in Erkuta in 2017 in the most common vegetation types. The percentage omission is calculated as the percentage of pellets not detected out of a known number of reindeer pellets in a plot. Different letters correspond to significant differences in percentage of omissions between vegetation types ($p < 0.05$). Outliers are indicated as open circles.
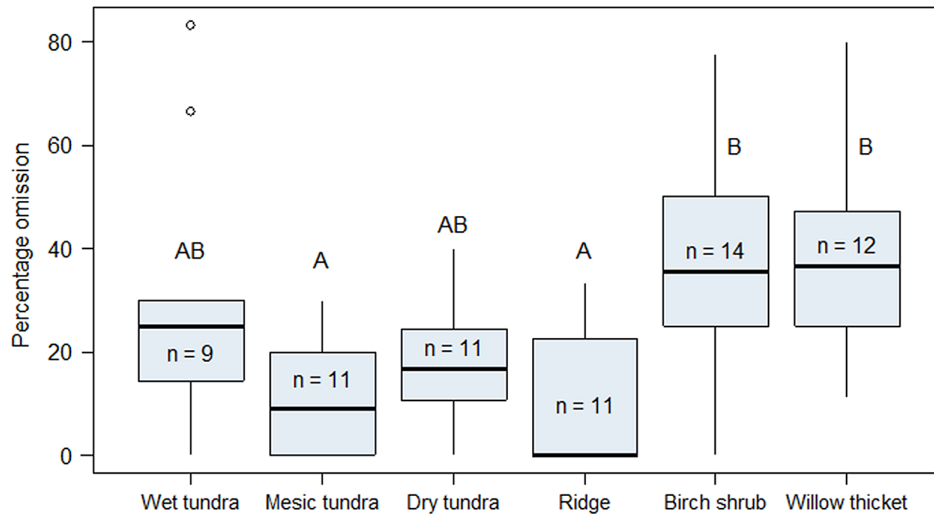

Fig. 6. Effect of transect area (m^2) on the mean (95% confidence interval, shaded areas) estimated number of pellets per m^2. Random locations for 2 m wide transects between 10 and 150 m long were simulated (transect area between 20 and 300 m^2) in a homogeneous habitat; for each transect length, the simulated sampling was carried out 500 times. Data is based on pellet counts at an International Tundra Experiment (ITEX) site in Endalen, Svalbard (concave moss tundra) for the most abundant herbivore taxa (reindeer, goose, and ptarmigan). Note the different y-axis scales by species. Transect area is presented here for comparability with Fig. 9.
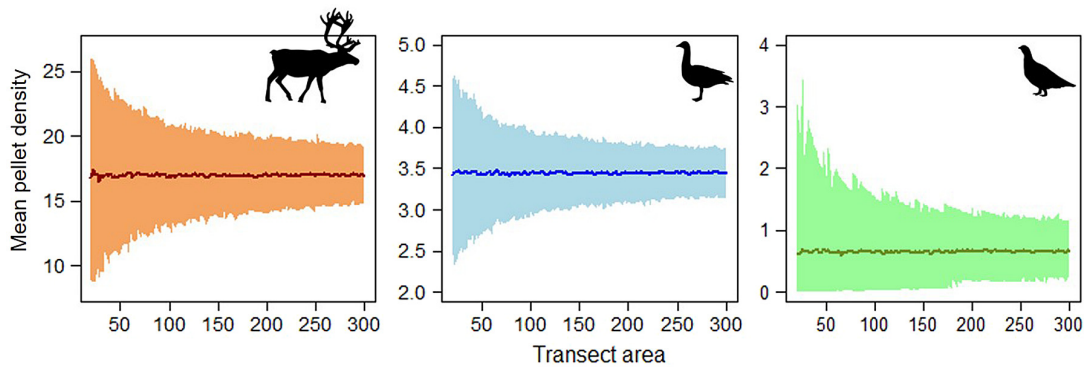
landscape, or whether the landscape was heterogeneous with respect to habitat and herbivore use. In a homogeneous landscape, the estimates of mean pellet densities were normally distributed around the true mean, independently of how the sampling effort was allocated (Fig. 9a). In a heterogeneous landscape, however, mean pellet densities were consistently below the true mean when using fewer, longer transects (two 150 m long transects; Fig. 9b), likely due to the higher probability of missing locations with high pellet density.

Fig. 7. Effect of transect length on the mean estimated number of pellets per m^2 and 95% confidence intervals (CIs, shaded areas) for the most abundant herbivore taxa (reindeer, ptarmigan, hare, and goose) in three selected study areas (Erkuta, Sabetta, and Isfjorden). Increasing transect length generally increased the precision of pellet density estimates (narrower 95% CIs). Random locations for 20 transects 1 m wide, between 5 and 100 m long were simulated on a heterogeneous landscape; for each transect length, the subsampling was carried out 500 times. Note the different *y*-axis scales by species.
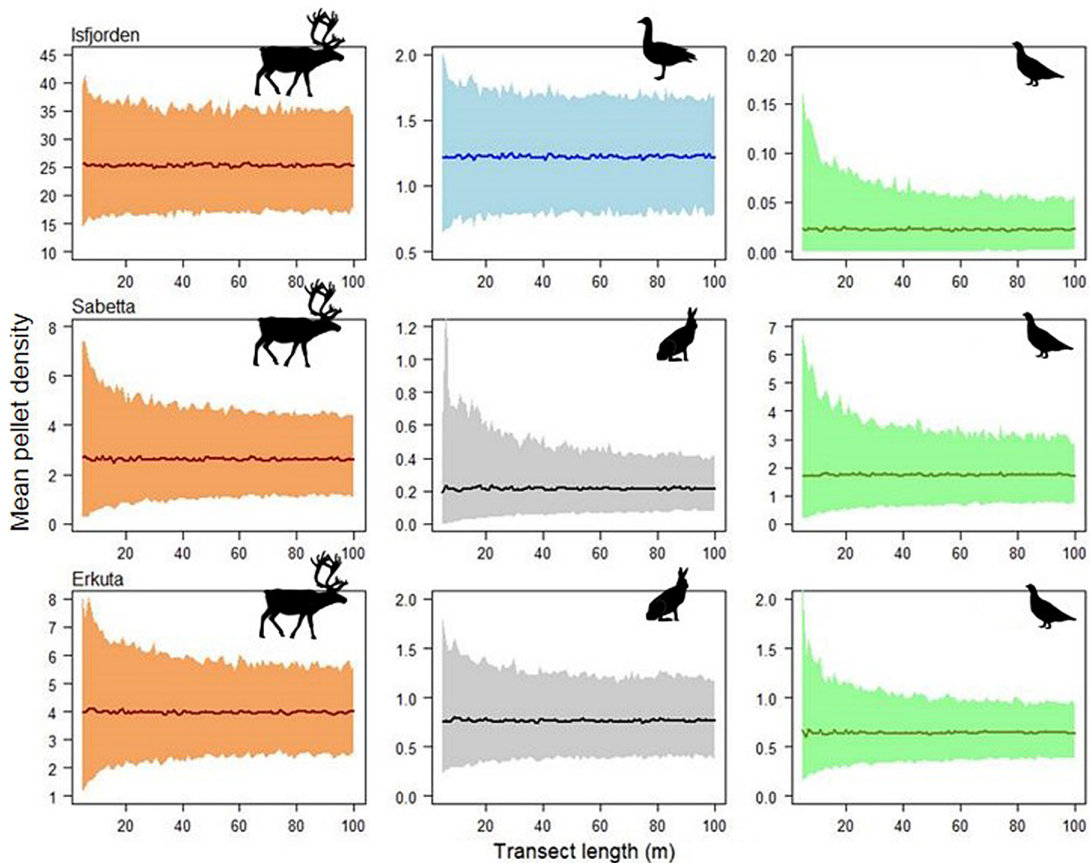
For a larger number of shorter transects (60 5 m long transects) the estimates improved and became normally distributed around the true mean, although variation was still wider than for the homogeneous landscape (Fig. 9b).

Assuming that only a fixed number of person days can be allocated to pellet counts during a field campaign and taking into account the time needed to walk between transects, a large number of shorter transects may not be the optimal solution, as an increasing amount of time will be used to walk between transects. In this case, the total area sampled decreased considerably, contributing to lower precision. Our simulated example based on a heterogeneous landscape suggested that for three person days, the best compromise between precision and bias is achieved with 38 transects of 20 m placed at random in the landscape (Supplementary Fig. S2[2]). By contrast, in a homogeneous landscape, the variant covering the largest amount of area (based on the longest transects) resulted in the best precision (data not shown).

Fig. 8. Effect of the number of transects on the mean estimated number of pellets per m^2 and 95% confidence intervals (CIs, shaded areas) for the most abundant groups of herbivores (reindeer, ptarmigan, hare, and goose) in three selected study areas (Erkuta, Sabetta, and Isfjorden). Increasing the number of transects generally increased the precision of pellet density estimates (narrower 95% CIs). Between 5 and 100 transects of 20 m were chosen at random on a simulated heterogeneous landscape; for each number of transects, the subsampling was carried out 500 times. Note the different *y*-axis scales by species.
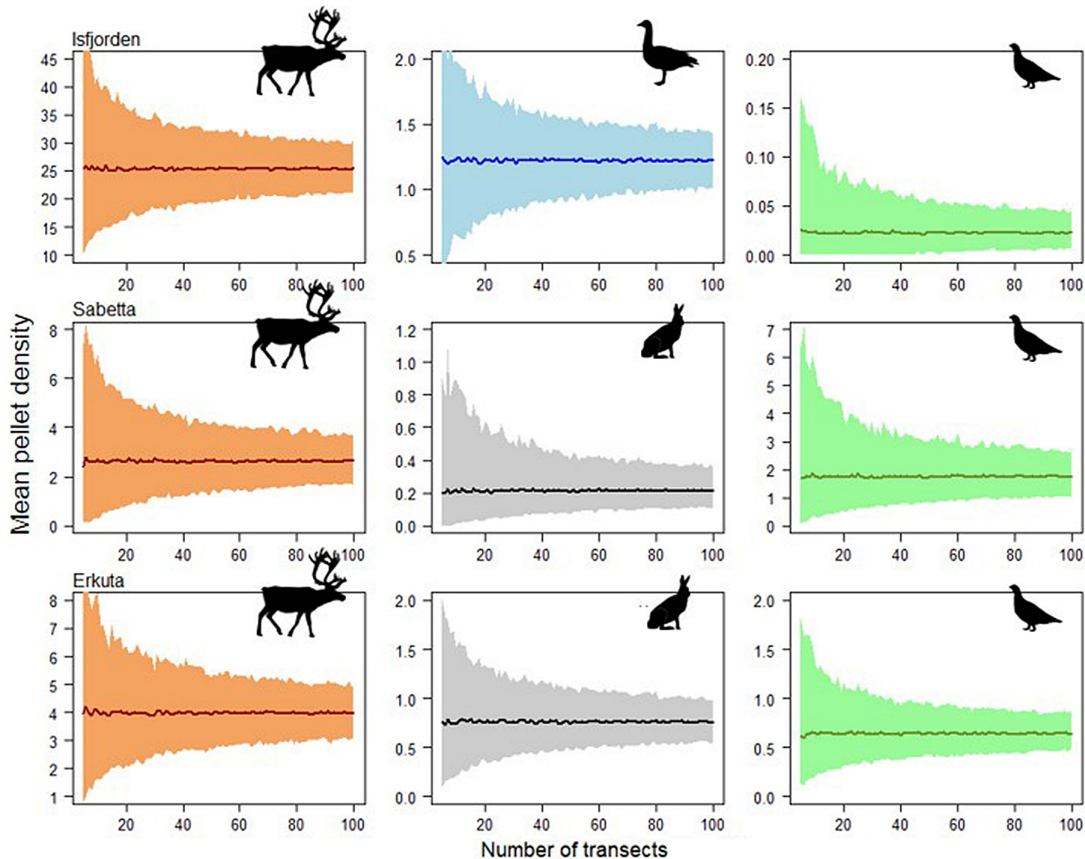
Can the protocols detect the occurrence of all herbivores and capture differences in herbivore communities across study areas and sites?

At the site level, the randomized species accumulation curves for the five ITEX sites at Endalen show that 30 m long transects were sufficient to detect the three herbivore taxa present in the study area (reindeer, ptarmigan, and geese). Moreover, the simulations suggest that even shorter transects (around 20 m) could be used in certain habitats (*Cassiope* and *Dryas* heath; Fig. 10) for detecting all herbivore taxa. The results of the observed species accumulation curves were similar to the randomized species accumulation curves (Supplementary Fig. S3[2]).

At the study area level, the species accumulation curves for Erkuta, Sabetta, and Isfjorden generally show that if transects are shorter, slightly more transects were needed to detect the common herbivore taxa known to be present within an area, although CIs largely overlapped (Fig. 11). At these three sites, the most common herbivore taxa were already detected with about 15 transects, regardless of their length.

Fig. 9. Effect of allocating sampling effort to fewer, longer transects (left side) or more, shorter random transects (right side) on the estimates of mean pellet density in homogeneous (*a*) and heterogenous (*b*) landscapes. Red vertical lines indicate the true pellet density (i.e., the mean of the whole simulated landscape). Simulations are based on data from Erkuta.
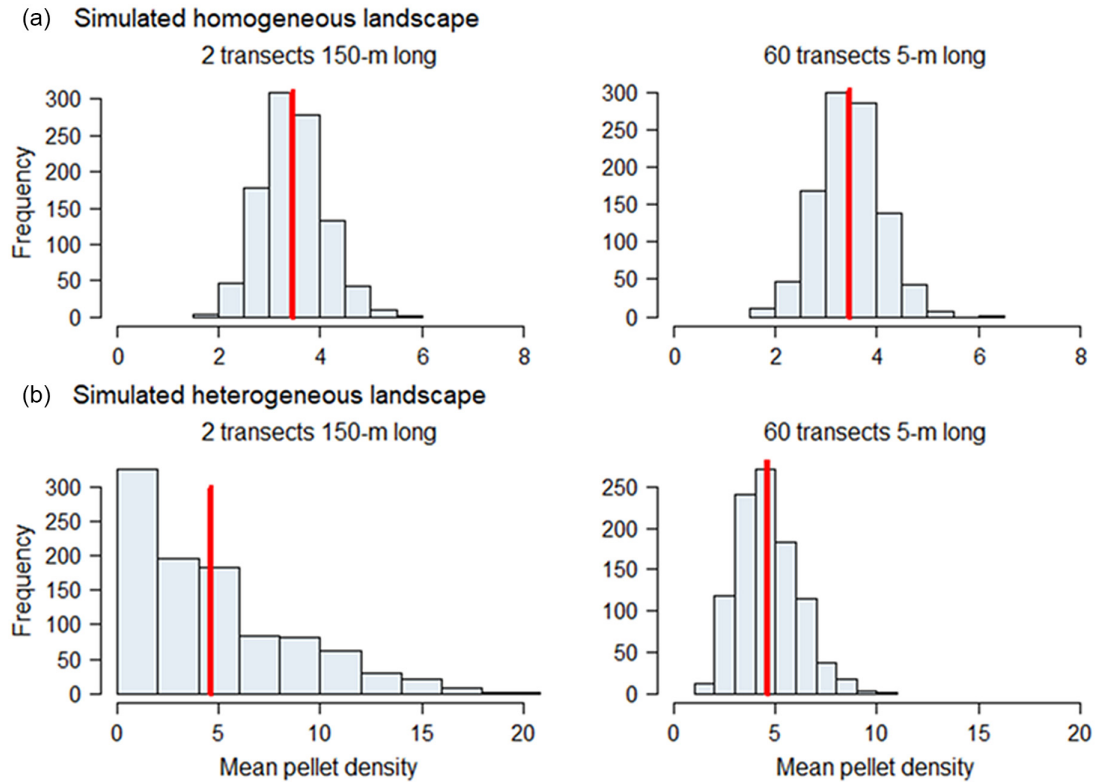

Fig. 10. Species accumulation curves for the five International Tundra Experiment (ITEX) sites in Endalen. The curves show the accumulation of herbivore taxa (mean and confidence intervals) detected based on pellet counts along transects of different lengths (m) and random resampling of transects. The horizontal red line shows the true number of herbivore taxa known to be present in the study area.
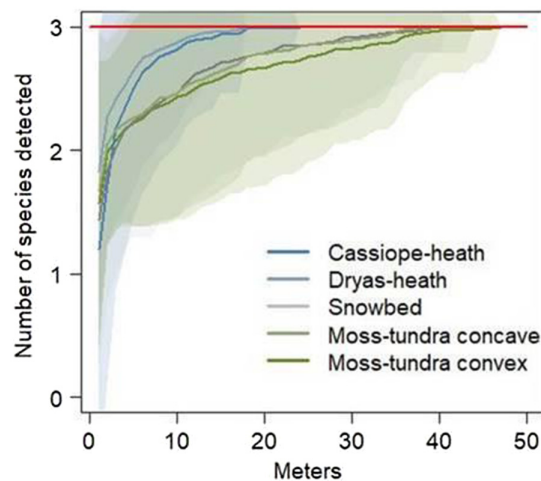
Fig. 11. Species accumulation curves for three selected study areas: Erkuta, Sabetta, and Isfjorden. The curves show the accumulation of herbivore taxa (and 95% confidence intervals (CIs), shaded areas) for which feces were detected based on increasing numbers of transects of different lengths (5–30 m), and random resampling of transects. The horizontal red line shows the number of herbivore taxa (including small rodents) known to be present in the study areas and the dashed red line shows the number of taxa excluding rare taxa.
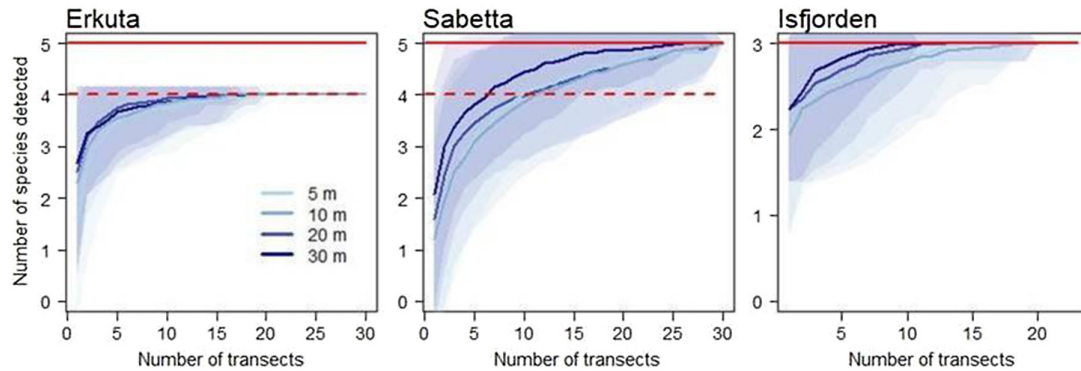
For 87% of the study areas ($n = 30$) all herbivore taxa that were reported as abundant by the research leaders were detected within the transects. Within six study areas, even species reported as rare were detected on the transects (Hochstetter and Karupelv in Greenland; Sabetta in Russia; East Bay, Arviat, and Cambridge Bay in Canada; Table 2). Three study areas (Arviat and Churchill in Canada; Ammarnäs in Sweden; Table 2) did not detect species reported as abundant. In all cases, the missing abundant species was ptarmigan; in Arviat, ground squirrels were also reported as abundant but were not detected in the transects.

The NMDS analysis (stress = 0.152) indicated that the composition of the medium and large vertebrate herbivore community varied among different tundra study areas, as estimated by the pellet counts along transects (Fig. 12). In the NMDS analysis, grouping of study areas based on the similarities of their herbivore communities roughly corresponded with geographical areas (Table 2). Broadly, herbivore communities in the study areas in Greenland were characterized by muskoxen and hare, study areas in the North American Arctic were dominated by geese, and the study areas in Svalbard and Scandinavia were characterized by reindeer/caribou. The presence of domestic sheep was unique to the study area in Iceland (Auðkúluheiði), and marmots and ground squirrels were characteristic of the study area in southwest Yukon (Kluane), Canada.

Discussion

In this paper, we report on the results of several studies using standardized protocols to assess herbivory, with the aim of leveraging coordinated efforts to address ecological questions at a biome-wide scale. We implemented standardized protocols for both vertebrate and invertebrate herbivory in the Arctic and evaluated their ability to detect differences in the intensity of herbivory at the plot level, and to effectively characterize herbivore communities at the site (habitat patch) and study area (landscape) levels. Our three-tiered assessment effectively captured the presence and activity of herbivores across different spatial scales. The plot-level assessments successfully reflected the frequency of invertebrate herbivory and detected differences between experimental manipulations but were often not effective at capturing vertebrate herbivore activity. In turn, assessments at the site and study area levels provided an overview of the use of Arctic landscapes by larger vertebrate

Fig. 12. Non-metric multidimensional scaling (NMDS) plot based on pellet densities of herbivore taxa (silhouettes) detected at each study area. Sizes of points indicate sampling effort at each site (area surveyed). Triangles indicate whether the study area consisted of International Tundra Experiment (ITEX) sites included in this study (excluding the alpine site in Val Bercla, Switzerland). Ellipses indicate 95% confidence intervals for broad geographical areas.
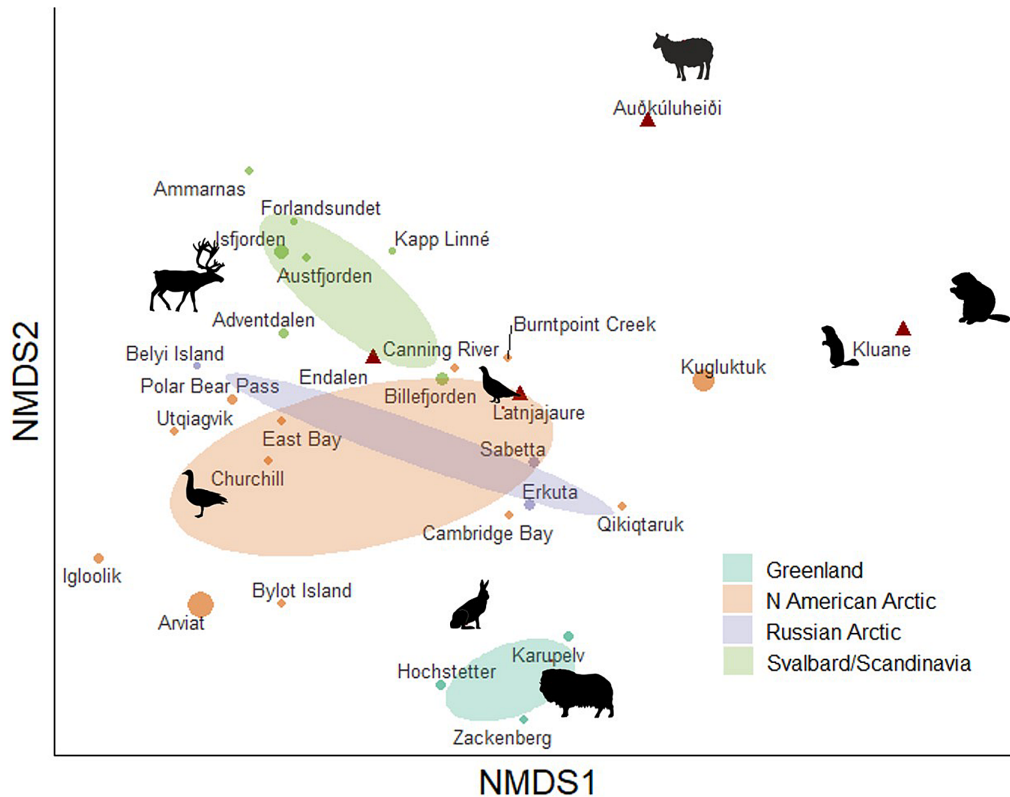
herbivores that was otherwise not captured by plot-level measurements. Based on our results, we discuss the limitations of these methods and make recommendations for best use practice of the protocols to obtain comparable estimates of herbivory at ITEX and other tundra study areas.

Plot-level assessments

The results from our plot-level assessments indicated that the ITEX herbivory protocol was sufficiently robust to locally identify the occurrence and intensity of invertebrate herbivory in tundra communities. Until recently, invertebrate herbivory had received relatively little attention in tundra ecosystems, particularly in non-outbreak situations (Kozlov et al. 2015). At background (non-outbreak) levels, leaf damage by invertebrate herbivores occurs at low rates but is widespread across the tundra biome (Barrio et al. 2017; Rheubottom et al. 2019). Other studies measuring invertebrate herbivory have reported more detailed measurements of defoliation, such as the percent leaf area damaged or the proportion of leaves affected for individual plants (Kozlov and Zvereva 2017). Here, we measured herbivory more coarsely by using a modified point–intercept method that estimates the percentage of sampling points affected by herbivory. Nevertheless, our results are within the same order of magnitude as other studies measuring invertebrate herbivory in the tundra (Barrio et al. 2017; Rheubottom et al. 2019). Additionally, the method described here bears some

similarity to the widely used transparent grid method (Coley 1983; Pearse and Hipp 2009; Henderson and Southwood 2016), which has been shown to accurately measure insect herbivory on plants (Getman-Pickering et al. 2020). The current protocol, therefore, provides a rather fast way of assessing the relative intensity of invertebrate herbivory that can be implemented at different sites. In addition, the point–intercept method is widely used in tundra vegetation studies, so information on plant damage can be combined with species composition data to obtain species specific damage and facilitate estimates of community-wide herbivory (Zvereva et al. 2020), and thus enhance comparability across sites (Rheubottom et al. 2019).

At the plot level, the protocol was also able to capture differences in the intensity of invertebrate herbivory between experimentally warmed plots and controls. Warmer temperatures have been associated with increased levels of herbivory in the fossil record (Wilf et al. 2001) and in other field experiments involving warming manipulations (Richardson et al. 2002; Roy et al. 2004; Li et al. 2019). However, other studies have reported variable responses across sites or for specific plant–herbivore systems (Dollery et al. 2006; Gillespie et al. 2013; Barrio et al. 2016a; Birkemoe et al. 2016). The responses to warming reported here were not consistent across sites, suggesting that the complexity of interactions in the responses of herbivory and warming reported by previous studies may not be solely related to the different methodologies used.

Assessments at larger spatial scales based on pellet counts

Counting fecal pellets of vertebrate herbivores is a rapid and easy way to obtain an estimate of presence and relative abundance, and our results support this view. Previous studies have found that herbivore pellet densities correlate with herbivore abundance (e.g., Krebs et al. 2001; Bråthen et al. 2007), and thus reflect overall herbivory pressure on plants in an area. Pellet counts have been widely used to assess variation of herbivore density and activity in space (Krebs et al. 2003; Bråthen et al. 2007; Ims et al. 2007) and time (Ehrich et al. 2012; Soininen et al. 2013; Ravolainen et al. 2014). Our recommended protocol for conducting pellet counts in tundra (Appendix B) provides new information on optimal sampling designs balancing sampling effort (number and length of transects) and the available sampling time, to allow for comparisons between different study areas individual sites within landscapes and between different study areas.

As the aim of the present study was to obtain estimates at the broader landscape scale, independent of the specific habitats available at each site, estimates were based on randomly placed linear transects (e.g., Bråthen et al. 2007; Ims et al. 2007). We carried out simulations based on real data collected for common tundra herbivores at three study areas distributed from Low Arctic shrub tundra to the low growing vegetation of the High Arctic to assess how increased sampling effort improves the precision of pellet density estimates. In a heterogeneous landscape, where herbivores use different habitats with different intensities and where pellets are not evenly distributed, our results show that increasing the number of random transects increased precision more than when increasing the length of each single transect. For the three study areas considered, little precision was gained by increasing transect length beyond 20–30 m, but precision increased with the number of transects up to approximately 40–50. In the real world, however, the total sampling effort in field studies will often be limited by the time available for sampling (Alves et al. 2013). Given a limited number of person days, there will be a trade-off between sampling many random points and the time spent walking between transects. For example, in a simulated 6 km × 6 km landscape with habitat patches of 1 km^2 and assuming 15 min to walk between random locations, the optimal use of three working days for estimating reindeer pellet densities was 38 transects of 20 m. An R script to run simulations of this

trade-off for other sites considering the implementation of this protocol is provided as part of the supplementary materials (Supplementary Text Files S1–S3[2]). To sample large study areas covering landscapes of several hundred square kilometres with rivers, hills, and fjords (as was the case, for example, for Isfjorden and Erkuta), it is not possible to use randomly distributed transects over the whole area. In this case, it will be more effective to select several sub-landscapes as replicated sampling units, within which transects are distributed at random. The number of transects within these sub-landscapes could then be optimized based on the time available for sampling each unit (Appendix B).

Depending on the objective of the study, targeted sampling of certain habitats might be preferable to sampling across broader heterogeneous regions. A decision tree for different study designs is provided in Appendix B. For example, when the aim is to estimate an index of herbivore activity in a small locality or for a given habitat, such as at an ITEX site, the habitat can be assumed to be homogeneous with respect to herbivore use. In this case, a large number of transects will not give better estimates of pellet densities than fewer yet longer transects (Fig. 9), as the precision of pellet density estimates will depend on the total area sampled. Thus, at the ITEX sites, where all counting was carried out in a small and relatively homogeneous habitat patch, increasing total sampling area increased the precision of estimates clearly up to approximately 300 m^2. This area should be recommended as a minimum sampling effort for the ITEX site protocol.

Similarly, pellet counts can be used as a low cost method to monitor herbivore populations over time on permanently marked plots, from which pellets are regularly removed (Ehrich et al. 2012; Soininen et al. 2013; Ravolainen et al. 2014; Fuglei et al. 2020). Removal plots have the advantage that the time over which pellets have accumulated is known (Krebs et al. 2001; Henden et al. 2011). Originally, the ITEX herbivory protocol recommended removal of pellets along the surveyed transects to estimate herbivore activity at the ITEX sites, but repeated sampling was only conducted at the study area in Endalen, so data have not been analyzed here. If the aim of the study is long-term monitoring of herbivore activity in a given area, removal plots can provide a reliable index (Appendix B).

Overall, our study suggests that pellet count transects might be a cost effective sampling method for most medium and large vertebrate herbivores in the High Arctic, as they allow covering large areas quickly. However, in the relatively lush vegetation of the Low Arctic, especially for medium and small vertebrate herbivores, smaller sampling plots provide better estimates than transects (Fig. 4). In addition, for smaller species, such as lemmings, it can be useful to record incidental observations and include other signs of activity such as the presence of runways or winter nests (Cadieux et al. 2015; Fauteux et al. 2018). At the same time, some caveats need to be considered when using pellet counts to characterize herbivore communities and compare them across sites, as the detectability of pellets of different herbivores will depend on the species of herbivore and their habitat choices, as well as on vegetation type and pellet decomposition rates (Davis and Coulson 2016).

This study, spanning a wide range of tundra herbivores, habitats, and sites, contributes to documenting the variation of herbivore communities across the tundra in a quantitative manner. The standardized protocols evaluated and presented here allowed for a broad characterization of the large- and medium-sized vertebrate herbivore community at each site. The protocols were able to detect all common large- and medium-sized vertebrate herbivore taxa, with a fairly low number of transects, which is useful for future sampling schemes in this remote biome. Information about the variation in herbivore community composition across the Arctic can help contextualize the interpretation of results from studies conducted at a single site. The composition of herbivore communities (Burkepile and Hay 2008; Burkepile and Parker 2017) can determine the impacts of herbivores on ecosystems (Wang et al. 2019), because different herbivores will have different feeding choices

and feeding modes, or will affect plants at different times. For instance, the combined effects of large and small mammalian herbivores on the biomass of tundra vegetation are stronger than would be predicted for each group of herbivores alone (Olofsson et al. 2004; Ravolainen et al. 2014). Our study provides a relatively simple way to measure single species and community level impacts of herbivory in this biome, a feat that was not previously possible.

Conclusions

Our standardized protocols provide an easy and relatively fast way of assessing the intensity of invertebrate herbivory at the plot level and characterizing the variation in vertebrate herbivore communities across the Arctic at larger spatial scales. We provide a road map to measure and track the impacts of herbivores at a biome-wide scale and to connect patterns of vertebrate and invertebrate herbivory. By assessing herbivore activity at various scales (plot, site, and study area) the protocols were able to detect different aspects of herbivory otherwise missed when treating each scale separately. A remaining challenge for future work is to integrate this information across the different spatial scales (Parsons 2016). Similarly, additional work is required to relate pellet densities to actual herbivore numbers; this could be done by adapting survey methods to record information about specific seasonal habitat use and diet, as well as movements and dynamics of herbivores at regional scales. Future standardized protocols will, therefore, be needed to achieve this aim. However, pellet counts require considerably less time and resources than other survey methods for estimating the abundances of tundra herbivores (e.g., Karels et al. 2004), hence standardizing and improving these methods for future herbivory monitoring will be well worth the effort.

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Author contributions

ICB and DE wrote the paper with significant contributions from EMS, VTR, CGB, AMK, JDMS, DSH, MM, and ISJ. ISJ, ICB, VTR, CGB, DSH, and MM conceived the original idea of this paper and developed the first versions of the ITEX herbivory protocol. OG, AMK, DE, EMS, CGB, JDMS, MM, AN, and AS actively participated in discussions in the HN meeting in Yamal. ISJ, DSH, JP, JMA, NBL, and IMS contributed data using the ITEX protocol. VTR and DE developed the protocol for pellet counts. AA, JB, LB, GSB, IE, MAG, BBH, JFL, JL, CL, NL, LMcK, ÅØP, JDR, STS, NMS, PS, CS, RVB, ØV, and PFW contributed data using the IWG pellet count protocol, coordinated by OG. VTR, PM, TH, and ØV contributed additional data from pellet counts in Svalbard, and NS, AS, and DE from Yamal. SPH and DE contributed data on the pellet detectability trial in Erkuta. ICB ran the analyses for the ITEX data. DE analyzed the data for the pellet counts, ran the simulations and provided the script. ISJ revised the updated ITEX protocol presented in Appendix A. DE wrote the recommended pellet count protocol presented in Appendix B, with input from VTR, EMS, and ICB. All authors provided comments on the manuscript and approved the final version for publication.

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Appendix A. ITEX herbivory protocol

Background and rationale

Herbivory is a main driver of tundra plant communities (Jefferies et al. 1994; Mulder 1999; Barrio et al. 2016), and recent studies have shown that herbivores can modulate the

Box A1. Outline of changes in the protocol since the previous version.

Changes since last version from 2016

A summary of the main changes since the previous version (Barrio et al. 2016) based on the evaluation presented in this paper are listed here. For more details, please read corresponding sections.

- *Pellet counts for large/medium sized herbivores:* We suggest conducting at least five 30 m long 1 m wide transects spread across the ITEX site to assess use of the area by large/medium sized (vertebrate) herbivores. These transect sizes provide similar levels of detection and density precision. See Section 2.
- *Pellet counts for small mammals:* In Low Arctic areas with relatively lush vegetation and (or) sites with high density of small mammals, pellet counts in smaller sampling plots placed systematically along a transect may be an option, in addition to counting all small mammal pellets present in the ITEX monitoring plots. More efficient estimates of small mammal use of the area would involve sampling specific habitats and (or) more time-consuming methods that are beyond the relative estimates proposed in this protocol. See Section 3.

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responses of tundra plants to warming (Post and Pedersen 2008; Olofsson et al. 2009; Speed et al. 2012; Kaarlejärvi et al. 2013; Post 2013). The International Tundra Experiment (ITEX; https://www.gvsu.edu/itex/) provides an experimental setting to test this idea across a large number of sites.

This protocol was designed specifically for the ITEX experimental set up, which includes passive warming manipulations using open-top chambers (OTCs) and unmanipulated control plots. The goal of the protocol is to provide guidelines for assessment of the occurrence and intensity of herbivory within ITEX plots (OTCs vs. controls) and among ITEX sites (controls at different sites) and has been updated from an earlier version (Box A1). In addition, the protocol for the site level assessment has been coordinated with a more general protocol for assessment of vertebrate herbivore communities using pellet counts in tundra ecosystems (see Appendix B). This information will allow a quantitative evaluation of herbivory, to address the following.:

- ✓ Whether herbivory occurs at different intensities within OTCs and in controls.
- ✓ Whether herbivory is similarly prevalent across tundra sites (by comparing control plots at different sites).
- ✓ Whether herbivory by vertebrates and invertebrates has a similar impact across tundra sites.
- ✓ To what extent are large/medium (vertebrate) herbivores using the ITEX site in a wider landscape context.

Although the measurements proposed in this protocol will undoubtedly benefit the ongoing studies at each site, the data obtained would be also extremely valuable for collaborative research, e.g., comparisons across sites within landscapes and between different study areas.

Because herbivores (both vertebrates and invertebrates) can affect plant communities directly, through plant biomass consumption, and indirectly, through trampling and increased nutrient availability via feces and urine (Van der Wal et al. 2004), it is relevant to quantify both, the signs of herbivory and the signs of herbivore presence.

In this document, we will refer to "ITEX sites" as a group of pairs of OTCs and control plots occurring in places with broadly similar environmental conditions. For example, if you have plots on wet tundra but grouped at two different elevations, your groups will be regarded as two separate ITEX sites. Similarly, if your plots are at the same elevation but in three markedly contrasting habitats, for example in wet tundra, heath, and dry tundra, your groups will be considered three separate ITEX sites.

Fig. A1. The protocol consists of three levels of assessment (photographs: David Hik and Isabel C. Barrio).

1. Overall characteristics of the herbivore community

Overall description of the site, and relevant management practices that may affect herbivore populations

2. Site-level assessment

Local estimates of (vertebrate) herbivore presence and abundance in the area

3. Plot-level assessment

Fine-scale measures of herbivory and herbivore activity that can be related to plant measurements

This protocol consists of three levels of assessment (Fig. A1): description of the overall characteristics of the herbivore community, site-level assessment, and plot-level assessment.

## 1. Overall characteristics of the herbivore community

A brief description of the ITEX site will help in framing the specific monitoring protocols for the site and plot level assessments (Fig. A2). General information on features of the site relevant to herbivore populations (e.g., if the area is under grazing management, hunting activities, etc.) will be requested. A preliminary list of potential herbivores should be prepared and updated using local information and consulting local experts, particularly regarding the presence of domestic herbivores. If available, data on densities of different herbivores, population fluctuations, status of populations (e.g., if migratory or resident) and accuracy of the observations would be highly desirable. Also, an indication needs to be made if the ITEX sites are within an exclosure fence that prevents access to any herbivore, either large or small mammals or birds. This site description should be completed once for each ITEX site.

Background data on the potential occurrence and densities of herbivores and their distribution will help in defining overall herbivore activity in the area. This information will also help in defining the methods for herbivory assessment at the site- and plot-level (Sections 2 and 3).

2. Site-level assessment of herbivory

For herbivores likely to have an impact at a scale larger than the ITEX plots (e.g., wide-ranging animals such as reindeer/caribou or muskoxen, or for smaller mammals whose home range is larger than ITEX plots, e.g., lemmings and voles), recording herbivore presence at the site level is critical, because herbivory might be spatially variable and, thus, more difficult to detect in the small plant measurement plots. This assessment includes vertebrate herbivores only, as invertebrate herbivores tend to have a more localized effect and will be assessed at the plot scale. In some cases, signs of herbivore presence are not easily assigned to a certain herbivore species, or they may only give an indication of relative abundance; nevertheless, this information is extremely valuable to approximate "herbivore pressure" at each site. Because we are interested in the effects of herbivores related to the

Fig. A2. Summary of proposed activities within this herbivory protocol. Ideally levels 2 and 3 will be conducted once in the season every year if possible, but "snapshot data" from different years will also be very useful.
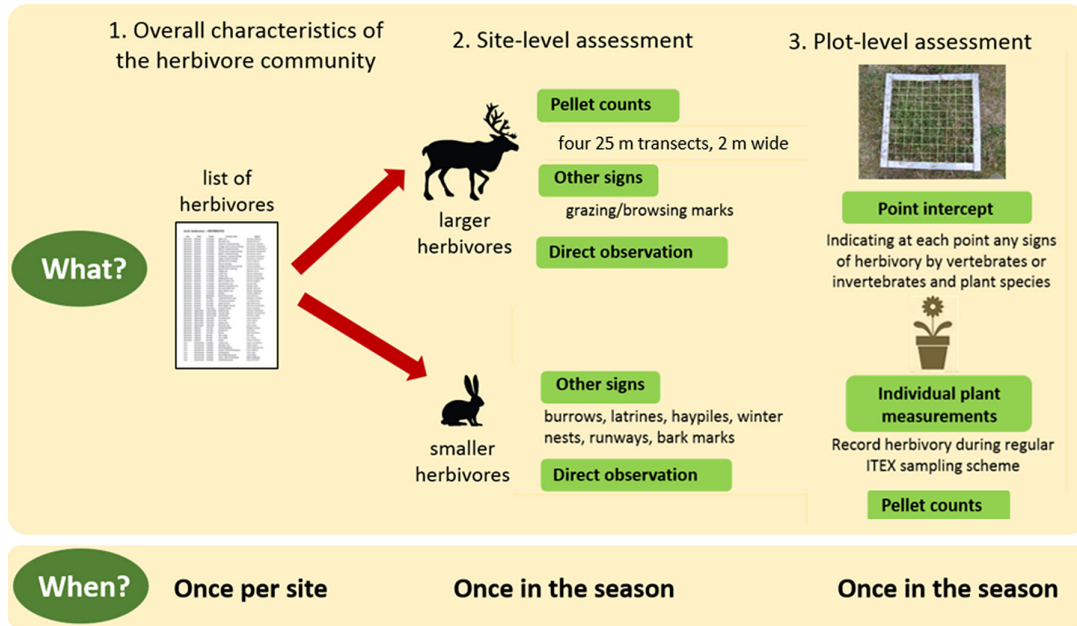
ITEX sites, assessments at the site level will be conducted roughly in a 100 m radius from the centroid of the group of ITEX plots at each site, but within the same habitat type as the plots.

Based on the list of potential herbivores in your site (Section 1), their density, and vegetation characteristics, you may need to use one or more of the following methods.

Transects for pellet counts: For most sites this method will be suitable. Establish the transects within the site (100 m radius from the centroid of the group of ITEX plots at each site but representative of the plant community of the ITEX plots), either at random (random origin and direction) or systematically. The number of transects will depend on their length and width, as this will determine the precision of the pellet count estimates. We recommend that at least five (ideally 10) transects 30 m long and 1 m wide are established at each site and should be at least 10 m apart. Alternatively, shorter and wider transects can be used. Ideally, these transects will be permanently marked, pellets removed in each visit, and repeated in different years to assess changes in herbivore activity over time. Pellet removal ensures that in the next visit, only pellets deposited in the time between visits are counted and allows more accurate estimates of recent herbivore activity. In the first visit to the site (i.e., when establishing the transects for the first time), all pellets will be counted and removed; this first assessment, although not strictly comparable with subsequent ones because of pellets of unknown "ages", gives an indication of herbivore activity in the area. It is, thus, very important to note in your field data collection if the visit corresponds to a first survey of a transect or not. When species identification is not possible from the pellets, pellets will be assigned broadly to groups of herbivores (e.g., large mammals, small mammals, birds); whenever possible, take a picture of the "unidentified" pellets.

For large mammals (caribou/reindeer, moose, sheep, muskoxen), some medium-sized mammals (marmots; Karels et al. 2004)) and birds (swans, geese, ptarmigan) walk the

Fig. A3. Transect for pellet counts of large mammals (sheep) and birds (geese and ptarmigan) at an ITEX site in Auðkúluheiði (Iceland). The transect is set up between ITEX plots to capture herbivore activity at the ITEX site (photograph: Isabel C. Barrio).

transect down slowly (Fig. A3), recording the presence of pellets within every transect metre. If you are applying 30 m transects, pellets can be recorded by one observer within a 1 m band (0.5 m to each side of the transect line). Alternatively, you could use 15 m transects with a 2 m band (1 m to each side of the transect; using a 2 m stick as a reference with two people walking on each side of the transect line is helpful). Make sure that this is clearly recorded in your notes. Pellets frequently occur as groups or clumps; each group will be counted as one "unit" (fecal event) and recorded as a "group" (as opposed to isolated "pellets"). This will allow later standardization for reindeer and some other ungulates whose "pellets" may differ between summer (one big clump) and winter (several small pellets) by using a conversion factor (one clump = 30 pellet equivalents) before analyzing the data. The distance along the transect at which each unit is found will be recorded; when there are a lot of pellets it is easier to count in segments of 1 m. Again, make sure this is clear in your field notes.

Plots for pellet counts: At Low Arctic sites with relatively lush vegetation and at sites with very high densities of small herbivores, we showed that pellet counts on quadrats of 50 cm × 50 cm gave more precise results than counts carried out along transects. Plots can be arranged as linear transects separated by a fixed distance (for example 2 m).

Other observations of herbivore activity (all species, including invertebrates): Record other evidence of herbivore activity, or the numbers of herbivores seen at the ITEX site.

Information at the site level will help update the list of potential herbivores (Section 1) and will give a more accurate estimation of actual herbivore activity in the area. This information will be valuable to evaluate the role of (vertebrate) herbivory at ITEX sites in a wider landscape context and across ITEX sites.

3. Plot-level assessment of herbivory

The aim is to determine the intensity of herbivory in the ITEX plots (OTCs and controls), both by vertebrate and invertebrate herbivores, by collecting quantitative information (point frame of occurrence of herbivory or observations on individual plants, and pellet counts for smaller herbivores) and qualitative (other observations of signs of herbivore

activity). This will evaluate the local impact of herbivores at the plot level, including also invertebrate herbivores. We are mainly interested in assessing the incidence of herbivory by vertebrates and invertebrates, without distinguishing species of herbivores because this might be more challenging; thus, damage on plants will be assigned only to either vertebrate or invertebrate herbivory. Because measurements of herbivory are typically cumulative, assessments of herbivory might be conducted only once in the season, preferably after the peak in biomass, and before plant senescence at the end of the season. This assessment can be done as part of your regular ITEX monitoring (see below), or as a standalone survey (point frame); if you are not planning on doing your ITEX monitoring in a given season but still want to assess herbivory, the point frame method should be used.

This part of the assessment does not depend on the type of herbivores or vegetation present at the site.

Point frame: Use a modified point–intercept method to assess the incidence of herbivory by vertebrates and invertebrates on the plant community. In a quadrat, with 100 evenly distributed points, record all signs of herbivory intercepted at each point with a 1 cm buffer, indicating the plant species eaten and if damage is due to vertebrate or invertebrate herbivores. The size of the quadrat can be the same as used for vegetation analysis (usually 1 m × 1 m or 75 cm × 75 cm) or any size down to 50 cm × 50 cm.

Herbivory (especially by invertebrates) might be very localized; by including the 1 cm buffer we maximize the chances of detecting herbivory in a standardized way. Record all distinct herbivory damages intercepted in each point and distinguish between leaf and floral herbivory, and between vertebrate and invertebrate herbivory if possible. Herbivory (total, by vertebrates, and by invertebrates) will be expressed as the percentage of points intercepting leaves with signs of herbivory. Points only intercepting bare ground or cryptogams (mosses and lichens — with potential herbivory that is hard to detect) will be subtracted from the total number of point intercepts. Using the point frame helps focus your attention to leaf herbivory that otherwise goes undetected; on average, each 100-intercept point frame will take around 5–10 min if only herbivory is recorded. This assessment can be combined with the regular ITEX vegetation monitoring.

Individual plants: When monitoring individual plants within the ITEX regular sampling schemes, herbivory can be recorded. This will provide an estimation of the incidence of herbivory on particular plant species. For each monitored plant, a visual estimation of the proportion of leaf herbivory using a scale from 0 to 6 (Kozlov et al. 2015) (where 0 is no herbivory, 1: <1% leaves eaten, 2: between 1%–5%, 3: 5%–25%, 4: 25%–50%, 5: 50%–75% and 6: >75%) will be used. For each plant, herbivory would be broadly classified as caused by vertebrates, invertebrates, or both. Where possible, floral herbivory should be recorded too (as presence/absence).

Other signs of herbivore presence or activity in the plot: As in Section 3, signs of herbivore activity and (or) presence in the plots should be recorded. Here, it is particularly important to pay attention to the presence of invertebrate herbivores (non-outbreaking), which might be overlooked when making the assessment at a larger scale, and to count pellets of small mammals within the plots. We could expect differences in herbivore use of plots with and without OTCs, for example by rodents, ptarmigan, or invertebrates, due to an exclosure effect or due to the passive warming effect.

Plot-level assessment of herbivory will allow comparisons between plots (OTCs and controls) and across ITEX sites. Ultimately, this information will help in evaluating the role of herbivory as a driver of plant community responses to warming across a large number of tundra sites.

Quality control: estimates of observer bias

Differences in estimates within and between observers can be a potential source of variation in the data collected. As an internal control procedure, we suggest that at each site some estimate of repeatability is conducted. This would involve repeating the same point–intercept or transects independently by different observers, or by the same observer for each set of measurements and would allow a quantification of observer bias and error.

Timing and time commitment

Herbivore data could be collected at the beginning of the field season (some signs might be only detectable early in the season, e.g., lemming winter nests), or at the end (cumulative signs of herbivory might be better assessed later in the season, before plant senescence). We expect sampling to take up to one day of work per ITEX site for two people over the whole season, preferably during the peak of the growing season. However, if you are able to do only part of the proposed activities, please do! And let us know dates. A rough estimate of time dedicated to each activity (needs to be adjusted to each site, depending on the herbivores present and the number of ITEX plots) is as follows.

- Transects for pellet counts: 3 h.
- Point frame (for herbivory only): 10 min per plot; with 10 OTCs and control pairs (20 plots): 3.3 h.

Materials

- An ITEX point frame with 100 equally spaced points for assessing leaf herbivory. The frame size can be 100 cm × 100 cm, 75 cm × 75 cm or smaller (Section 3). This same frame can be used for pellet counts of smaller vertebrate herbivores (Section 2).
- One 30 m tape measure, for establishing the transect (Section 2).
- One 1 mm stick (or any other reference) to estimate the 1 m strip along the transect, or 2 m stick if you choose to use shorter and wider transects (Section 2)
- Wooden stakes, to permanently mark the beginning and end of the pellet transects (Section 2).
- Plastic bag, for removing pellets from the transect and pellet plots (Section 2). Pellets do not need to be kept but must be removed from the surroundings of the transect.

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Appendix B. Recommended protocol to assess herbivore communities in tundra using fecal pellet counts

Herbivores are a key component of tundra ecosystems, but assessing their presence and abundance is challenging. Here, we suggest a general framework for how to carry out fecal pellet counts in tundra ecosystems to characterize vertebrate herbivore communities. Our recommendations are based on the results of this paper and previous experience using this method in tundra ecosystems for multi-scale spatial assessments (e.g., Bråthen et al. 2007) or temporal monitoring (e.g., Krebs et al. 2001; Ehrich et al. 2017). Due to the spatial movement of vertebrate herbivores, this method is not easily applicable at a plot level scale and we therefore focus on a site (habitat) and study area (landscape) levels. A plot level assessment may, however, be useful for studies addressing invertebrate herbivory; a recommended protocol to assess the intensity of herbivory at that level are also presented in Appendix A.

Depending on the aim of the study, the recommendations for how to best design the fecal pellet count study will differ, as presented in Fig. B1.

Background and rationale

Counts of fecal pellets have long been used in wildlife ecology as an easy and low cost method to assess abundance, trends, distribution, co-occurrence and habitat use of mammal and bird herbivores (e.g., Bennett et al. 1940; Eberhardt and Etten 1956; Neff 1968; Putman 1984). Earlier studies have assessed protocols and given recommendations particularly in relation to monitoring large ungulates in temperate areas (e.g., Robinette et al. 1958; Alves et al. 2013) and tropical systems (e.g., Camargo-Sanabria and Mandujano 2011). Pellet counts have also been successfully applied to study different aspects of northern herbivore ecology, but discussions among Herbivory Network members showed the need for a protocol with specific recommendations for how to carry out pellet counts to obtain comparable data for tundra herbivores.

Pellet counts have been used in long-term studies to estimate changes over time in herbivore density. For instance, Krebs et al. (2001) studied snowshoe hares in Canada over a period of 10 years, demonstrating a strong relationship between pellet counts and population density estimated from capture–mark–recapture data. Pellet counts have also been shown to correlate well to herbivore density at larger spatial scales, using spatial contrasts. For instance, Bråthen et al. (2007) showed that the occurrence of pellets of semi-domestic reindeer was correlated with reindeer densities across different herding districts. Evans et al. (2007) documented a good correlation between roost piles of red grouse in Scotland and grouse density estimates based on counts with pointer dogs both in space and time. However, other studies have obtained varying results and poorer congruence (e.g., Härkönen and Heikkilä 1999 for moose in Finland).

Pellet counts have also been used for large scale assessments of herbivore loads at different sites. For example, Krebs et al. (2003) used estimates from the literature of pellet persistence in different habitats, defecation rates, and average weight of different herbivores, to convert pellet count data to biomass of several herbivore species per area. Furthermore,

Fig. B1. Recommended protocols for pellet counting in tundra, in terms of spatial design depend on the aim of the study: (A) one-time surveys aimed at comparing herbivore communities across study areas, (B) monitoring of herbivore activity in a study area, and (C) habitat use or species co-occurrence studies.

	AIM OF STUDY		
	A One-time survey aimed at comparing herbivore communities across study areas	**B** Long-term monitoring aimed at assessing changes in herbivore activity in a study area	**C** Herbivore habitat selection or species co-occurrence
PELLET REMOVAL	No (one-time survey)	Yes (temporal variation) – sampling units should be **permanently marked**	Yes or no (depending on the time frame of the study)
SPATIAL DESIGN	**Random placement** of sampling units allows comparisons across study areas independent of habitat composition	**Habitat stratification** targeting habitats known to be used by herbivores is more efficient to determine year to year variation. A **block design** where replicates represent different independent groups of herbivores could be adequate in most situations.	**Habitat stratification** targeting the habitats of interest or potential focal habitats for species interactions. A **block design** where replicates represent different independent groups of herbivores could be adequate in most situations.
SAMPLING UNITS (plots or transects)	In the **low Arctic**, where vegetation is dense, smaller **plots** (e.g. 50x50 cm) arranged in groups allow better detection of pellets and more precise counts		
	In the **high Arctic**, where vegetation is short and density of feces low **transects** allow covering more area		
NUMBER OF REPLICATES	In heterogenous landscapes it is important to **maximize the number of random sampling points** (at least 30). Common species should be detected in ca 30% of the random points.	A rule of thumb regarding the number and size of sampling could be at least 20 sampling units where feces are detected.	Number of sampling units will depend on obtaining sufficient detections in each block to evaluate the targeted interactions (herbivore-habitat or herbivore-herbivore)

many studies have used pellet counts to investigate habitat use of bird and mammal herbivores (e.g., Klein and Bay 1994) for the herbivore community in High Arctic Greenland; (Henden et al. 2011 for ptarmigan; Ehrich et al. 2012 for ptarmigan and hare; Skarin 2007 for semi-domestic reindeer), or to infer possible ecological interactions from species co-occurrence (e.g., Ims et al. 2007).

Herbivore feces counts have, thus, been used in many studies to assess patterns of occurrence or abundance both in space and time, for single or multiple species of northern herbivores. The specific details and protocols used depend on the aims of the study and the resources available, but the lack of a general protocol for pellet counts in tundra areas prevents meaningful comparisons across studies.

General recommendations for pellet counts in tundra ecosystems

The study design for using pellet counts will be determined by the aim of the study. Our first decision point will be if the study aims to: (A) be a one-time survey covering multiple sites across large areas, (B) monitor changes in herbivore abundance or activity over time in a study area, or (C) address specific questions regarding habitat use or species interactions (Fig. B1). In the following sections we provide specific recommendations for each of these broad study aims separately, specifically on the planning, configuration, and main activities to be conducted in the field. Finally, we complement this information with examples from the published literature.

A. One-time survey aimed at comparing herbivore communities across study areas

In many studies where the goal is a large-scale comparison of the herbivore community across sites (Fig. B1A), a one-time survey will be enough. In such cases, pellet counts will be performed once at each site, sampling units do not need to be permanently marked and pellets will not be removed after counting. Pellet numbers reflect accumulation of pellets

over an unknown amount of time. To compare counts across different habitats or larger areas, some assumptions need to be made regarding pellet persistence, which may differ among herbivores, vary by habitat or with latitude (e.g., Klein and Bay 1994; Krebs et al. 2003). To address this issue some studies differentiate between fecal pellets of different ages or seasons (e.g., Härkönen and Heikkilä 1999; Evans et al. 2007), but in some cases age identification of pellets is not feasible.

When the aim of the study is to compare herbivore communities between different locations in the tundra, it is important that the study design is independent of the specific habitat configuration, as it is difficult to obtain a consistent habitat stratification that spans over large biogeographical regions. Without an accurate habitat map, which is usually not available when doing large scale comparisons between different relatively little studied areas, it is not easy to a priori obtain a choice of locations within each study area that would be representative for the study area (Krebs et al. 2003). Therefore, to be comparable, herbivore pellet counts should be carried out at random locations across the heterogeneous landscape in each study area.

Planning the study design

1. *Identify the study area* by using available information (GIS maps, paper maps, knowledge of the locality). The study area can be located around an existing study site, such as an ITEX site or other stationary long-term project site, it can be an area where other ecosystem components are being surveyed (e.g., Arctic fox den survey, small rodent trapping), or a little-studied area that is investigated. The study area can also be delimited by using available information on herbivore home ranges, density estimates or other boundaries such as pasture districts.

2. *Define vegetated areas within the study area and any other potential further stratification.* Although some non-vegetated areas might be used by herbivores sporadically or during parts of the season, we suggest leaving out barrens, boulder fields, glaciers, water bodies, etc. Although not limiting the sampling to specific habitat types, we recommend restricting the area for random point distribution to vegetated areas, which could be used by herbivores for foraging. If the study area is large and good prior information is available (e.g., vegetation map, bedrock, other GIS layers), it is possible to make other kinds of rough stratification of the area such as vegetated slopes vs. vegetated flats or bedrock categories, and decide to focus on certain strata. However, it is important to keep in mind that the pellet density estimates as a proxy for herbivore activity will be representative only for the selected strata.

3. *Consider logistic limitations* and if necessary, delimit the study area further to what will be realistic to cover and where it is feasible to do the sampling. In a very large study area, it can be an effective approach to identify several sub-areas in which sampling at random points will be carried out. Each sub-area should be large enough to cover the landscape heterogeneity of the total area. Strictly speaking, the estimates of herbivore abundance will only be representative of these sub-areas. Record the exact delimitation of the study area, and all criteria and arguments used to exclude specific components and restrict the definition of the study area.

4. *Locate random points* that will serve as transect starting points in the study area. This can be done in a GIS software, or manually using maps and Global Positioning System (GPS). Any approach that ensures that the random points are independent of subjective decisions in the field will work.

5. *Randomly draw points for pellet counts.* Make a list of starting points and draw a number that may be feasible to visit. Based on the results of this paper we recommend at least 30 pellet count sites in a study area encompassing a heterogeneous landscape at least several square

km large (simulations were carried out for an area of 6 km × 6 km). This should allow capturing the most important herbivore species present in a given location and comparing their relative abundance across locations. For a very heterogeneous landscape, more sites will be necessary to provide a good coverage. The simulation script provided in Supplementary Text File S1[2] can give some ideas about bias and variance of pellet counts given different sampling designs.

6. *Determine criteria for exclusion of starting points in advance*. Not all starting points will be suitable and criteria for excluding them should be defined in advance. For example, it can be decided a priori that starting points for transects where more than 50% of the transect would be covered in water can be discarded.

Configuration of the counts at each point — which type of sampling units to use

Typically, pellet counts are conducted along linear transects or in smaller sampling plots. The choice between these two types of sampling units will largely depend on vegetation characteristics, the herbivore species present and their densities (this paper). In rather lush vegetation in the Low Arctic, we showed that pellet counts on quadrats of 50 cm × 50 cm gave more precise results than counts carried out over 1 m wide and 30 m long transects. Plots can be arranged as linear transects separated by a fixed distance, or for example as two parallel lines. In High Arctic areas, by contrast, where vegetation is low, pellets are easily detected and often occur at much lower densities, thus, the counts need to cover a larger area. In this case, 1 m wide transects of 5 m or longer may be more effective. Another consideration will be the type of herbivore present. For smaller herbivores, smaller sampling plots yield better pellet count estimates than transects (Fig. 4). Decide in advance the layout for a group of plots or a transect at each starting point. Any predefined rule is suitable, but it is important that it is independent from subjective or ad hoc choices in the field. For example, you may decide to always orient transects in a certain direction (e.g., always to the north) or to use a random direction at each point, choosing for example a random number between 0 and 360 for the heading.

Sampling effort — number of replicates

The number of sampling units (number of small plots or the length and number of transects) will also depend on herbivore density in the study area. The effort at each point (number of plots or length of transect) should thus reflect the density of feces of the focal herbivore species. To obtain reliable estimates of pellet densities a sufficient amount of non-zero data are needed. As a rule of thumb, we recommend that the presence of common herbivores is detected in at least 30% of the sampling points. The results of this paper show that to obtain more precise and unbiased estimates of pellet counts from random locations and to make the counts representative for the whole study area, it is better to increase the spatial extent by conducting counts at more random points, and use shorter transects or fewer plots at each point. There will be a trade-off between the number of sampling points and the amount of time necessary to travel between points, but an indication about the optimal number of sampling points can be obtained from the simulation script provided in Supplementary Text File S1[2].

Practical recommendations in the field

- Visit each starting point. If the point must be excluded based on the predefined criteria (see above), go to the next possible starting point. Visit as many points as needed to fulfil the minimum number of sampling points.
- For counting on small plots, it is convenient to make a frame of the size of the plot, which can be put on the ground at each sampling point and clearly shows the delimitation of the plot.

Frames can be made very simply for example from wire, or using more durable materials, like aluminum or wood. You can use a measuring tape rolled out in the predefined direction to place the frames along it at a predefined distance from each other.

- For pellet counting on transects, it is convenient to roll out a measuring tape in the predefined direction, and to walk along it with a stick (ruler) of the width of the transect to make it easy to see what is within the transect and what is outside of it.
- Along the transect, counts of pellets of each focal herbivore species or group are recorded for each small quadrat frame or for each 1 m segment along the transect. See the section on counting and data recording.
- Additional measures of signs of herbivory (e.g., grazing or browsing marks, grubbing signs, and rodent activity) and the dominant plant communities can also be recorded, as they provide also useful data for assessing the importance of herbivory.
- Suggested covariates to be recorded for each transect:

  ○ slope along transect,
  ○ general vegetation type,
  ○ microtopography,
  ○ elevation (given by GPS –remember to verify that the GPS elevation is calibrated correctly; alternatively, elevation can be obtained from a good Digital Elevation Model),
  ○ GPS coordinates of start and end of the transect,
  ○ height of vegetation,
  ○ date,
  ○ observer.

Literature examples

An example of large-scale pellet count study is presented in Bråthen et al. (2007) and Ims et al. (2007). The study covered 20 reindeer herding districts in Finnmark, northern Norway, representing more than half of the available summer pastures in the region. Pairs of reindeer herding districts were chosen as pairwise comparisons for their contrasting reindeer densities and the aims of the studies were to assess the effect of reindeer densities on vegetation and on other vertebrate herbivores, respectively. To cover such an extensive area, a hierarchical stratified sampling design was developed. Vegetation strata were delineated based on vegetation type classifications and satellite images, and sampling was restricted to low alpine zone areas dominated by mesic and wet vegetation types, where herbivores were expected to concentrate their activity.

The procedure for choosing sampling points was as follows: a 2 km × 2 km grid of landscape areas was laid over the areas in each district laying in the low alpine zone. From these, landscape areas with more than average amounts of mesic and wet vegetation were chosen. By doing so, the counts obtained in this study are only representative for the more vegetated parts of the study area, and not the dry heath, boulder fields or barren rock uplands. Landscape areas that included more than 50% of forest, lakes, sea, glaciers, or included a major road, were excluded because such factors may influence the presence of herbivores. From all remaining landscape areas, between 4 and 14 were chosen at random in each district (number proportional to the area of the focal stratum in each district).

The selected 2 km × 2 km areas were further subdivided into 100 quadrats of 0.4 km^2, among which 25 were selected at random for pellet counting. The centre of the selected quadrat was the starting point for a 50 m sampling line, the direction of which was given by a random GPS position on a circle with a 50 m radius. Along the sampling line, pellets of different herbivores were counted every 5 m in 11 small triangular plots (40 cm side).

Together with the herbivore pellet counts, the vegetation was described in the same sampling triangles using the point intercept method (Bråthen et al. 2007).

B. Long-term monitoring aimed at assessing changes in herbivore activity in a study area

When the aim of the study is to assess temporal changes in herbivore abundance or activity within a study area, a different approach is recommended (Fig. B1B). Pellet counts should be carried out on permanently marked plots, and pellets are removed after each count. Pellet removal presents the advantage that the time over which pellets have accumulated is known and pellet abundance can be related to a specific year or season (Krebs et al. 2001; Henden et al. 2011).

Most herbivores do clearly not use the landscape at random and prefer certain habitats to forage, often with seasonal patterns of use. Therefore, if the aim is to monitor trends in herbivore activity over time, it is best to establish the sampling units in the habitats used by the herbivores. That prevents investing time on counting plots with very few occurrences.

Planning the study design

1. *Delimit the study area* by using available information (see A).
2. *Identify the focal vegetation or landscape strata*. If habitat preferences of herbivores in the study area are not known, data from the literature or expert knowledge from other areas can be used. Alternatively, an initial survey could be performed to assess habitat preferences. In this aim, pellet counts should be organized in a random stratified design, where all potentially important habitats are represented, and an even number of counts are carried out at each site. If available for the study area, this design can be made based on GIS layers or topographic, vegetation or bedrock maps. Otherwise, a classification in landscape elements or habitat types can be made during exploratory walks in the study area. Herbivores often prefer the most productive parts of the landscape, which may be a good starting point for a habitat stratification (e.g., Bråthen et al. 2007). It is likely that herbivores use different habitats in different seasons. If seasonal monitoring is to be included in the study, these differences in habitat preferences need to be taken into account when determining focal landscape strata.
3. *Number of focal strata*. For statistical analysis it is not effective to have too many strata, and often better to have more replicates in each stratum. This consideration needs, however, to be balanced against representativeness. If monitoring is only carried out in one rather narrowly determined stratum, some aspects of herbivore abundance fluctuations may be missed. For example, if monitoring focuses only on summer habitat, variation in presence of herbivores over winter, which may result from changes in migration patterns may not be detected by the monitoring data.
4. *Block design*. Within the study area, a block design is an effective way to arrange replicated counts. Based on maps of the study area or on previous knowledge, a series of landscape blocks or elements are identified. These could be roughly similar valleys, similar hills, or areas of several square kilometres, which contain all the focal landscape strata. The blocks should be far enough from each other to be used by different groups of herbivores to serve as independent replicates for monitoring herbivore abundance over time. On the other hand, logistic constraints usually require that blocks are close enough to each other to be reached by foot/boat/vehicle and can be surveyed within a reasonable unit of field time, often a day or some days.
5. *Random choice of sampling points*. Also, in a block design with focal habitat strata, it is important to choose the exact sampling locations according to a stratified random scheme (Mörsdorf et al. 2015). This is best done by choosing several replicate locations in the focal

stratum (strata) within each block among which the locations to use as sampling points will be drawn at random. If GIS layers or good maps are available, this can be done in advance. Otherwise, possible locations can be mapped in the field, and then a random subsample can be drawn to be used as monitoring sites.

6. *Timing and frequency of counts.* Pellet counts should be carried out at the same time of the year each year. In the aim of monitoring herbivore populations, usually annual or bi-annual removal counts (spring and fall for example) are carried out.

Configuration of the counts at each point — which type of sampling units to use

As for (A) above, the best configuration for the counts at each point will depend on the vegetation, and the type of herbivores and their density in the area. Pellets can be counted in small plots or along transects. Plots can be arranged in groups or in a predefined configuration such as a line or around a quadrat, or in a collection of randomly chosen plots.

For repeated counts on removal plots, the plots or transects must be permanently marked with markers that will not be removed by herbivores. Colored metal or wooden sticks can be used, but care should be taken that markers are not too conspicuous, as some animals avoid marked plots (e.g., Nugent et al. 1997) or use them as marking spots. In low High Arctic vegetation, large nails can be put in the ground through a small square of coloured tape, to create a mark that is less conspicuous. Avoid using plastic ribbon that will be ripped into many small plastic fragments by wind and weather in the course of the year, and can also be attractive to some herbivores. Having two to four marking sticks at each small plot, around which a sampling frame can be placed, usually works well.

Removed pellets from each sampling unit can be kept for further analysis, as they can provide important information on herbivore diet (Soininen et al. 2009, 2015), population structure (Kohn and Wayne 1997), or nutritional quality (Leslie et al. 2008).

Sampling effort — number of replicates

The number of blocks and the number of replicated counting locations for each stratum within blocks will depend on the time allocated to pellet counting at each sampling occasion. As for A) above, there will also be a trade-off between more replicated locations and more counting plots at each location. To obtain annual or seasonal estimates, non-zero data in most seasons are needed. The density of the focal herbivores will also matter; for rare species more replicates are needed. A rule of thumb could be at least 20 sampling units where feces are detected in an average year/season.

Practical recommendations in the field

- For counting on small plots, it is convenient to use a frame of the size of the plot, which can be put on the ground at each plot and clearly shows the delimitation of the plot (see A). Place the frame on the permanently marked plots.
- For counting on transects, it is convenient to roll out a measuring tape between two permanent marking pins, and walk along it with a stick (ruler) of the length of the transect width to make it easy to see what is within the transect and what is outside of it. Alternatively, two measuring tapes or ropes can be laid out, one on each side of the transect.
- Count fecal pellets of each species and remove them from the plots and their immediate surroundings to avoid wind and water from drifting pellets from adjacent areas onto the plots.
- Additional measures of other signs of herbivory (e.g., grubbing signs, rodent activity), and information on the dominant plant communities can also be recorded, as they provide also useful data for assessing the importance of herbivory.

Literature examples

Krebs et al. (2001) compared pellet counts of snowshoe hares with hare abundance esti-
mated from live trapping in the Canadian boreal forest. They used ten different snowshoe
hare live trapping areas and annual removal counts for the comparison. Each of these areas
can be considered one block in a block design. In each block, pellet counts were carried out
on 80 sampling quadrats that were arranged systematically in each live trapping area. The
quadrats were long and narrow (5.2 cm × 302 cm) and 20 quadrats spaced by 30 m were
arranged in four parallel lines spaced 120 m apart. Quadrats were marked with permanent
markers at each end and in the centre. Pellets were counted each year in June and removed
from the plots.

Another example is presented in the study by Henden et al. (2011), who counted fecal
pellets of ptarmigan in removal plots twice per year in eastern Finnmark, Norway. Counts
were carried out shortly after snow melt to reflect ptarmigan presence in winter (the period
with snow cover), and a second time in the end of August to represent ptarmigan presence
in summer. In this Low Arctic region, willow thickets are a hotspot of productivity and
biodiversity in an otherwise low productive tundra landscape. Therefore, the focal stratum
for pellet counts was the edge of willow thickets and the productive meadows often
surrounding the thickets on the riparian sediment plains of the valleys. The study design
consisted of several levels of nesting. At the largest scale there were three study regions
with the same main vegetation and landscape characteristics that were chosen to contain
fertile riparian plains. This scale was convenient for having separate field teams staying in
different camps. Within each region, three valleys or valley segments with riparian plains
were chosen, and within each of them four to six sampling locations were chosen in the
focal habitat. This scale was convenient for members of a field team to reach on foot from
their camp. At each sampling location, a 15 m × 15 m plot was delimited with one side of
the plot following the edge of the willow thicket. Pellet counts were carried out in eight
small 50 cm × 50 cm quadrats arranged around the 15 m × 15 m plot.

C. Studies focusing on herbivore habitat selection or species co-occurrence/interactions

Herbivore pellet counts have also been used to answer more specific questions, for
instance about habitat use or co-occurrence of different species of herbivores (e.g., Skarin
2007; Ehrich et al. 2012 for habitat use; Klein and Bay 1994 for co-occurrence. For such stud-
ies, one-time pellet counts will often be appropriate. However, if seasonal use or density-
dependent use are to be addressed, removal plots should be used.

Spatial designs will depend on the specific aims of the study. For habitat choice studies,
two main different approaches have been taken: the sampling can be stratified according to
a series of a priori defined focal habitats (as described under method B; e.g., Ehrich et al.
2012), or a random sampling approach can be used (as described under method A), or the
habitat can be described at each of a series of random location to determine habitat factors
associated with increased pellet numbers (e.g., Skarin 2007)). Based on statistical considera-
tions, we recommend the first approach as it allows to have a balanced design regarding the
number of plots sampled for each habitat category. A block design taking into account
larger landscape features of the study area can also be used to minimize correlations of dif-
ferent factors, such as for example altitude and exposition, which the study might want to
analyze as separate explanatory variables.

For species interaction studies, focal habitats as well as random locations may be used.

Planning the study design

1.   *Delimit the study area* by using available information (see method A).

2. *Identify the focal vegetation or landscape strata.* When doing a habitat selection study, habitats should be described based on explicit criteria that allow researchers to classify most points of the study area in a certain habitat type.

3. *Block design.* As for method B, a block design is an effective way to arrange replicated counts. Based on maps of the study area or on previous knowledge, a series of landscape blocks or elements are identified. These could be similar valleys, similar hills, or areas of several square kilometres, which contain all the focal landscape strata. The blocks should be far enough from each other to be used by different groups of herbivores to serve as independent replicates to assess habitat choice or herbivore species interactions.

4. *Random choice of sampling points.* Also, in a block design with focal habitat strata, it is important to choose the exact sampling locations according to a stratified random scheme (Mörsdorf et al. 2015). This is best done by choosing a number of replicate locations in the focal stratum (strata) within each block among which the locations to use as sampling points will be drawn at random. If GIS layers or good maps are available, this can be done in advance. Otherwise, possible locations can be mapped in the field, and then a random subsample can be drawn to be used as monitoring sites.

5. *Timing and frequency of counts.* Habitat choice studies can be performed as one-time surveys. They can also address seasonal variation and (or) multi-annual variation depending for instance on the small rodent cycle or on spring phenology. This will depend on the aim of the study and should be carefully thought through in the planning phase.

Configuration of the counts at each point — which type of sampling units to use

As for method A (above), the best configuration for the counts at each point will depend on the vegetation, the type of herbivores, and their density in the area. Pellets can be counted in small plots or along transects. Plots can be arranged in groups or in a predefined configuration such as a line or around a quadrat, or in a collection of randomly chosen plots.

Sampling effort — number of replicates

The number of replicates and the area of the counting units will depend, in the first place, on the density of the focal herbivores. To be able to detect differences in the preferences of the herbivores for different habitats and interactions between the species a considerable amount of detections in each stratum will be required. A pilot study can give an indication of pellet densities and such preliminary estimates can be used to plan the final design. In general, considerably more replicates will be required for such specific studies than for the survey or monitoring pellet counts outlined above.

Practical recommendations in the field

See practical recommendations in the field for methods A and B above.

Literature examples

An example of a study using pellet counts to assess habitat use by different species of herbivores is provided by Ehrich et al. (2012). In their study, they investigated the use of willow patches by two specialized herbivores, the willow ptarmigan (*Lagopus lagopus* (Linnaeus, 1758)) and mountain hare (*Lepus timidus* Linnaeus, 1758), spanning three subarctic shrub tundra regions in northern Norway, northern European Russia, and western Siberia. Willow thickets provide food and shelter to herbivores, and are, thus, an important habitat. To quantify habitat use by the focal herbivores, the authors used pellet counts in a hierarchical spatial design. At the largest scale they compared the three study regions. Within each study region they established two to four units, at least 2 km apart and 15 m × 15 m in size. Within each unit they established permanently marked sampling plots (0.5 m × 0.5 m) in

willow thickets and the adjacent tundra. Units and plots were selected to cover the existing variation in willow thickets at each study region. Each plot was sampled twice per year, shortly after snowmelt and by the end of summer. Pellets of both species of herbivores were counted and removed from the plots in each visit.

An example of a study using pellet counts to infer co-occurrence of different species of Arctic herbivores was presented by Klein and Bay (1994). At their High Arctic Greenland study site, they investigated habitat use by muskoxen (*Ovibos moschatus* (Zimmermann, 1780)) and hares (*Lepus arcticus* Ross, 1819) using pellet counts. In addition, they assessed habitat use by collared lemmings (*Dicrostonyx groenlandicus* (Traill, 1823)) using other presence signs (e.g., active burrows and winter nests). Their study area (15 km^2) consisted of four main vegetation types relevant to herbivores: sedge fens dominated by *Carex stans* Drejer, sedge grasslands dominated by *Eriophorum triste* (Th. Fr.) Hadac & Á. Löve, hummocky willow (*Salix arctica* Pall.) slopes, and open willow flats. These habitats were mapped, and pellet count transects were established on each habitat. For muskoxen and hare, winter and summer pellets were differentiated based on their morphology.

Data recording and analysis
Counting single pellets or groups/clumps

Although some herbivore species such as hares or geese leave single fecal pellets, others can produce both single pellets, groups or piles of pellets, or clumps. Single pellet counts and counts of pellet groups have been used in different studies, and a feces counting protocol needs to specify how pellets should be counted in each case.

One possibility is to record separately single pellets and clumps or pellet groups and apply a conversion factor to single pellets when processing the data. Such conversion factors are based on the approximate average number of pellets in a clump to convert clumps to pellets. For example, we used 30 as a conversion factor for clumps of reindeer feces and 20 for clumps of muskoxen feces.

Another possibility is to count only groups of feces, each group of pellets representing a single defecation event (e.g., Eberhardt and Etten 1956). Evans et al. (2007) counted roost piles of grouse and assessed how well roost pile density reflected abundance as estimated with pointer dogs. A similar approach was used by Skarin (2009), who counted the number of reindeer pellet groups in a survey in Sweden and used the approximate number of pellets in a group as a conversion factor. When using this approach, an arbitrary limit should be set for the minimum number of pellets that are counted as a group.

Counting presence only

In some cases, especially for small rodents, fecal pellets can be extremely abundant. It is then worth considering recording these as presence only or counting single pellets until a given threshold (e.g., 20) and then recording "more than 20".

Species identification

The identity of the species should be recorded to the lowest taxonomic level possible. In many cases identifying pellets will not be possible for closely related herbivore taxa, so broader categories such as ptarmigan (willow ptarmigan and rock ptarmigan) or goose may be needed (Fig. B2). Pellets for each herbivore or herbivore group should be recorded separately.

Analyzing pellet counts or presence absence

Fecal pellet data can be analyzed as pellet densities, i.e., the average number of pellets for a given surface, or they can be analysed as presence–absence data. In the first case, the data can be modelled using generalized linear models or generalized linear mixed models

Fig. B2. Examples of pellets of Arctic herbivores: (*a*) lemming, (*b*) caribou, (*c*) ptarmigan, (*d*) muskoxen, (*e*) hare, (*f*) goose. (Images (*a*), (*c*), and (*e*) courtesy of Siri Lie Olsen, Norway; images (*b*) and (*f*) courtesy of Hanna Christoffersen, United States; and image (*d*) courtesy of Cornelia Schütz, Germany).
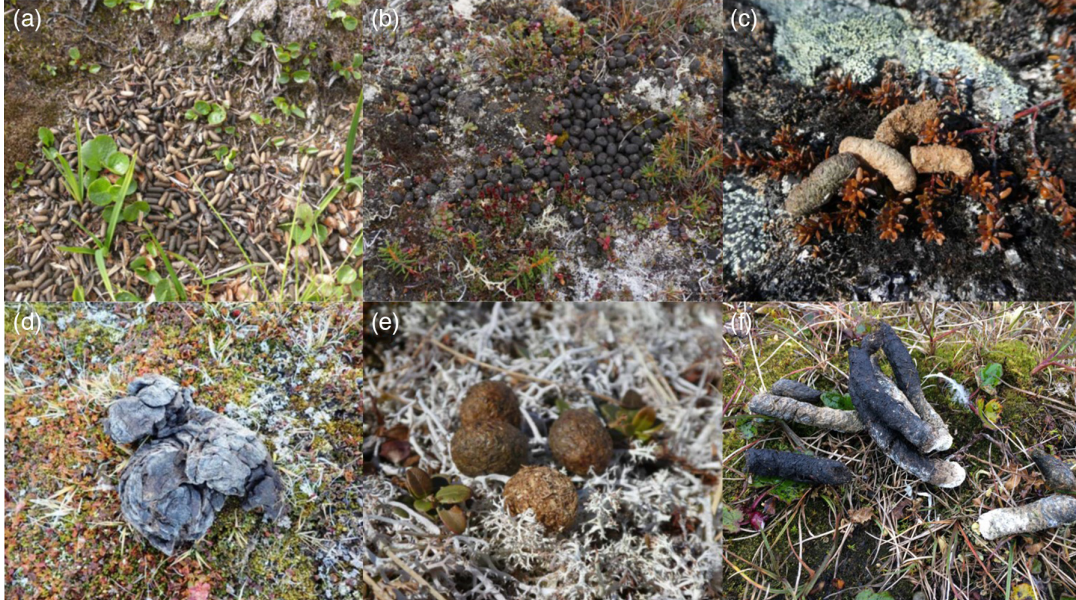
with a Poisson error distribution, whereas in the second case similar models with a binomial error distribution will be appropriate. More advanced data analysis approaches taking into account imperfect detection and measurement error in hierarchical models can also be used (Forsyth et al. 2007; Alves et al. 2013).

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