OrgBalt LIFE project

EU LIFE Programme project
"Demonstration of climate change mitigation potential of nutrients rich organic soils in Baltic States and Finland"

REPORT

ON IMPLEMENTATION OF THE PROJECT

DEMONSTRATION OF CLIMATE CHANGE MITIGATION MEASURES IN NUTRIENTS RICH DRAINED ORGANIC SOILS IN BALTIC STATES AND FINLAND

WORK PACKAGE

FILLING KNOWLEDGE GAPS ON GHG EMISSIONS FROM ORGANIC SOILS (C.1)

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"LIFE OrgBalt compiled the first regional Baltic/ Finnish GHG emission factors for managed nutrient-rich organic soils (current and former peatlands), which have been made available for the customary scientific review and further verification for national GHG inventories in the hemiboreal region in Finland and the Baltic countries. While the project analysed selected CCM measures for drained organic soils in agriculture and forestry and developed spatial models and tools, it also identified remaining knowledge gaps. To bridge the remaining limitations and fill the gaps, it is essential to continue GHG measurements and model development, as well to broaden and complete the scope of the evaluated CCM measures in the after-LIFE-project period, notably by including rewetting and restoration of peatlands that are currently considered to be among the most recommended CCM measures on drained peatlands in the EU. In addition, the developed Simulation and PPC models still include limited macroeconomic considerations and lack assessment of all environmental impacts. For all these reasons, these models should be used carefully in CCM strategy development for identification of gaps in climate neutrality transition policy and funding frameworks and need further optimization for broader applicability as decision-making tools."

SUMMARY

One of the central goals of the Life OrgBalt project is to estimate the annual soil carbon (C) balance for different types of, and differently managed, forests on drained organic soils in the Baltic states and Finland. The aim of this report is to examine the state-of-the-art of litter-input and fine-root data, and to present the protocol for collecting new data in the Life OrgBalt project. For this purpose, we monitor the soil C-stock change by combining measured or modelled biomass data, litter input rates, litter decomposition rates, and gaseous C-losses from the soil based on GHG flux monitoring on the sites. Litter inputs both aboveground and belowground form the "C added" component of the soil C balance. Aboveground litter inputs can be roughly divided into two categories: tree litter, and ground-vegetation litter. Annual litter production will be calculated as the sum of aboveground litter of tree stand, vascular ground vegetation and mosses, and belowground litter of roots and rhizomes.

Most of the previously collected tree litterfall data have been collected in forests growing on mineral soils, and a thorough evaluation of whether models based on such data are applicable also on forests growing on organic soils has not been done. Another caveat is that the data do not cover different management options that affect stand structure and therefore also litterfall patterns in forests growing on drained organic soils. Thus, also tree litter inputs are to be monitored in the Life OrgBalt project. Existing ground vegetation litterfall data are scarce and partly complex to collect, and measurements done in the Life OrgBalt project will also here contribute a significant amount of novel data with an afterlife well beyond the project period.

The root systems of trees and shrubs can be roughly divided into fine roots and coarse roots. Coarse roots are a longer-time C investment, while fine roots turnover is faster and thus, fine roots form a significant annual C input into the soil. There is still considerable uncertainty in estimates of fine-root litter inputs, especially in forests growing on organic soils because production, and turnover (renewal rate) are notoriously laborious to quantify, especially so in organic soils that may largely consist of root and rhizome remains. The approach that we have chosen for the Life OrgBalt project is estimating fine-root biomass from soil cores and fine-root production using ingrowth cores, and estimating fine-root turnover, which is an estimate of the belowground litter inputs, based on those as production per biomass.

We also aim to use data collected in Life OrgBalt for calibrating such models and for developing new models that we can use. Models are used for estimating some of the C-stock components, because the project lifetime is relatively short compared to C-stock changes (e.g. tree biomass production) and some are too laborious to measure directly (e.g. belowground tree biomass C-stock). To achieve the set goals, we have formed protocols for data collection in the partnership. These data collection protocols are presented in this document. Measurements done in the project will contribute a significant amount of novel data from organic soils that can be used for modelling and will thus have an afterlife well beyond the project period.

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1. INTRODUCTION

The goal of the Life OrgBalt project is to estimate the annual soil carbon (C) balance for different types of, and differently managed, forests on drained organic soils in the Baltic states and Finland. For this purpose, we monitor the soil C-stock change by combining measured or modelled biomass data, litter input rates, litter decomposition rates, and gaseous C-losses from the soil based on GHG flux monitoring on the sites. Our evaluation of existing data reported in peer-reviewed publications indicated that in earlier work that focused on drained organic forest soils, not enough attention has always been given to the "mass-based", litter and fine-root C fluxes (Jauhiainen et al., 2019). However, they are critically needed in addition to the monitored GHG data for forming the annual soil C-balance. Litter inputs both aboveground and belowground form the "C added" component of the soil C balance (Figure 1). That is why they are paid specific attention to in the Life OrgBalt project. The aim of this report is to examine the state-of-the-art of litter-input and fine-root data, and to present the protocol for collecting new data in the Life OrgBalt project. In the following, we will use the collective terms "aboveground litter inputs" and "fine-root litter inputs" to describe the mass-based C fluxes to the soil through aboveground litter and fine roots, respectively.

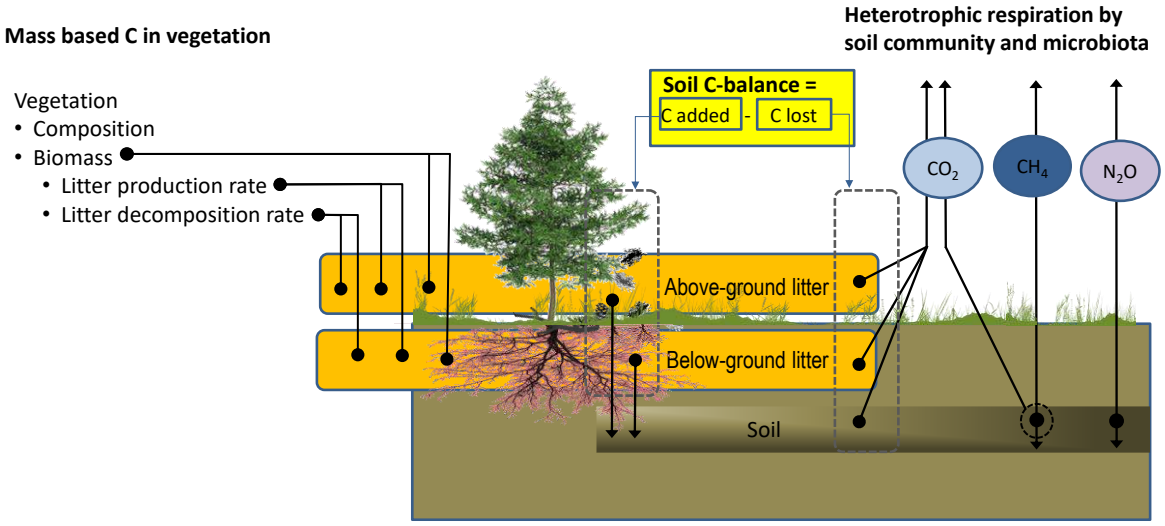

Figure 1 Data needs for estimating the annual soil carbon balance.

2. ABOVEGROUND LITTER INPUTS

Aboveground litter inputs can be roughly divided into two categories: tree litter, and ground-vegetation litter. Tree litter inputs have of old been recognized as an integral part of the element cycles of forest ecosystems (e.g., Nordén 1993; Hansen et al. 2009), and standard measurement protocols exist for collecting foliar litter (e.g., Pitman et al. 2010). In addition to mediating element fluxes, foliar litters also affect the composition of ground flora and fauna (e.g., Sydes and Grime 1981; Stoler and Relyea 2010). A limitation of the standard foliar litter traps is that they do not reliably capture the input of woody litter types: twigs and branches. These may be important carbon inputs, even though they may not carry significant nutrient inputs and have therefore received less attention than foliar litters. Woody litter inputs can be captured with traps placed on the ground (e.g., Dearden et al. 2006; Straková et al.

2010) as opposed to the standard foliar litter traps that are recommended to be fixed above ground to ensure good drainage of the samples before collection, and to reduce disturbance (e.g., Pitman et al. 2010). Based on long-term monitoring, models and conversion coefficient have been developed to estimate tree litterfall based on more readily measurable stand parameters (e.g., Lehtonen et al. 2004; Matala et al. 2008). Distinguishing between foliar litter types and tree species that they originate from is essential, since these control the litter decomposition rates and thus the contributions of the litters in the soil C balance (e.g., Laganière et al. 2010).

Most of the tree litterfall data have, however, been collected in forests growing on mineral soils, and a thorough evaluation of whether models based on such data are applicable also on forests growing on organic soils has not been done. Another caveat is that the data do not cover different management options that affect stand structure and therefore also litterfall patterns. Thus, also tree litter inputs are to be monitored in the Life OrgBalt project. Existing data from forests on organic soils (e.g., Finér et al. 1996; Straková et al. 2010; Ojanen et al. 2013) will be evaluated for applicability as complementary data. Measurements done in the Life OrgBalt project will contribute a significant amount of novel data from organic soils that can be used for modelling and will thus have an afterlife well beyond the project period.

Existing ground vegetation litterfall data are scarce and partly complex to collect (e.g., Straková et al. 2010). Measurements done in the Life OrgBalt project will also here contribute a significant amount of novel data with an afterlife well beyond the project period. Also for ground vegetation litter, it is critical to record the specific litter type (plant species and organ), since their chemical quality and thus decomposability vary widely (e.g., Straková et al. 2010).

Annual litter production will be calculated as the sum of aboveground litter of tree stand, vascular ground vegetation and mosses, and belowground litter of roots and rhizomes. For all litter components, C content of 50% will be applied for the conversion from dry mass to C.

3. FINE-ROOT LITTER INPUTS

The root systems of trees and shrubs can be roughly divided into fine roots and coarse roots. Coarse roots are a longer-time C investment, while fine roots turnover is faster and thus, fine roots form a significant annual C input into the soil (Gower et al. 1996; Vogt et al. 1996; Leppälammi-Kujansuu et al. 2014; Jackson et al. 1997). In boreal forests, on average FRP accounts for 73 % of the total root production and 32 % of the total forest production (Marschner and Rengel 2007). Diameter limits for separating coarse and fine roots have varied to some extent, but roots with diameter ≤ 2 mm are generally considered as fine roots (e.g., Finér et al. 2011). Ground vegetation root systems generally consist of fine roots and, depending on species, rhizomes, horizontally growing belowground stems from which fine roots emerge and which facilitate vegetative reproduction through emergence of new shoots. Fine-root and rhizome biomass, production, and turnover (renewal rate) are notoriously laborious to quantify, especially so in organic soils that may largely consist of root and rhizome remains (e.g., Sjörs 1991). That is why there is still considerable uncertainty in estimates of fine-root litter inputs, especially in forests growing on organic soils (e.g., Ojanen et al. 2014).

Methods that are most often used for fine-root studies include ingrowth cores (e.g., Laiho et al. 2014) and minirhizotrons (e.g., Iversen et al. 2012). Of these, minirhizotrons are generally preferred for quantifying fine-root turnover but their use has been limited due to the long monitoring period needed and the laboriousness of treating the digital picture data. While methodologies for automating the analysis are advancing fast, the time demand still remains. Ingrowth cores, in turn, are relatively easy to use and require less time (e.g., Bhuiyan et al. 2017). The approach that we have chosen for the Life OrgBalt project is estimating fine-root biomass from soil cores and fine-root production using ingrowth

cores, and estimating fine-root turnover, which is an estimate of the litter inputs, based on those as production per biomass.

The chemical quality, and thus decomposability, of fine-root litter inputs differs between species or species groups (e.g. Straková et al. 2020) in ways that cannot be estimated based on aboveground parameters (Hobbie et al. 2010). Consequently, it needs to be estimated specifically. Species group identification may be sufficient for that purpose (Straková et al. 2012, 2020). For that purpose, an indirect methods utilizing infrared spectroscopy has recently been developed, which speeds up the identification and increases its reliability as compared to visual identification (Straková et al. 2020).

4. PROTOCOLS FOR ESTIMATING ABOVEGROUND AND FINE-ROOT LITTER INPUTS IN THE LIFE ORGBALT PROJECT

4.1 Aboveground litter inputs

Tree litterfall

For estimation of tree stand fine litter, i.e. **foliar, cone and other small-sized litter**, standard forest litter traps can be used (5-6/site).

For the estimation of the aboveground **fine woody litter** (twigs, branches) from tree stand and shrubs, specific litter collectors will be placed at the surface level on the ground (frames sized 50 cm x 50 cm, 5 or 6 per site, two on each subplot or two on others, one in the middle subplot). They do not need to have mesh bottoms, but those may be useful. They should not avoid shrub-growing areas so that they also capture woody litter of shrubs. **Frame locations are cleaned of older woody litter upon installation**. Tree and shrub **twigs and branches longer than 10 cm** and all **dead shrub stems** of shrub species that are not counted in the tree inventory fallen on the collector are collected (others are removed and discarded). If they stretch over the collectors are cut so that **only the part inside the collector area** is harvested (Figure 2). The staff needs to carry shears or secateurs for this.

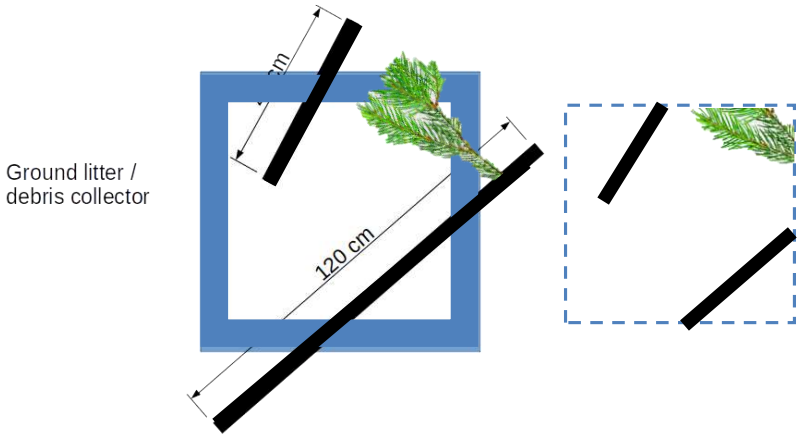

Figure 2 Sampling woody litter materials from the ground. If woody litter fallen on the ground litter collector crosses the frame, only the materials inside the collector frame should be harvested for drying and weighing.

Branches and twigs with differing size enter both into fine litter collectors and inside the framed area on the ground, some branches may have also foliar litter attached. In order to avoid double counting or neglecting litters with specific characteristics, the following rules apply:

- fine-woody litter collector on the ground:
    - Tree and shrub twigs and branches longer than 10 cm fallen on the collector are collected (shorter ones are removed and discarded)
    - Only the parts that are inside the collector with are included in the sample
    - Dead woody shrubs falling over the collector frame should also be collected (e.g. raspberry shoots) for the parts inside the frame.
    - If the collected part has foliar attached, it is included in the sampled material
        - minor foliar proportion included in the wood mass can be included (and subsequently become dried and weighed together with the woody litter)
        - large proportion of foliage in the sample material should be separated, dried and weighed, and added to the value obtained from the foliar litter collector for the same time period
- foliar litter collector above soil surface:
    - loose small-sized materials (e.g. bark, lichen, small woody pieces), foliar litter (needles, leaves), and cones are collected
    - branches and twigs longer that 10 cm are removed from the collected materials
        - small woody pieces with length ≤ 10 cm are included in the foliar litter collector harvest
        - If the larger (discarded) twigs and branches have foliage attached, it is also excluded from the sample together with the branch/twig

Partners may choose between two options in monthly litter harvesting at field; either (1) treat materials in each litter collector separately or (2) pool all harvested materials from different traps in one site materials together. If the litter collected from the different traps within a site is put into separate bags and treated as separate samples, we will get information about within-site variation. That may be interesting information for other purposes but is not necessary for this project. Consequently, the option 2 for litter harvesting is pooling materials from similar replicate traps in one site together in one bag.

The litter collected from the fine litter traps is divided in the lab into main types (Figure 3): foliar litter (further to species if possible), cones (to species if possible), fine woody debris (twigs and branches, length ≤10 cm; further to species if possible; lichen is not removed). All litter fractions are dried in 60-

70°C and weighed, and the litter dry mass values are recorded. As the separation is time consuming, you may do the separation of the fine litter only for year one, and use the proportions of total litter per each litter type to estimate the fraction of each litter type in year two total litter mass.

The fine woody litter (twigs and branches) from the woody litter collector should be roughly separated into two diameter classes (which will be utilized then also in the decomposition experiment for woody litter). This is best done of the pooled sample of the whole year (see below). Based on the composition of fine woody litterfall in the Finnish sites, we suggest division to litter with diameter ≥1 cm and litter with diameter <1 cm.

Sample fractions from both collector types in one-year long period are pooled together (per each fraction and subplot) to represent annual litterfall for each subplot and litter fraction, and the samples will be sent to Silava for CN analyses.

In case you have started fine litter collection before start of winter 2020 but are able to setup woody litter collection frames only after snow melt in spring 2021 (by cleaning the soil surface from debris), you have a different starting time for the two litter collector types. Parallel-time litter sampling from both collector types will then be until autumn 2022, and thereafter the ground frames are sampled one more time in spring 2023 to get a full year data. Most important is to get two full annual input estimates from both trap types. If they represent the exact same periods – excellent, if not – they are still usable annual estimates.

Litter collectors are emptied monthly during the snow-free season for 2 full years.

Figure 3 Litter fractions from fine litter collector (pine needles, deciduous leaf litter, woody fragments, unspecific), cones were not present in this catch.

Ground vegetation litterfall

Mosses

Moss litter input is determined specifically on sites that are characterized by an abundant moss cover. For other sites, biomass data from the ground vegetation sampling plots (Annex 1) is used as an estimate.

In sites with abundant moss cover, it is first evaluated which species or species groups are found, and what are the most abundant patch types. Patch is a separate moss-covered area dominated by the same species or species mixture all over (see Figure 4). Different patch types have different forest mosses or *Sphagnum* as the dominant moss. The projection cover (% of area) of each moss patch type in each subplot is roughly estimated visually, the estimates are recorded, and the main species or patch types are chosen for the analyses.

Moss production, which is assumed to equal moss litter production, is measured as follows. Square-shaped nets (each about 20 cm x 20 cm) will be placed on patches of (maximum) three most common moss species or moss patch types (five nets per species/patch type) at each site (i.e., not at each subplot) in the autumn of the first GHG monitoring year. The nets are firmly hooked on place e.g., with metal hooks used with tents (Figure 4). Biomass grown through the nets is harvested in the autumn one year later, from the middle of the net, using a 10-cm diameter circular sampler (e.g. cylinder-shaped section cut from empty water bottle or yoghurt container can be used). One sample taken from each net (i.e. aime is to get total of 5 samples per species/patch type from the site).

- Place the sampler on top of the moss net in a homogeneous spot.
- Remove the moss left on the outside of the sampler. We are not interested of these.
- Estimate the coverage of different moss species that have grown through the net from the sample area.
- Harvest the mosses grown through the net inside the circular sample area. Cut along the upper net surface, not underneath it.
- Put the sample from each net in a separate bag and make good markings on the bag.
- If you want to continue collecting moss biomass production samples also next year (not necessitated in Life OrgBalt), remove the harvested nets and set & anchor on new undisturbed moss surfaces.

For forest mosses, the biomass grown through the net represents production as such and is simply dried at 60-70°C and weighed. For *Sphagnum*, a capitulum correction needs to be done (explained below), since the production estimate should cover only stem length increment, as the capitula remain more or less constant over time.

Figure 4 Moss growth monitoring. Example of a Sphagnum moss patch (left), a moss production net anchored on top of a forest moss (Hylocomium) patch (right).

For *Sphagnum* samples, the mean **total length** of the moss (length from the cut to the tip of the capitulum, see Figure 5) is first determined. This can be done with a subsample of about 10-20 randomly chosen shoots. Then the capitula are cut off and the mean **stem length** is determined accordingly. The *mean length of the capitula* can then be calculated as *total length - stem length*. Next, the capitula of the whole sample are removed. The total dry mass of the stems only (including the stems that were used as subsample!) is measured, and *mass per unit stem length* is calculated as *total stem mass / (mean stem length without capitulum x number of shoots in the total sample)*. The production estimate is then calculated as *total stem mass + (mean length of the capitula x mass per unit stem length)*. These values are transformed to represent one m^2 (based on the area of the sampler).

Figure 5 Moss growth measurement. Illustration of natural markers showing forest moss stem growth, which we will use for estimating the limit between live biomass and dead part of the moss (from Pouliot et al., 2010) (left). For Sphagnum, similar color identification will be used (drawing modified from Weston et al. 2015) (right).

For each moss patch type of the site, average patch-level production values are calculated. Subplot-level estimates of moss produc will then be calculated by multiplying the site-level patch-level averages with the respective projection coverage for each patch type at the subplot: (*projection cover of the patch type (%) x average patch-level production)/100*. These patch-type-specific values are summed up for each subplot, and further for each site.

For the species/patch types not sampled for biomass production at the respective site, species/patch-specific production of the same species/patch at another site representing the same vegetation type, or a close species at the same site can be applied.

The site-level production estimates are used as estimates of annual moss litter input.

Annual herbaceous plants

The ground vegetation biomass samples (Annex 1) are used for estimating biomass production. For annual plants, the harvested maximum biomass is used as such to represent annual aboveground biomass production and the amount of litter input.

Perennial plants

The ground vegetation biomass samples (Annex 1) are used for estimating biomass production. For deciduous shrubs (<50 cm high species), leaf production, and litterfall, is estimated as leaf mass obtained from the biomass samples. For evergreen shrubs, leaf production is estimated as leaf mass of the current-year shoots. Stem production is estimated as the mass of current-year shoots.

For the >50 cm high species, all of the above-mentioned mass values are multiplied with the number of stems recorded for the plot (Annex 1).

4.2 Fine-root litter inputs

Fine-root biomass

For the estimation of belowground biomass, 9 volumetric soil samples at each site will be cored down

to 50 cm (Figure 6). Roots and rhizomes will be separated and identified to main species or species groups either visually, or based on infrared spectroscopy (IRS) with the models of Straková et al. (2020).

- Nine (9) peat cores taken from each experimental plot at the end of the growing season 2021
- Zero-level estimated in the field, subsamples cut as 10-cm pieces down from that; surface vegetation are kept in the topmost sample
- Samples down to 50 cm depth in deep organic soils
- Shallow organic soils may be tricky if roots reach down to mineral soil; somehow the coring should be extended down to the desired depth (either by a different corer, or some more laborious method such as shoveling…)
- Samples are stored frozen if not processed within one week
- Roots are washed out using, e.g. soil sieves, to prevent loss of roots
- Roots with diameter ≤2 mm are harvested as 'fine roots'
- Roots with diameter >2 mm and ≤2 cm are harvested as 'small roots'
- Shrub and sedge rhizomes are separated to form specific biomass components, separate from the actual roots
- Roots are dried in 40°C for 72 hours, weighed, and powdered if planned to include in IRS analysis

Figure 6 Example of a corer that can be used for taking the belowground biomass samples.

Fine-root production

Fine-root production is estimated using one of three optional methods: the **mesh ingrowth-core method** for peat soils (Laiho et al. 2014, Bhuiyan et al. 2017), the **mesh-free ingrowth-core method or** the **root mesh method**. The amount of ingrown roots represents fine-root production over the incubation period, which will be generalized into annual production.

Method 1. Mesh ingrowth core method

Preparation of mesh ingrowth cores

- Prepared to a diameter 3.2 cm (10 cm perimeter) and effective length of 50 cm, using 1 mm mesh polyester fabric
- Filled with **local soil that is roughly sieved** to remove living and freshly dead roots and rough woody or *Eriophorum* material (as shown here for mineral soils in Figure 7)
- The cores need to be packed tightly - too loose cores lead to poor root growth and underestimates
- 15 cores per site – 5 per each GHG measurement subplot (3 cores, 1 per subplot, are for bulk density measurement only)

Figure 7 Preparation of root-free peat material for ingrowth cores by sieving and manual picking of live roots.

- In late autumn before soil frost
- Easy in deep-peat soils with the corer-installer; problems only if peat is dense and dry, or there is a mat of thick roots, then probing is needed (Figure 8)
- Soil auger is needed for mineral soils or shallow peat soils
- Soil contact in the surface need to be secured by hand after installation
- A plastic stick is put next to each core, and the part of the core remaining above ground is fixed in vertical position in the stick with a cable tie
- Recovered after two years

Recovery of mesh ingrowth cores

- In root core recovery (lifting out from the soil) it should be avoided pulling out roots grown through the fabric mesh and changes in peat core length and material composition
- By using a long knife, such as insulation cutter (see, Figure 8), cut peat around the root core as deep as you can reach from the surface level
  - avoid immediate vicinity of the core when cutting, so as not to damage the core
  - this is to detach any aboveground plant parts attached to or growing through the cores, and to cut the root systems, especially rhizomes and any hard lateral expansion, to avoid risk of pulling out roots from the cores
- Gently pull the core out of the soil
- Either mark in the core, or record separately, the distance from the core top to the soil surface (this will be used as zero-line when treating the cores in the lab)
- Lay the core horizontally on plastic foil, wrap into the foil, mark with site, subplot and core # identifier, and set in container for transport

- Keep cores in freezer until further treatment

Post-recovery treatment of mesh ingrowth cores

- Remove gently any attached plant/soil material that is outside the mesh, also all roots found outward from the core segments are cut and discarded.
- Each core is cut into 10-cm segments, starting from the zero-line of soil surface (marked or recorded in the field during recovery).
- Measure the diameters of the segments (two measurements at right angle of both the top and the bottom of each segment).
- One core per subplot is used to determine bulk density: after measurements, it is dried in 60-70°C and weighed
- Of the other cores, the roots inside the cores are gently washed clean with water and recovered.

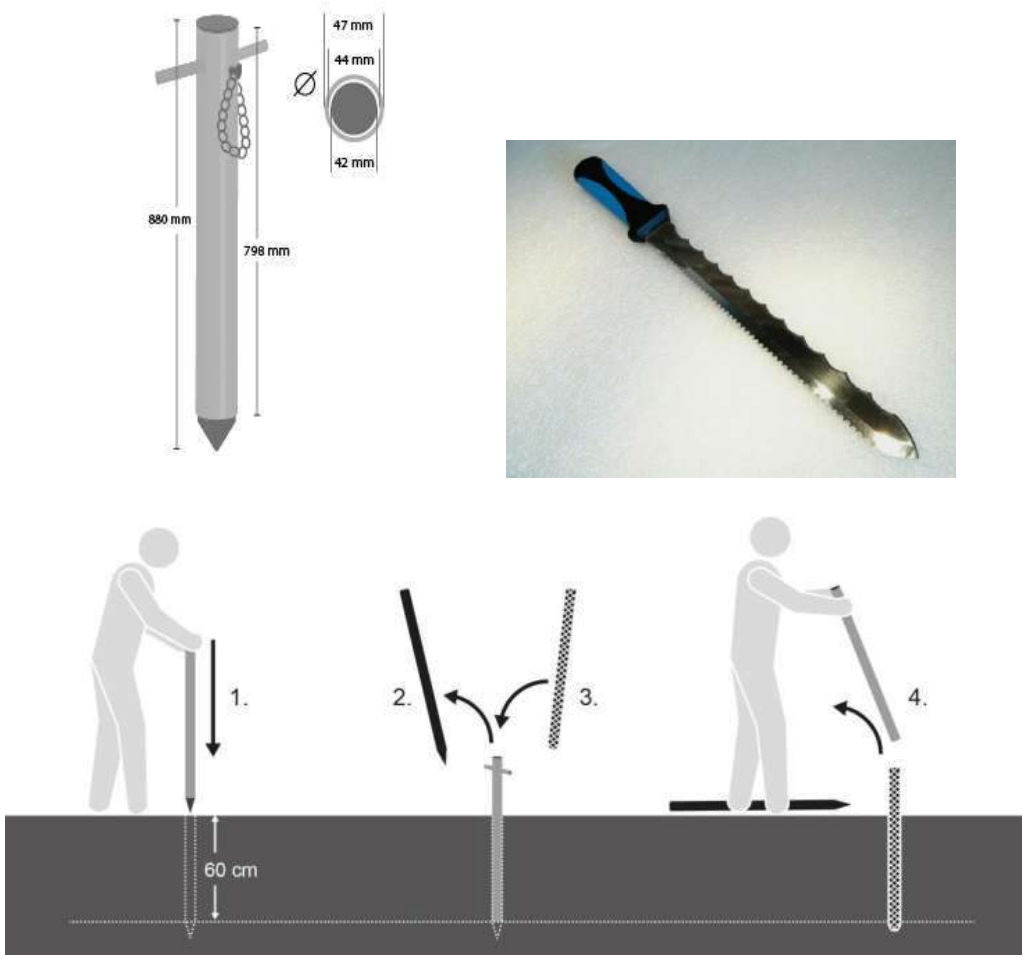

Figure 8 Installing mesh ingrowth cores. Corer-installer used for installing (top left), the installation procedure (bottom) (from Laiho et al. 2014), and long knife (e.g. insulation cutter) used during the core recovery (top right).

- Estimation of whether the roots are living or dead is based on colour and friability; dead and live roots are separated into different fractions if there are abundant dead roots; also species/functional type separation can be done at this stage if that option is chosen.
- The roots are oven-dried to constant mass at 40°C (note the low temperature) and then weighed.

Method 2. Mesh-free ingrowth core method

Local soil is used and prepared as described above for mesh ingrowth cores. Number of cores to be installed in the site is also the same. The difference is that no mesh cores are prepared in the lab. Soil corer is used to remove the original soil in the core installation point. The hole is firmly marked with sticks, and the hole is tightly filled with the prepared soil. At recovery, the same corer is used to carefully remove the installed soil only, from between the sticks. Laboratory treatment also follows that of the mesh ingrowth cores. (Note that foxes may like to pull out the sticks and play with them, so thin metal sticks may be preferable.)

Figure 9 Installation of mesh-free ingrowth cores. Marking core position by sticks (left) and cores installed and marked (right).

5. SCHEDULE OF LITTER INPUT MONITORING

Timing plan for the litter input studies is presented in Table 1.

Table 1 Suggested timing of data collection.

Topic	Timing (start)	Notes
Tree biomass	2021	Done once
Tree biomass production	2021	Based on modelling and use of existing data
Ground vegetation biomass (aboveground)	2021 or 2022 Jul./Aug.	Ground vegetation biomass, including projection cover; plant functional types and shrub species separately; done once
Ground vegetation production (aboveground)	Moss biomass production nets set 2020 Moss biomass production nets harvested 2021, 2022	For other ground vegetation production is estimated base on biomass data
Litter inputs (aboveground)	Traps set 2020 or 2021 Litter harvested monthly to reach two full years	Two types of traps: foliage + other "small litter", and fine woody litter
Belowground biomass	2021 Jul./Aug.	Fine-root biomass (from peat cores) should be collected simultaneously with above-ground biomass. Plant functional types separated, if possible, otherwise infrared spectroscopy can be used to determine proportions. Tree small and coarse root biomass is calculated using biomass equations, noting the diameter limit of roots separated from cores
Belowground biomass production	Setup 2020-2021 Collection 2022-2023	Ingrowth cores or nets in other sites, peat cores in croplands
Litter decomposition	Aboveground litter materials collected 2020/2021; setup 2021; collection 2022, 2023,.. belowground litter materials collected 2022; setup 2022; collection 2023, 2024,..	

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ANNEX 1. GROUND VEGETATION BIOMASS AND BIOMASS PRODUCTION ABOVEGROUND

This data is also used for estimating annual biomass production and litter inputs from ground vegetation, except for mosses in sites where there is an abundant moss cover. If abundant moss cover, sampling of moss nets and total moss biomass sampling is done and mosses

Ground vegetation biomass is harvested from six 30 x 30 cm (area = 900 cm^2) sample plots per site, two at each GHG measurement subplot. Biomass will be harvested in July-August, during the period of maximum ground vegetation biomass. The collected biomass is separated by species or plant functional types, and further separated as listed below.

The concept of 'flagship sites' can be adopted for easing sampling and/or dividing workload to take place over two years. If there are several sites relatively comparable site types and land uses in monitoring in the Baltic states, flagship sites can be chosen to be included in sampling first year as priority, or for representing all comparable sites in certain measurements in the database (in cases where excessive work could be avoided by use of modelling).

Guidance:

- Timing of sampling
    - Once at peak biomass in July/August (annually only if vegetation type changes, or if weather conditions are dramatically different)
    - All sites 2021, or alternatively 1st summer at flagship sites and 2nd summer at the rest of the sites
- Ground vegetation biomass sample plots (Figure A1)
    - 6 plots per site (2 plots at each GHG monitoring subplot at locations comparable with the GHG monitoring points)
    - Equipment needed: frame (30x30 cm) for delineating the sample plot, plastic bags for the harvested biomass (different sizes), markers, scissors, secateurs (pruning shears), form for recording projection cover estimates
    - Each sample plot is a 30 cm x 30 cm square area (delineated e.g. with a wooden frame)
    - The locations of the two plots are chosen near the gas measurement plot group (max 10m from it) in spots that have similar vegetation as the gas measurement plots
    - Select a spot with similar kind of vegetation as in the gas measurement plot group and drop the sample frame to that spot without selecting the exact spot too carefully. Remember that tall trees (trees included in tree stand measurements) and large shrubs (tree-like shrubs included in tree stand measurements) are not included in the sampling. Make sure that the key species of the measurement plot group are included in at least either one of the samples. Remember that if the gas measurement plot group has little vegetation, it's okay to have a little vegetation in the biomass plot as well.

Give a descriptive name to the plot so that you remember the gas measurement plot group where the sample is from

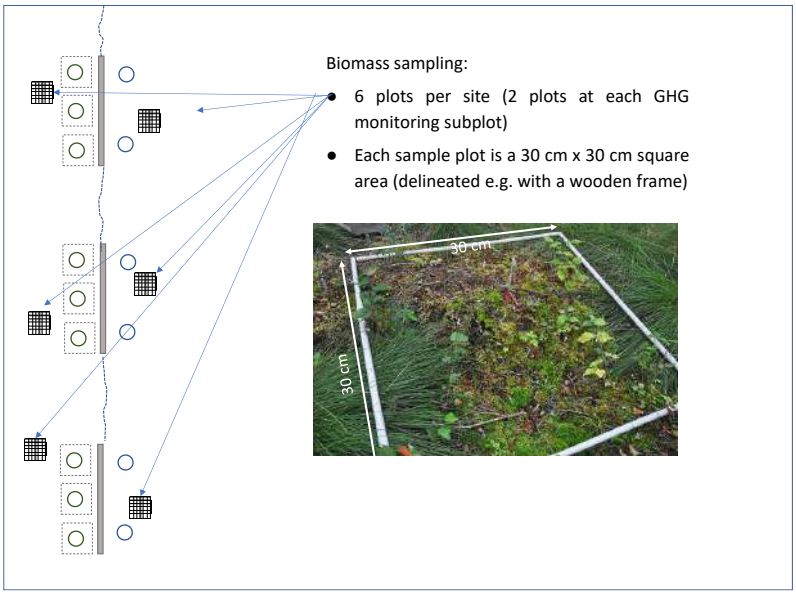


Figure A1 Biomass sampling plot locations at the soil GHG monitoring site.

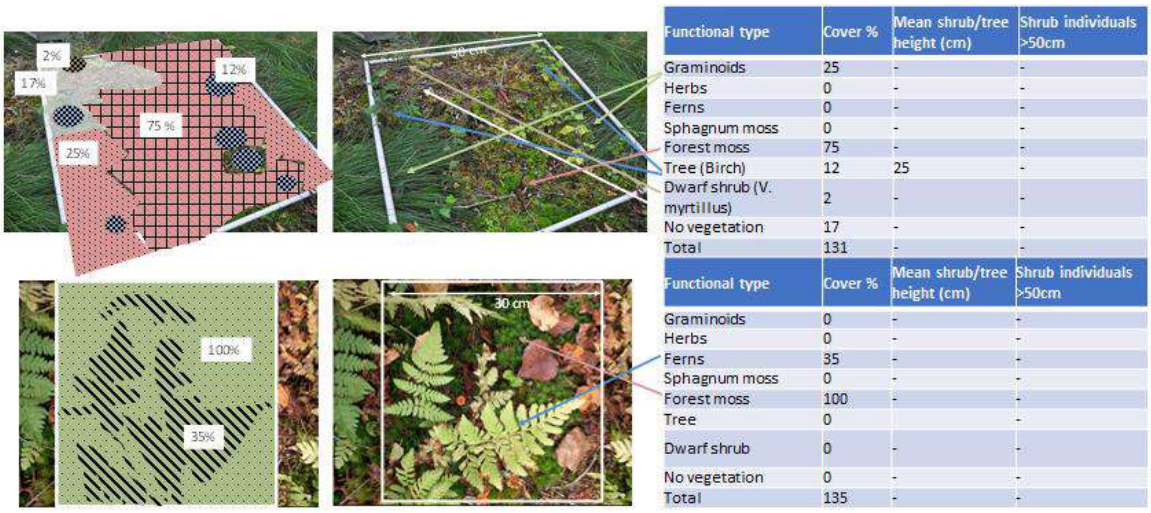
- First, the projection cover (% of area) of the vegetation inside the sample plot is estimated for plant functional types:
    - Trees: only trees that are not taken into account in tree stand measurements. For example, *Picea abies*, *Pinus sylvestris*, *Betula pubescens*
    - Shrubs: perennial plants with woody stem and not included in tree stand measurements and not dwarf shrubs. Shrubs by species, and the cover proportions are recorded. For shrubs also the average height per species is estimated and recorded (Figure A2). For example, *Rubus idaeus*, *Salix* species.
    - Dwarf shrubs: plants with woody stem and height mostly less than 50cm, perennial plants that can either drop all the leaves in the autumn or have overwintering leaves. For example, *Vaccinum* species, *Linneae borealis*, *Rhododendron tomentosum*
    - Graminoids: Grass and hay-like species. For example, *Carex* species (sedges), *Calamagrostis* species and *Luzula* species
    - Herbs: Herbaceous plants that are not graminoids (= non-grass-like plants) For example, most flowering plants like *Anemoe* species or *Melampyrum* species.
    - Ferns: Vascular plants that produce spores instead of seeds. Ferns and Lycophytes. For example, *Dryopteris* species, *Lycopodium* species and *Diphasiastrum* species
    - Moss (*Sphagnum* and other mosses separately)

    - Exclude all the vegetation that ends up inside the plot but grows outside of it. However, if the species can grow horizontally (like *Vaccinium oxycoccos*) then all the parts that are inside the frame are included.
        - Recognize the species groups growing in the plot
        - Estimate the coverage of the functional species groups for graminoids, herbs and ferns. Estimate the coverage by species for dwarf shrubs, shrubs and trees. Write down the estimates.

- The total summed coverage of all the species can be more than 100% if the vegetation is growing in different layers. For example, a young birch tree (about 50cm) can cover 40%, blueberries 60% and a sedge 10% (=110%).
- You can divide the plot in half (50%), and then in half (25%) and half (~12%) and so on in your mind to help to get the coverage estimate as accurate as they can get.
- Estimate also the total moss coverage and the coverage of litter. Litter can be above the moss so the summed coverage of mosses and litter can sometimes be more than 100%. Coverage of bare ground or tree trunks can be marked separately.
- Only the coverage of living vegetation is included. By living, we mean the vegetation that has been living in this growing season. For example, a small flowering plant that is already brown because it's late growing season, is included but grass that is from last year, is not included in the coverage estimates.

- Note that the total projection cover can be over 100% because plants may form several layers partly overlapping each other.

- Second, taking the biomass sample
  - What is included in the biomass sample?
    - The plants that are estimated as this year's living vegetation (and whose coverage is already estimated) are included in the biomass sample.
      - Dead last year's biomass is left there as well as mushrooms.
      - Mosses are included only if there is no separate moss sampling done in the site due to relatively small moss cover.



Functional type	Cover %	Mean shrub/tree height (cm)	Shrub individuals >50cm
Graminoids	25	-	-
Herbs	0	-	-
Ferns	0	-	-
Sphagnum moss	0	-	-
Forest moss	75	-	-
Tree (Birch)	12	25	-
Dwarf shrub (V. myrtillus)	2	-	-
No vegetation	17	-	-
Total	131	-	-

Functional type	Cover %	Mean shrub/tree height (cm)	Shrub individuals >50cm
Graminoids	0	-	-
Herbs	0	-	-
Ferns	35	-	-
Sphagnum moss	0	-	-
Forest moss	100	-	-
Tree	0		-
Dwarf shrub	0	-	-
No vegetation	0	-	-
Total	135	-	-

Figure A2 Estimating projection cover in different plant functional groups.

- Only one shoot of each shrub individual >50cm is sampled by species (if two different shrub species have individuals of >50cm, sample one shoot of both species). Measure also mean height separately for >50cm individuals and <50cm individuals.

- If part of the plant is dead (for example a branch of a dwarf shrub, or last year's grass leaves) you should consider if the part really is dead and has not been living this growing season. Parts that have suffered from drought but have been living in the beginning of the summer are included. Also, all the vegetation that is already brown because it's late growing season is included. Dead parts of shrubs and swarf shrubs are separated, dried and weighted in the lab.
- If some plants have dropped their leaves, also include the dropped leaves in the sample.
- Basically, include all the living vegetation inside the frame that doesn't end up in root ingrowth cores/other kinds of root measurements or in moss sampling. If you pick up a grass or dwarf shrub branch that has roots, remove the roots but include everything else except dead parts.
- If there are berries in dwarf shrubs/shrubs, you can include them in the sample and dry and weight them in the lab later. Berries are not included in the model if the models are used.
- Tall trees/shrubs should be avoided already when choosing the spot for the plot so there is no need to exclude too tall trees/shrubs from the biomass sample. Take also the tree seedlings/shrubs that may be a bit taller than the 0,5 cm limit completely without excluding the tallest parts.

○ Vegetation clipping

- Use hand or scissors to clip the vegetation. Cut the vegetation from the ground level but if some parts are growing in the moss layer or in the litter layer, you should include them. If it doesn't end up in root socks or other measurements, include it.
- Trees (trees that are not included in the tree stand measurements): Count the number of shoots of each species. Collect all shoots and place them into separate bags by species.
- Shrubs (≥50 cm high species that are not considered as trees, tall shrubs or tree seedlings in the tree survey!): Count the number of shoots of each species with height more than 50 cm. Different species separately. Take one shoot that represents the average shoot and put it into one plastic bag. Do this for all shrub species >50cm. Make good markings to the bag and include the number of shoots >50cm of that species that were growing in the plot but not included in the sample.
- Shrubs (<50cm) and dwarf shrubs are collected into marked bags, each species separately (alternatively, all can be put in one bag, and species separation can be done in the laboratory – consider full time consumption and potential errors done in both field and lab!)
    - The dead looking lower parts of dwarf shrubs are also taken if the upper parts are living because the lower part is living too even though it may have had too little light to grow leaves/green leaves.
- Other vascular plants: Graminoids, herbs and ferns (including *Lycopodiaceae*), above-ground parts are collected into marked bags by functional types.

- Mosses: If there's are no separate moss samples taken in the site (moss net samples and total moss biomass samples) due to small moss coverage, the living parts of mosses are also collected into marked bags. Living parts are separated based on change in color or visible beginning of decomposition. Preferably take too much and trim the dead parts off in the laboratory.
- If a plant has dropped its this year's leaves and you notice them on the ground, pick them up and include them in the sample.
- Separate clipped vegetation into different species groups already in the field and put them into separate plastic bags. You get one bag of biomass from one plot if there is only one species group growing (for example dwarf shrubs) in the plot, or more bags (about up to 5) if there are several different species groups growing (for example dwarf shrubs, graminoids, forbs, trees, shrubs)
- Use plastic bags at least those times when dwarf shrub models are made because separating annual growth from the samples is only possible when the sample is fresh. It may be wise to store all the species groups similarly in similar kinds of bags. Plastic bags keep the sample fresh for longer and they don't break so easily if they get wet in the field. Paper bags can work well in dry conditions and when there is no need to make/calibrate dwarf shrub models.
- Write site name, gas plot group name and biomass plot name as well as date and the species group on the bag.
- Keep the samples in the fridge if they are dried and weighed approximately within a week of the sampling date. Otherwise, store the samples in a freezer.

- Third, laboratory preparation
  - Preparation of everything else than shrubs and dwarf shrubs:
    - Make sure that there are no dead parts included in the samples.
    - IF species is perennial (like, e.g., *Lycopodium annotinum*), separate the species from rest of the functional group sample, further separate the individuals of that species to annual growth and old parts. Dry different parts and rest of the functional group sample separately.
    - Otherwise, trees and other vascular plants are ready for drying.
  - Preparation of shrubs and dwarf shrubs
    - Shrub separation is first done only for flagship sites, or for 20 samples per species altogether from different sites. After that, we will see whether the proportions can be modelled based on those data!
    - Shrubs (>50 cm and <50 cm high species) and dwarf shrubs are prepared the same way.
    - If the species is deciduous (drops all leaves every autumn): the shoots are separated into dead stem, old stem, new stem (part of annual growth) and leaves (part of annual growth)

        - If species is evergreen: the shoots are separated into dead stem, old stem, old leaves and total annual growth (current-year stem with leaves)
    - Mosses: separate the samples roughly to *Sphagnum* mosses and other mosses ("forest mosses"). If there are just a few shoots of either group, they may be pooled to the more abundant group. Check the cutting done in the field, and cut off the potential dead and decomposing lower parts of the moss growth. NOTE: this is not done per moss shoot but per the whole clump of moss.

- The samples are dried at 60-70°C and dry mass of each sample is recorded (the default drying temperature of each lab, within this range, can be used)
    - Dry mass records from each ground vegetation biomass plot: graminoids, herbs, ferns, possibly moss, trees by species, total annual growth of shrubs and dwarf shrubs by species, current year leaves of deciduous shrubs and dwarf shrubs by species, current-year stem of deciduous shrubs and dwarf shrubs by species, old stems of both deciduous and evergreen shrubs and dwarf shrubs by species, old leaves of evergreen shrubs and dwarf shrubs by species, dead stems of shrubs and dwarf shrubs by species.

- Dwarf shrubs of those species that will have an annual growth model (most common species):
    - For building the models, you need test-dwarf shrubs that will be taken from the biomass samples to build the models. Annual growths of those test dwarf shrubs are separated, dried and weighted separately. See instructions below.
    - The rest of the dwarf shrub samples of the species that will have annual growth models are weighted without separation to annual growth or old parts but different species separately to get the total dry weight of each dwarf shrub species in the plot. First make the models and let rest of the samples from other than test plots wait in freezer/fridge until you have the model and you know what you can model
    - Dwarf shrub species that will not have an annual growth model (less common species): Those dwarf shrubs in the sample that will not have a model to estimate annual growth, have to be prepared similarly as explained above (deciduous and evergreen species differently)

- Story behind the dwarf shrub models:
    - Annual growth models: When we are collecting and weighting biomass samples, we are estimating the production of biomass but also the amount of litter that is produced every year on the forest floor. Dwarf shrubs, shrubs and trees are perennial plants and the total above ground biomass is not equal to the annual growth. Some forbs and graminoids also have parts that remain over winter but those are usually underground. If some forb species in the sample is perennial with annual growth and older parts, the annual growth should be separated and weighted separately. Otherwise, we assume that above-ground biomass represents the annual growth of forbs and graminoids. For dwarf shrubs, we must separate annual growths by hand (not so common species in the samples) or estimate the annual growth by using simple models that are created using a small subset of the dwarf shrubs taken from the biomass samples (common dwarf shrub species). To make the preparation in the lab quicker, we use models for the most common dwarf shrub species.
    - Leaf production models: There are at least two kinds of dwarf shrubs: those that have overwintering leaves and those that have not. Dwarf shrubs that make all their leaves again every

year also drop all the leaves every year. This means that the species is producing leaf litter that must be estimated either by separating all the leaves from all the samples (species with only some individuals in the samples) or by using a model to estimate leaf litter production (common species). For this kind of dwarf shrubs, we also make a model to estimate the amount of leaf biomass from the total biomass.

- Models are species specific. If there are dwarf shrub species that have only a few individuals in the samples, the annual growth can be separated, dried and weighted by hand without making models for those species. Make models for the most common dwarf shrub species to make preparation work easier and quicker next year.
- You don't need to make a new model every year and you can use the model from this year in the following years (maybe do some calibration with a few test individuals) --> less work next year.

- How to make an annual growth model for one species of dwarf shrub?
  - What are the most common dwarf shrub species in the site/sites? Note that it may be wise to do the models for all the most common dwarf shrub species to save time (in Finland three species). Decide the species you want to build the annual growth models.
  - Step 1: Select 3-5 biomass plots that had the species growing in it. If there are different treatments, different nutrient status or some other spatial differences in the site, try to include plots from all kinds of environments. If the site is very homogenous and plots have a lot of the chosen species, fewer plots (3) is probably enough.
  - Step 2: Take dwarf shrub sample from one of the chosen plots and pick all of the individuals of that species as test dwarf shrubs. If there are a lot of that species in the biomass sample, take only part of them (for example 10 individuals, include individuals with different sizes).
  - Step 3: Take half of the individuals first. This will be subset number 1 from that plot (the rest are subset 2 from that plot). Separate different plant parts as explained above (deciduous and evergreen species differently)
  - Step 4: Place different parts of each plots' subset in separate paper bags
    - Make good markings to the bags (site name, gas measurement group name, biomass plot name, species name, annual growth/old parts/leaves, number of individuals in the bag AND subset number 1 or 2). NOTE that the test dwarf shrubs will be part of the total biomass of the biomass plot and must be summed with the rest of the biomass later. Do not mix samples from different plots and make sure you know where the sample dwarf shrubs are from.
  - Step: 5 Do the steps 3 and 4 for the rest of the chosen individuals in the plot (subset number 2 of the plot).
  - Step 6: Do the same for other selected biomass plots until you have enough test dwarf shrubs to make a simple linear regression of the ratio between total dwarf shrub biomass and annual growth.
    - Minimum is probably 3 biomass plots with individuals from all those plots always separated into two halves making in total 6 bags of old parts and 6 bags of annual growths (and 6 bags of leaves if they are separated). If you include more plots, you have more data for the regression.

- Step 7: Put test dwarf shrubs to the oven to dry similarly as other biomass samples (70 degrees, 2 days)
- Step 8: Weigh the samples
- Step 9: Calculate total living weight of each sample: old parts + annual growth (+ leaves if those have been separated)
    - If the leaves have been separated (dwarf shrub species drops all the leaves every year), calculate also the total annual growth: annual growth of the stems + leaves
- Step 10: Make a linear regression for annual growth: Annual growth (stem + leaves) as a predictor variable (y-axis in scatter plot) and total mass of the sample as the explanatory variable. The mass of the annual growth is expected to increase linearly with the total mass of the dwarf shrub (old parts + annual).
    - If the leaves have been separated (dwarf shrub species drops all the leaves every year), make a linear regression also for leaves: Leaf mass as a predictor variable and total mass of the sample as the explanatory variable.
    - Set the intercept as 0
    - Find the slope of the linear regression
    - Now you have a simple model to estimate the mass of annual growth of that dwarf shrub species from the total mass of the sample that has only that species in it. You can also estimate the amount of leaf litter produced using the model and total mass of the species in the sample.
    - Include more test dwarf shrubs in the model if needed and compare models from different parts/treatments of the site and different sites to see if the same models work for all sites and treatments.
    - When you have done models for different sites, compare them and consider using a common model for all the sites if the models are similar