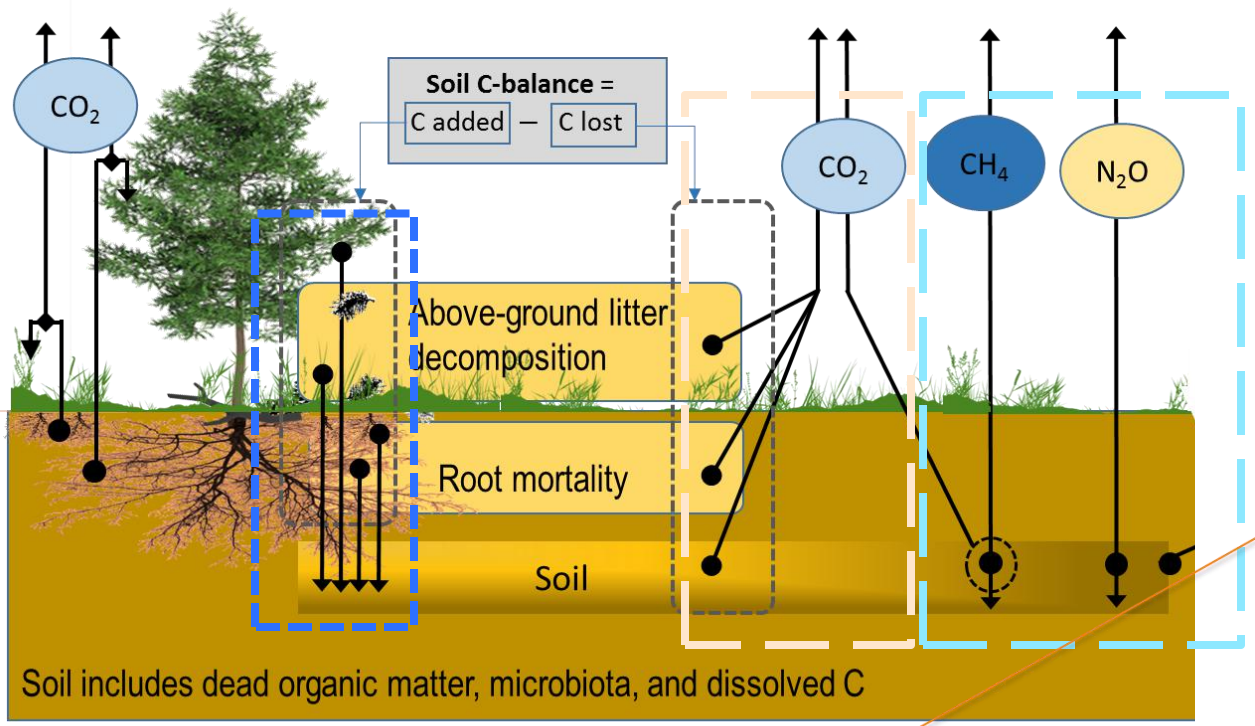
Project implementation (Actions C1 – C5)
Action C1 “Measurements”
Measurements - what and how

Presentation on GHG monitoring followed by partners discussion

Autotrophic gas exchange by plants

Heterotrophic respiration by soil community and microbiota



'Method-1' by using closed static chambers. CH_4, N_2O (& CO_2)

- 5 replicates
- Collars (Ø 50 cm) form permanent bases for chambers
  - Vegetation within the collar enclosed soil surfaces is not disturbed
  - In grasslands and croplands collars will be used to assess net ecosystem exchange ('Method-2')
  - Deployment period in gas sampling 40-60 minutes per collar
  - Lab analysis for samples

Biomass-related measurements quantifying annual plant biomass, biomass and litter production and litter decomposition

'Supplementary method-2' by transparent closed dynamic chambers. CO_2

Other options?

'Method-2' by closed dynamic chambers. CO_2

- 25 permanent flux monitoring spots per site
- Monitoring & analysis by infra-red gas analyzer (IRGA)
- Flux monitoring
  - Deployment period up to 3 min.
  - Trenched and root-ingrowth preventing cloth
  - Surface kept free from litter
  - Further data needs
    - belowground litter deposition and decomposition.
    - aboveground litter deposition and decomposition

-> flux & litter data forms soil CO2 balance

Initial land use	Land use after completion	Vegetation	Potential GHG monitoring/	Notes	Notes	Sites
Cropland	Cropland (D)	Legumes & crops rotation	M1, M2, M2s	Collar removals		1
		Fast growing species (poplars and willows)	-			1
	Grassland (D)	Cereal production (30+ cm GWT)	M1, M2, M2s	Collar removals		1
	Forest land (D)	Agroforestry – fast trees & grass (poplars & reed canary grass)	-		Poplars not planted. Reed site fluxes?	2?
Grassland	Grassland (D)	Controlled drainage	M1, M2, M2s	Collar removals		1
	Forest land (D)	Afforestation (conventional, 30 – 40 years old)	M1, M2	Trenching	Preparations & timing of measurements?	1?
	Rewetted (W)	Paludiculture (alders, birch of age 20 – 30 years)	M1, M2	Trenching	Preparations & timing of measurements?	1?
Forest land	Forest land (D)	Thinning & applied wood ash (spruce)	M1, M2	Trenching	Timing related to management?	X regions x 3 (C, s1, s2)
	Forest land (D)	Continuous forest cover	M1, M2	Trenching	Sites ready	2 regions x 3 (C, CF, SL)
	Naturally wet (W)	Black alder and birch	M1, M2	Trenching	Preparations & timing of measurements?	1
	Rewetted (W)	Grey alder and birch (20 – 30 years old)	M1, M2	Trenching	Rewetted when? Preparations & timing of measurements?	1

Sites not mentioned in the proposal?

Sites already in monitoring?

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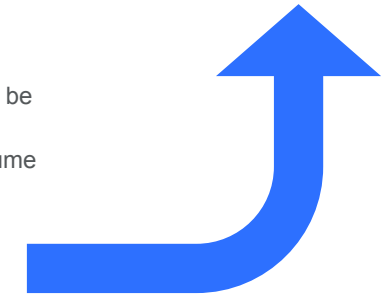
Other GHG monitoring related studies

Biomass-related measurements quantifying annual plant biomass and production

- **Tree stand above-ground and below-ground biomass** (coarse root)
  - Tree stand diameter distribution of all trees, and tree height and length of the live crown for sample trees
  - Allometric functions for separating biomass in stem, branches, foliage, stump and coarse root systems
- **Tree stand biomass production**
  - Biomass production as the difference between biomass values of 2 consecutive years in sample treees.
- **Ground vegetation above-ground biomass**
  - By harvesting the vegetation of small plots at the time of peak biomass
    - shrubs, graminoids, forbs, mosses separated and annual biomass production determined

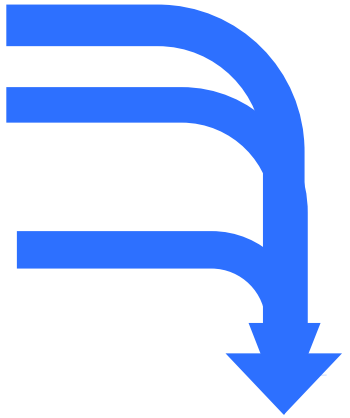
- **Fine root biomass (<2mm)**
  - From volume-exact soil cores, analyzed down to the rooting zone lower limit
  - Soil bulk density will be used to generalize root mass per sample volume to values per square meter.
- **Fine-root production**
  - by the ingrowth-core method for peat soils, or the root mesh method for annual plants.
    - The amount of ingrown roots represents fine-root production over the incubation period, which will be generalized into annual production
    - Roots grown through the strips during incubation period and thereafter measured for a known volume both sides of the strip represent production
- **Fine-root turnover** (below ground input) will be estimated as production per biomass.
  - Roots in both biomass and ingrowth core samples are separated into tree and ground vegetation roots to the extent possible

Fine-root litter input & decomposition

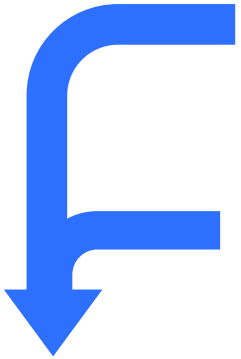


Carbon inputs with dead biomass and carbon loss rates in each site

- **Forested sites**
  - Annual tree mortality estimates is based on monitoring data from other projects, or tree mortality models (e.g., Jutras et al. 2003), where applicable.
  - Perennial plants' annual above-ground litter (carbon input) is based on a repeated collection of litter from litter traps of known area set at the sites (e.g., Ojanen et al. 2013, Uri et al. 2017),
    - following the litter classification and analysis by methodology defined for ICP forest monitoring.
  - For annual plants, the annual biomass production equals also the amount of litter input.
  - Annual fine-root litter input rates will be based on the production/biomass ratio as described in Chapter 2.
  - Decomposition of these C pools will be estimated using
    - Decomposition models
      - coarse woody debris of on conifer and deciduous trees (e.g., Tuomi et al. 2011a, Pearson et al. 2017)
      - fine litter (e.g., Tuomi et al. 2011b, Straková et al. 2012) in different climatic conditions.
    - Thee litterbag method (Straková et al. 2012)
      - for estimating litter decomposition rates in cases where no applicable models exist
        - A known mass of air-dried litter sampled from the site is placed in a mesh bag
        - Sets of filled bags will be placed on site / Incubated a set period /Harvested for analysis.
        - Remaining litter mass in each bag will be weighed
        - A time sequence of such data can be used to estimate mass and C loss dynamics.



Above-ground litter input



Litter decomposition

Open sites ?

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Other field studies described in the proposal

Characterizing soil microbial communities

- One-time soil sampling will be done for characterizing the microbial communities that participate in CH_4 and N_2O fluxes (in case of CO_2 there are too many groups involved to make such analysis realistic)
- Sampling will be targeted on a subset of sites, chosen based on the first-year results of flux measurements on the sampling sites

Soil screening with infrared spectroscopy (IRS)

- IR spectra summarize the whole chemical composition of the sample.
- The LIFE OrgBalt project will test IRS as such solution for cool temperate moist climate zone.
    - If the comprehensive description of soil chemistry with IRS proves to have predictive power for soil GHG exchange, the methodology could revolutionize the estimation of these emissions.

Soil properties

- At all gas fluxes measurement plots
- Samples from litter layer (in forest land), 0-10 cm, 10-20 cm, 20-40 cm and 40-80 cm depth
- Sampling events
    - during the establishment
    - repeated at a topsoil layer 3 times per vegetation season
- Soil sampling and analyses will be performed according to ICP Forest guidelines (Cools & de Vos 2010, Konig et al. 2010). Exchangeable nitrate (NO_3^-) and ammonia (NH_4^+) iones will be determined in potassium or sodium chloride solution.

Water samples

- At all gas fluxes measurement plots
- 4 times during the vegetation season from permanent wells
- used for measurement of the groundwater level.
- Only 1 of 2 wells installed in every site will be used for water sampling
- Water analyses will be done according ICP Forest guidelines (Konig et al. 2010, Nieminen, 2011), rough estimation of soil water permeability

Questions for discussion

- Sites
  - What is “organic soil” in this project?
  - What site types are most relevant for our countries?
  - How to avoid unnecessary duplication in studies?
  - Who works and where?
- Monitoring activities
  - Which auxiliary data will be collected (ground water table, temperatures, …)?
    - Loggers and manual vs. manual monitoring only (modelling)
  - Seasonal climate
    - Warm season monitoring period length and definition?
    - Is there cold season monitoring planned?
  - Monitoring intervals in warm season monitoring in M1 and in M2?
  - Length of monitoring at field sites
    - Prep. 2019 ; Monitoring Year 1: 2020; Monitoring Year 2: 2021
    - Data analysis and publication 2021,2022,2023
      - If preparations start 2019 – are sites ready for monitoring 2020?
- Methods
  - Method 2 (on site CO_2 monitoring)
    - Is litter removal necessary on M2 surfaces?
    - Root trenching on sites growing perennial vegetation
  - Method 1 (collars/gas samples analyzed by GC)
    - 5 replicates/ Collars (Ø 50 cm)
    - Deployment period in gas sampling 40-60 minutes per collar
    - How many monitoring rounds are done each year?
      - 1 site c. 6 hours work by 1 chamber – can we operate by 2 or 3 chambers simultaneously?
  - Biomass/litter/litter decomposition data collection in forest/perennial/annual systems
- Supplementary activities
  - Transparent chamber monitoring by who and where?
  - Transparent chamber monitoring, what complementary data will be produced?
  - Should we include some non-listed (cheap) activities, e.g. adding subsidence poles to some of the sites?

Thank you!

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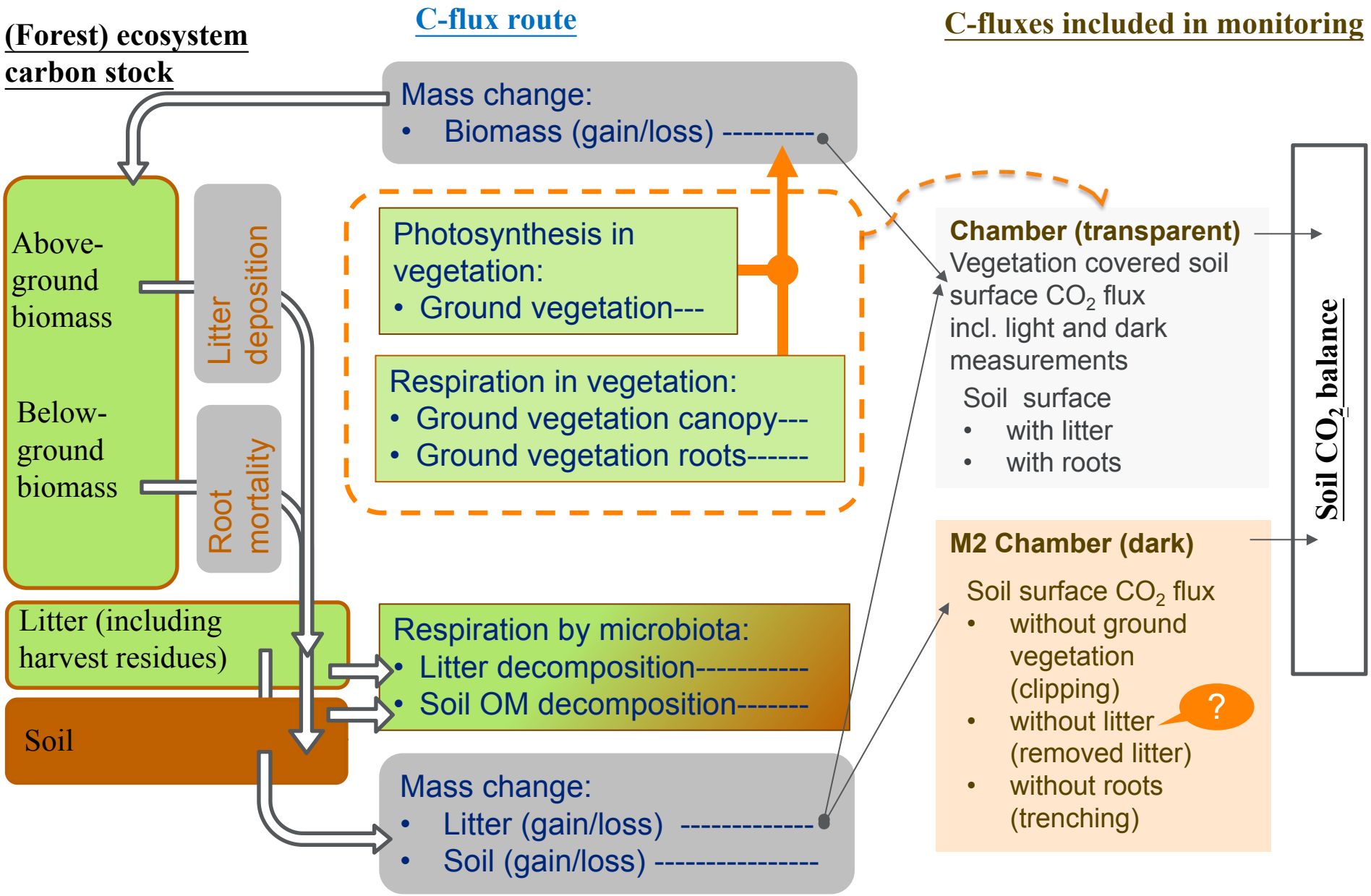
(Forest) ecosystem carbon stock
C-flux route
C-fluxes included in monitoring
Mass change:
• Biomass (gain/loss) ---------
Above-ground biomass
Below-ground biomass
Litter deposition
Root mortality
Photosynthesis in vegetation:
• Ground vegetation---
Respiration in vegetation:
• Ground vegetation canopy---
• Ground vegetation roots------
Litter (including harvest residues)
Soil
Respiration by microbiota:
• Litter decomposition----------
• Soil OM decomposition-------
Mass change:
• Litter (gain/loss) ----------
• Soil (gain/loss) ----------
Chamber (transparent)
Vegetation covered soil surface CO_2 flux incl. light and dark measurements
Soil surface
• with litter
• with roots
M2 Chamber (dark)
Soil surface CO_2 flux
• without ground vegetation (clipping)
• without litter (removed litter)
?
• without roots (trenching)
Soil CO_2 balance

Annex C1.1 Analytical methods for GHG flux and microbial diversity measurement sites

- Flux monitoring at each site will be continued on monthly basis for **24 months**.

GHG flux monitoring methods

- **'Method-1'** by using closed static chambers for monitoring CH_4, N_2O (& CO_2)
  - 5 replicates
  - Collars (Ø 50 cm) form permanent bases for chambers
    - Vegetation within the collar enclosed soil surfaces is not disturbed
    - Collars in cropland- and grassland sites will be removed during field management operations
    - In grasslands and croplands collars will be used to assess net ecosystem exchange ('Method-2')
  - Sampling & analysis
    - Deployment period in gas sampling 40-60 minutes per collar
    - Four air samples will be drawn into pre-evacuated glass bottles.
    - CH_4 and N_2O concentration will be analysed in the lab using gas chromatography
    - As the final outcome, gaseous flux monitoring data will provide directly soil net balance for CH_4 and N_2O fluxes over monitoring period.
  - Questions:
    - ~~pre-installed to form permanent bases – Are there new established forest plots where root trenching is needed?~~
      - ~~Decomposition of freshly cut tree roots form unwanted emission source.~~
    - ~~Collars in cropland- and grassland sites will be temporarily removed during field management operations –~~

https://life-peat-restore.eu/en/fieldwork-progress-at-soorsoo-restoration-area/

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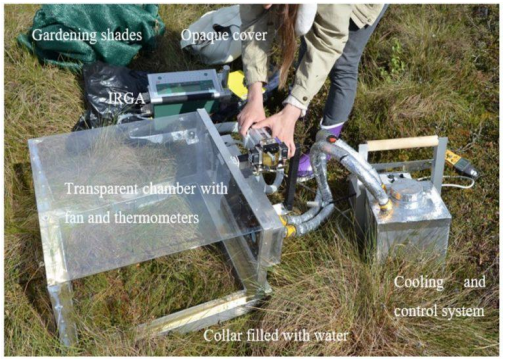
Annex C1.1 Analytical methods for GHG flux and microbial diversity measurement sites

GHG flux monitoring methods

- **'Method-2'** for in-situ heterotrophic soil CO_2 flux monitoring by using closed dynamic chambers
  - 25 permanent flux monitoring spots per site
  - Concentration change and flux will be determined using infra-red gas analyser (IRGA)
  - Flux monitoring surfaces
    - Trenched and root-ingrowth preventing cloth will be installed beforehand
      - belowground litter deposition and carbon loss as CO2 will be determined separately (see 'DDE field').
    - Kept free from litter during monitoring
      - litter deposition and emissions from litter decomposition will be determined separately (see 'DDE field').
  - Sampling & analysis
    - Respiration chamber will be set gas-tightly on the soil surface and during each flux measurement,
    - CO_2 concentration and temperature inside the chamber will be recorded
    - Deployment period up to 3 min using 'method-2'.
    - Heterotrophic CO_2 fluxes will be combined with relevant biomass based C-flux flows (i.e. litter deposition, above-ground and below-ground litter decomposition) for providing soil net CO2 flux balance.
    - In addition, soil net CO_2 balance in non-forested sites will be estimated from modelled net ecosystem CO_2 exchange based on in-situ collected data.
  - Questions:
    - ~~pre-installed to form permanent bases – Are there new established forest plots where root trenching is needed?~~
      - ~~Decomposition of freshly cut tree roots form unwanted emission source.~~
    - ~~Collars in cropland- and grassland sites will be temporarily removed during field management operations –~~



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Annex C1.1 Analytical methods for GHG flux and microbial diversity measurement sites

Biomass-related measurements quantifying annual plant biomass and production

- **Tree stand above-ground and below-ground biomass** (coarse root)
  - Tree stand diameter distribution (BHD) of all trees on the sample plot, and. tree height and length of the live crown for sample trees.
  - Allometric functions for separating biomass of different stand components (stems, branches, foliage, stump and coarse root systems)
- **Tree stand biomass production**
  - Based on annual diameter growth of measured sample trees
  - The allometric functions will be fitted
    - Annual biomass production as the difference between biomass values of 2 consecutive years.
- **Ground vegetation above-ground biomass**
  - By harvesting the vegetation of small plots at the time of peak biomass in late summer
    - shrubs, graminoids, forbs, mosses separated as applicable
      - For deciduous shrubs, the biomass will be separated into leaves and stems.
      - For all shrubs, current-year shoots will be separated.
      - Shrub stem radial growth will be estimated using literature data
    - Otherwise, deciduous leaves and current year shoots will be considered as annual biomass production.
    - Herbaceous plants' total biomass will be considered as annual above-ground production.
- **Fine root biomass (<2mm)**
  - From volume-exact soil cores, analyzed down to the rooting zone lower limit in 10-cm sections
  - Roots will be separated from soil by hand, washed, dried and weighed
  - Soil bulk density will be used to generalize root mass per sample volume to values per square meter.
- **Fine-root production**
  - by the ingrowth-core method for peat soils, or the root mesh method for annual plants.
    - In the ingrowth-core method, mesh cores filled with peat free of live roots installed into the sites in late autumn,
      - incubated for one (sites with annual crop plants) or two years (sites with perennial plants),
      - harvested, and separated into ingrown roots and peat.
      - The amount of ingrown roots represents fine-root production over the incubation period, which will be generalized into annual production
    - In the root mesh method, 2-dimensional mesh strips inserted in soil will be used.
      - Roots grown through the strips during incubation period and thereafter measured for a known volume both sides of the strip represent production.
      - This simpler method is enough where branching and radial growth of existing root systems need not be considered.
- **Fine-root turnover** (litter input) will be estimated as production per biomass.
  - Roots in both biomass and ingrowth core samples are separated into tree and ground vegetation roots to the extent possible; this task is labor intensive and requires expertise.

Annex C1.1 Analytical methods for GHG flux and microbial diversity measurement sites

Carbon inputs with dead biomass and carbon loss rates

- Current carbon stock in litter and dead wood estimates will be by the area-based sampling in each site
- **Forested sites**
    - Annual tree mortality estimates is based on monitoring data from other projects, or tree mortality models (e.g., Jutras et al. 2003), where applicable.
    - Perennial plants' annual above-ground litter (carbon input) is based on a repeated collection of litter from litter traps of known area set at the sites (e.g., Ojanen et al. 2013, Uri et al. 2017),
        - following the litter classification and analysis by methodology defined for ICP forest monitoring.
    - For annual plants, the annual biomass production equals also the amount of litter input.
    - Annual fine-root litter input rates will be based on the production/biomass ratio as described in Chapter 2.

    - Decomposition of these C pools will be estimated using
        - decomposition models
            - coarse woody debris of on conifer and deciduous trees (e.g., Tuomi et al. 2011a, Pearson et al. 2017), and
            - fine litter (e.g., Tuomi et al. 2011b, Straková et al. 2012) in different climatic conditions.
        - Thee litterbag method (Straková et al. 2012)
            - for estimating litter decomposition rates in cases where no applicable models exist
                - A known mass of air-dried litter sampled from the site is placed in a mesh bag (mesh ca. 1 x 1 mm to prevent physical loss and allow entrance of soil mesofauna participating in decomposition).
                - Sets of filled bags will be placed on site in conditions where litter decomposition takes place,
                - Incubated a set period
                - Harvested for analysis.
                - Remaining litter mass in each bag will be weighed after careful cleaning of possible ingrown plant parts and other alien material.
                - A time sequence of such data can be used to estimate mass and C loss dynamics.

Annex C1.1 Analytical methods for GHG flux and microbial diversity measurement sites

Characterizing soil microbial communities

- Due to the high expenses of these methods, sampling will be targeted on a subset of sites, chosen based on the first-year results of flux measurements on the sampling sites to capture the variation from high to moderate/low emissions
- Soil and sediment sampling strategy in each trial will be adjusted according to the data on methane emissions to evaluate activity of certain groups of bacteria in soil.
    - Similar emissions across all chambers in a trial -> collect a pooled soil sample from the entire trial.
    - Differences in emissions -> the sampling strategy will be adjusted in order to compare sites within trials.
        - Thee plots with the highest heterogeneity of CH_4 measurements will be selected for analysis
- One-time soil sampling will be done for characterizing the microbial communities that participate in CH_4 and N_2O fluxes (in case of CO_2 there are too many groups involved to make such analysis realistic)

- Methane oxidizing and reducing processes may occur in different soil layers, it is planned to take separate samples
    - the topsoil (upper 10 cm)
    - deeper soil layers (approx. 50 cm depth, representing anaerobic conditions in the most of the sites).
- DNA and RNA extraction from dried and sieved soils samples
    - The soil collection methods considers minimized exposure to air and instantaneous freezing of samples for RNA extraction.
- Microbial diversity will be determined in all soil samples by
    - HTS of 16s fragments amplified from total soil DNA.
    - Subset of samples by HTS of 16s fragments amplified from extracted RNA (to compare active microbes with the DNA analyses).
    - In addition, process-specific gene primers will be used to quantify these genes in the DNA and RNA samples analysed by 16s fragment sequencing.

Annex C1.1 Analytical methods for GHG flux and microbial diversity measurement sites

Soil screening with infrared spectroscopy (IRS)

- IR spectra summarize the whole chemical composition of the sample.
    - In this method, an IR beam of a known range of wave numbers is passed on the sample, and the absorption of the radiation by the sample is registered for defined wave number intervals.
    - IR absorbance spectrum, showing for each wave-length or wave-number the proportion of radiation absorbed by the sample, shows the relative abundance of different chemical bonds in the sample.
- The spectra can be
    - (1) used for direct interpretation of the absorbance intensities at different wave-lengths,
    - (2) reduced into a smaller number of variables that contain summarized information on the systematic variation in the spectra by, e.g., Principal Component Analysis or other multivariate methods (Adamczyk et al. 2016).
        - Such summary variables may then be used as predictive variables (e.g., Vavřova et al. 2008), in our case for GHG emissions.
    - These two approaches can be combined by first seeking for the characteristics of the spectra that have the best predictive power, and then interpreting them (Adamczyk et al. 2016).

- Lack of scientifically approved, simple and inexpensive methods for characterization of peat properties affecting GHG emissions from organic soils is one of the main issues challenges hampering the development of unified GHG accounting and projections models for organic soils.
- IRS is a fast and cheap method, once the spectrometer is available, as is for this project in both Luke and UT.
- The LIFE OrgBalt project will test IRS as such solution for cool temperate moist climate zone.
    - If the comprehensive description of soil chemistry with IRS proves to have predictive power for soil GHG exchange, the methodology could revolutionize the estimation of these emissions.

Annex C1.1 Analytical methods for GHG flux and microbial diversity measurement sites

Soil and water analyses at all gas fluxes measurement plots

- **Soil properties**
  - Samples from litter layer (in forest land) and from soil at 0-10 cm, 10-20 cm, 20-40 cm and 40-80 cm depth
  - 4 repetitions (combined sample from different depths for chemical analyses).
  - Sampling events
    - during the establishment of the reference and demonstration sites down to 80 cm depth or down to a mineral layer
    - repeated at a topsoil layer 3 times per vegetation season at 0-20 cm depth (to estimate seasonal variations)
  - Soil sampling and analyses will be performed according to ICP Forest guidelines (Cools & de Vos 2010, Konig et al. 2010). Exchangeable nitrate (NO_3^-) and ammonia (NH_4^+) iones will be determined in potassium or sodium chloride solution.

- **Water samples**
  - collected 4 times during the vegetation season from permanent wells used for measurement of the groundwater level.
  - Only 1 of 2 wells installed in every site will be used for water sampling, another will be left as a reference to obtain undisturbed time line of the groundwater level measurements.
  - Data from both wells will be used for rough estimation of soil water permeability.
  - Water analyses will be done according ICP Forest guidelines (Konig et al. 2010, Nieminen, 2011).

Table 1: Parameters and reference methods of soil analyses

No.	Parameter	Reference method	Application[1]
1.	Sample pre-treatment	ISO 11464	IR
2.	Soil Moisture Content	ISO 11465	IR
3.	Bulk Density	ISO 11272 (adopted to organic material)	I
4.	pH	ISO 10390	IR
5.	Organic Carbon (C)	ISO 10694	I
6.	Total nitrogen (N)	ISO 13878	IR
7.	Aqua regia extractable phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg)	ISO 11466	IR[2]
8.	Ash content	ISO 1171	I
9.	NO_3^-, NH_4^+	ISO 14256	IR

Table 2: Parameters and reference methods of water analyses

No.	Parameter	Reference method
1.	Sample pre-treatment	ISO 10523, ISO 7888
2.	pH	ISO 10523
3.	Electrical conductivity	BS EN 27888
4.	NH_4^+	ISO 7150/1
5.	Total N, NO_3^-, DOC, TOC	ISO 10304-1, ISO 12260, BS EN 1484
6.	Dissolved K, Ca and Mg	ISO 7980, ISO 9964-3
7.	Total P	ISO 6878