Herbivory Network Pilot Soil Protocol –field season 2016

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Introduction

All over the globe, herbivores can have strong effects on soils over spatial and temporal scales [1-3]. These effects are direct, such as trampling, excretion of waste products (faeces, urine, frass), biomass and litter removal, as well as nutrient translocation, or indirect effects mediated by changes in e.g. plant litter quality and quantity or root exudation, caused by changes in plant and microbial community composition and plant fitness [4-6]. These changes can feedback to herbivores through shifts in vegetation productivity, forage quality and quantity, and can also alter ecosystem function, resilience and development [7-10]. The effect of herbivory on soils may be especially pronounced in tundra ecosystems, where herbivory can limit vegetation biomass significantly [11] and the majority of biomass is situated belowground [12]. Despite their potential importance, the effects of herbivory on tundra soils are largely unknown.

Aim of the Pilot Soil Protocol (PSP)

The aims of this pilot soil protocol (hereafter PSP) are to provide guidelines for testing a selected group of chemical and physical properties of soils (hereafter 'soil traits') and to analyze the spatial variation of the effects of herbivory on these soil traits. The PSP consists of two levels: the site assessment and the plot-level assessment. All suggested measurements will be conducted only at the plot-level. The PSP focuses on soil traits that are relevant for understanding herbivore-soil interactions (Figure 1).

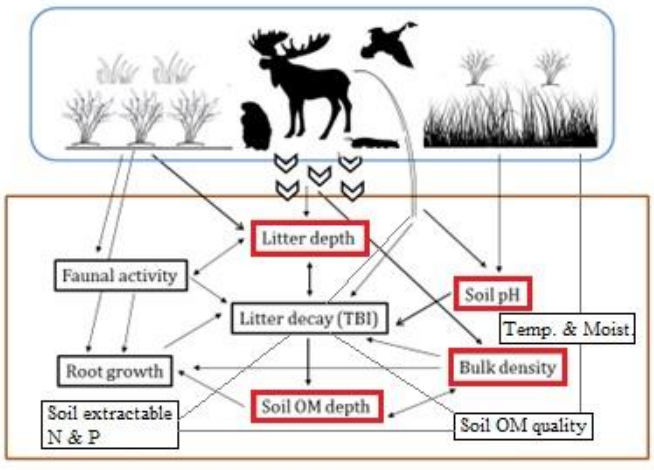

Figure 1. Direct and indirect (through altering vegetation) relationships between herbivores and the selected soil traits. The relationships among soil traits are also indicated. The selected soil traits presented in the PSP are highlighted in red.

The data obtained with PSP will be used to evaluate the use of these selected soil traits to provide improved field and laboratory guidelines for the final Soil protocol (SP), which will be an all-inclusive add-on for the general Herbivory Network protocol. All soil traits listed in PSP are such that they are known to influence microbially mediated release of nutrients from litter and soil organic matter. Thus, all soil traits measured here are important mediators for soil nutrient availability that may ultimately feedback to herbivores via altered plant productivity and forage quality. Due to the complexity of soil nutrient analyses, these analyses are not included in PSP at the moment (but see Materials and methods).

PSP also suggests an intensive sampling design for the selected soil traits (Figure 2). This initial sampling design aims at resolving the spatial scales relevant at understanding site-specific characteristics in terms of herbivore-plant-soil interactions. This information will allow the optimization of sampling resolution and effort for the final SP.

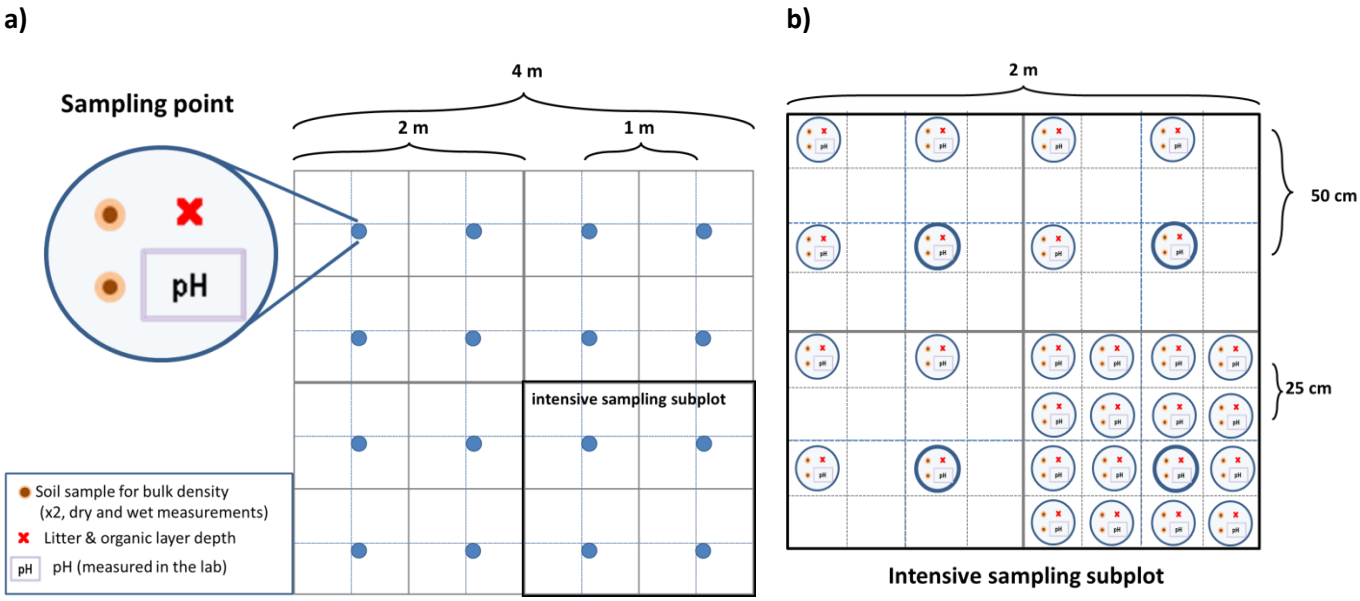

Figure 2. Sampling plot of 4x4 m for the measurements of three main soil traits: bulk density, soil depth (litter and organic matter layers) and pH, at three different scales: small (25 cm), medium (50 cm), and large (1 m), with 16 sampling points each scale (total 40 sampling points). a) Sampling plot unit with the large sampling points indicated with blue dots. b) Details for the medium and small sampling points *intensive sampling subplot* of 2x2 m containing 28 sampling points). Darker blue circles in the *intensive sampling subplot* (b), indicate the sampling points shared with the large scale.

Material and methods

Site assessment

The overall assessment of the site captures all relevant site-specific climatic, geological and physical conditions and provides valuable metadata for between-site comparisons. Optimally, it includes a brief description of:

- ✓ Site location (GPS coordinates; longitude, latitude)
- ✓ Type of lithology and soil.
- ✓ Cryogenic soil processes (effect and extent/abundance of ice-related processes altering the soil).
- ✓ Topography (hill-valley, ridge-brook, tussock-inter tussock variations, slope, aspect)

- ✓ Annual and summer temperature, precipitation, and any remarkable extreme event, such as flooding.
- ✓ Main herbivores and herbivory presence (list of herbivores observed, presence/absence of herbivory, number of droppings in the plots of each vertebrate herbivore if possible).
- ✓ Vegetation type (for classification, see Shaver et al. (2007) [13].

Plot-level assessment

The major aim of plot-level sampling is to capture within-site variation in soil traits, thus providing an unbiased mean and variability estimates. In PSP all site level measurements are based on a 4 × 4 m plot with 40 sampling points (Figure 2). The 4 × 4 m plot is repeated 2 – 3 times within a c. 100 m^2 area with similar vegetation and topography. If the study site includes herbivore exclosures, it is highly recommended to conduct sampling in both grazed and ungrazed sites. For the ungrazed sites only one 4 × 4 m plot is sampled. This will add, a good basis to understand how tundra soil heterogeneity is modified by herbivores. As a first approach, the current sampling scheme is recommended regardless of herbivore types and their densities. It is important to remark that in order to assess soil spatial heterogeneity, **each soil sample and soil trait measurement from different sampling points has to be identified/labelled and recorded individually.**

PSP gives instructions for analyzing three soil traits. Soil traits have been selected based on their feasibility and relevance for the system and their potential sensitivity to herbivore activities (Table 1). Detailed instructions for conducting the measurements for each soil trait are provided below. It is strongly recommended that the remaining of the soil samples are kept frozen; if not possible, please contact us. These samples will be invaluable for complementary chemical analyses in the near future.

Table 1. Proposed soil traits to be measured once in the growing season and tested with the pilot version of SP. The relevance and the potential sensitivity of each soil trait to herbivory is addressed.

Soil Trait	Relevance	Sensitivity to herbivory	Method
(1) Litter/organic layer depth	Root depth limitation. Indirect measurement of soil development	Descriptive; herbivory may reduce litter thickness	-probing, non-destructive
(2) Bulk density (root biomass)	Soil hydrology, porosity, oxygen levels and movement of dissolved nutrients	Tramping may increase soil compaction that can be detected measuring bulk density	-weighing dried soil cores/roots with known volume
(3) Soil pH	Crucial for some plant nutrient uptake and for key soil processes	Affected by urine, feces and vegetation shifts	-pH-meter

(1) Litter and organic layer depth

Litter and organic layer depth is measured using a probing stick. Ideally, the stick is sharp and has a scale (mm). Alternatively a long metal pin of around 2-5 mm diameter can be used in combination with a tape measure. The litter and organic layer thickness are measured on 40 points (see **Figure 2**). First, the litter layer thickness is measured by inserting the probing stick until it touches the soil ground. This measure is then recorded as the littler thickness. Then, at the same spot, press the stick until you hear a crunching sound or hit a rock and record the thickness again for the organic layer thickness. Repeat for all points and plots. If the spot has moss layer, then follow the same procedure, but annotating that the organic layer corresponds to the moss cover. Preferred sampling time: once around the peak of the growing season.

Materials: 1) probing stick; 2) Field sheet (see field sheet attached to this protocol)

Time estimation: 5-10 min per plot

(2), (3) Soil sampling

Measuring soil bulk density (2) and pH (3) requires destructive soil sampling. Soil samples are collected using a soil corer (Suppl. Fig 1) following the sampling scheme (Fig. 2). We recommend using a 2-3 cm diameter soil corer, but any size will do. Just make sure you use same soil corer for all sampling and write down the corer diameter (mm). Each soil sample (i.e. soil core) is treated as an independent sample and thus collected and stored separately (each soil core is stored in a separate plastic bag). Preferred sampling time: once in or around the peak of the growing season.

Step-by-step instructions for soil sampling and storage:

1. From the sampling point [same as for (1)], push aside vascular plants, but leave cryptogams and litter untouched. Set the soil corer over the sampling point and press/twist the corer into the soil. Core until you encounter a rock or mineral soil horizon. Encounter with mineral soil can be detected by a "scrunching" sound.
2. Lift the soil core from the ground. Remove the litter layer from the soil core. Detect and remove from the soil core the possibly included mineral soil horizon. Because separation of horizons is subjective, it is advisable that the same person conducts this part of the sampling every time, and subjective decisions are documented in pictures. After separation of organic and mineral horizons measure and record again the organic layer thickness [comparison with probing (1) approach]. Repeat the procedure, as you need to take two soil cores from each soil sampling point, one core for bulk density (2) and the other for pH (Fig. 2). For reliable comparison of results, soil sampling should be limited to the uppermost 10 cm organic soil horizon. So, if the organic horizon is > 10 cm deep, collect only the uppermost 10 cm of the core; if soil organic layer is < 10 cm, collect only the organic layer and record the thickness.
3. Store each soil sample in a separate labelled plastic bag, one for each of the 2 replicates from the same sampling spot. Seal the bags. Soil traits (2) and (3) are not sensitive to changes in storage conditions and long (1 – 4 weeks) storage times before processing. If possible, store the samples in darkness at low temperatures (+2 – +8 °C), and record the approx. storage temperature and time before processing.

Materials: 1) soil corer (diam. 2-3 cm; Suppl. Fig. 1), 2) plastic bags (various suppliers, normal household plastic bags, such as mini grip etc. for freezing and storing food), 3) meter/ruler, Field sheet

Time estimation: 1 min per point with 2 soil cores & probing

(2) Bulk density

For measuring bulk density you need to know a) the diameter of the soil corer (recorded above), b) the thickness of the organic soil horizon (recorded above) and c) the weight of the fresh and dried soil sample. The difference in core weight between fresh and dried soil can be used as an estimate for soil moisture and later corrected for accurate soil moisture measurement. The soil samples are dried 48 hours at 105°C (to 'constant weight') in labelled aluminum trays. After drying soil dry mass is recorded (at 0.1 g accuracy). For weighing the core, first

tare the balance with empty aluminum tray and then weigh the dried soil core, which is in another aluminum tray. Calculation of the bulk density (g/cm^3):

$$Bulk\ density = \frac{\text{soil dry weight (g)}}{\pi \times \text{core radius (cm)}^{\wedge}2 \times \text{organic } layer\ thickness\ (cm)}$$

And volumetric water content estimate (g/cm^3):

$$Volumetric\ water\ content = \frac{\text{soil fresh weight (g)} - \text{dry weight (g)}}{\pi \times \text{core radius (cm)}^{\wedge}2 \times \text{organic } layer\ thickness\ (cm)}$$

Materials: drying oven, balance

Time estimation: active 1 min per sample; 48 hours drying time

After determining bulk density, the dried soil core can be sieved (2 mm mesh). The homogenized dried soil material can be stored in a plastic bag at room temperature in darkness for further analyses (bulk C and N) by arranged with the Soil working group. After sieving, root biomass can be recovered and used to determine dry root biomass (g per cm^{-3} and/or per cm^{-2}).

$$Dry\ root\ biomass = \frac{\text{dried root weight (g)}}{\pi \times \text{core radius (cm)}^{\wedge}2\ [\times \text{organic } layer\ thickness\ (cm)]}$$

(3) Soil pH

The other soil sample from each soil sampling point is freshly homogenized (sieve, mesh size 2 mm). In homogenization soil is pushed through the sieve, while roots, stones and debris are retained in the sieve. Soil pH is measured from the homogenized soil sample as follows:

1. Measure 15 mL of homogenized soil into a Nalgene tube (approx. 50 mL). For measuring the soil use a table spoon sized meter. “Scoop” soil, then compress the sample by tapping the spoon slightly, take some more soil to fill the meter and remove the extra soil by drawing e.g. with a ruler.
2. Add 25 mL distilled water (i.e. 3:5 soil – water solution) and shake vigorously for a couple of seconds. Let the samples stand overnight. In cases, where there is not enough soil, you can decrease the volume of soil, but keep the soil: water ratio constant.
3. Shake the samples for 5 min, after which measure the pH with a pH meter. Record the value.

Materials: 1) sieve (2 mm mesh size), 2) 15 mL (metal) scoop, 3) plastic tubes with lids,
4) distilled water, 5) shaker, 6) pH meter

Time estimation: active; 10-15 min per sample (incl. sieving)

After determining soil pH the remaining soil samples can be stored frozen (-20°C) for further analyses by the Soil working group (accurate soil moisture, OM-%, soil extractable nutrients). However, these analyses **are sensitive to drying, long storage times and variations in storage temperatures.**

If you are interested in storing and shipping samples for further analyses by the Soil working group and are in doubt about storing etc. please do not hesitate to contact us (see contact info at the front of this protocol)

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