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























EU LIFE Programme project

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

WORK PACKAGE

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(D1)

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

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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."























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Abbreviations

ABBREVIATION DEFINITION

AFOLU agriculture, forestry and other land use

C carbon Ca calcium

CCM climate change mitigation measures

CO2 carbon dioxide CH4 methane

DNA deoxyribonucleic acid EF emission factor

GHG greenhouse gas or greenhouse gases
GLOSOLAN Global Soil Laboratory Network

IPCC Intergovernmental Panel on Climate Change

IPCC Guidelines

2006 IPCC Guidelines for National Greenhouse Gas Inventories

IPCC KP 2013 Revised Supplementary Methods and Good Practice Guidance

Supplement Arising from the Kyoto Protocol

IPCC Wetlands 2013 Supplement to the 2006 Guidelines for National Greenhouse Gas

Supplement Inventories: Wetlands

IR infrared

IRS infrared spectroscopy
ITS internal transcribed spacer
IRGA infra-red gas analyser

K potassium

LSFRI Silava Latvian State Forest Research institute "Silava"

Luke Natural Resources Institute Finland "Luke"

LULUCF land use, land use change and forestry

MEPRD Ministry of Environmental Protection and Regional Development

Mg magnesium
N nitrogen
N2O nitrous oxide

NEE net ecosystem exchange

NO3 nitrate

OTU operational taxonomic unit

P phosphorus

PCR polymerase chain reaction pH potential of hydrogen rRNA ribosomal ribonucleic acid

UT University of Tartu WOM without measures

WAM with additional measures

Introduction

The aim of monitoring the implementation of activities is to evaluate the impact of the implemented measures on greenhouse gas (GHG) emissions in the 17 demonstration sites and 36 reference sites established under action C3 and compare the identified impacts against the target indicators set out in the monitoring guidelines. In total, 53 sites are investigated.

The implementation of activities is monitored through three reports: initial, mid-term, and final.

The presented final monitoring report includes the description of the different field measurements used within the project to quantify greenhouse gas emissions from nutrient-rich organic soils on the two continuous years of measurement activities.

One of the main tasks of the LIFE OrgBalt was, in fact, the improvement of methodologies for the calculation (Action C1) and projections (Actions C2 and C5) of GHG emissions from drained nutrient-rich organic soils (grassland, cropland, forest land and managed wetlands), thus contributing to the development of national GHG inventory systems and to the implementation of national and global CCM targets. The main indicators of the success of Actions C1, C2 and C5 will be that key sources of GHG emissions or CO₂ removals on organic soils are reported according to Tier-3 methodology as preferred to Tier-1 or Tier-2 level reporting by the Intergovernmental Panel on Climate Change (IPCC) guidelines, as well as the impact of the climate change mitigation (CCM) measures implemented in managed cropland, grassland and forest land on organic soil.

GHG emissions in demo sites were monitored using GHG measurement methodologies applied in Action C1, including supplementary data on biomass production, weather conditions, soil and water properties. The long-term impact was modelled using the scenario analysis tool elaborated within the scope of Action C2 and C5. Monitoring data will be used to update the scenario analysis tool for short-term actions like changes in crop rotation and the application of wood ash. However, the continuation of the measurements after completing the project is of special importance to elaborate accurate impact assessment curves of climate change mitigation (CCM) measures.

The gas measurements in all sampling sites (reference sites established within the scope of C1 and demo sites established within the scope of C3) are used to improve GHG emission factors (EFs) elaborated in Action C1 and will be utilised in the final revision of the catalogue of CCM measures calculation and projections for WOM (Without measures) and WAM (With additional measures) projections, including the recommendation for application of CCM measures for the management of organic soils depending on land use, soil properties and climate projections.

Furthermore, considering high research value of the established demo sites, they will be used for monitoring GHG emissions from lands under transition period within the scope of the national CCM related research projects, as well as in training and education activities. Scientific outputs of the project will be monitored by the success of the implementation of the proposed methodologies and the publishing of project results.

The benefits, results, and effectiveness of the LIFE OrgBalt project actions are measurable and are evaluated and documented under the monitoring actions, compared with initial data, and checked if they are online with the project objectives and expected results. Specific indicators (measurements of CO_2 , CH_4 and N_2O fluxes or emissions, Tier-3 level methodology for emission from relevant sources calculation under national GHG reporting, content of national reports related to international environmental policy agreements) to detect the impact of the project activities at local (demonstration site level) and national level, are selected and regular monitoring was carried out.

The methodologies applied to evaluate the project results are described in further chapters. Due to the rapid developments in this field, the methodologies may be updated according to up-to-date best practices. The impact of the project climate change mitigation targeted activities implemented within demonstration sites will be assessed by collecting and analysing the values of the reduction of the GHG emissions in the demonstration sites.

1 Monitoring methodology of the impact of activities

1.1 Field measurements

Organic soils contribute to the atmospheric greenhouse gas (GHG) concentrations, as they can either remove or emit GHG and perform as globally extensive carbon (C) and nitrogen (N) stores. Currently, both the IPCC (2006) agriculture, forestry and other land use (AFOLU) guidelines and the IPCC (2014) Wetlands Supplement may be used for reporting the annual GHG emissions or removals for soils under anthropogenic land uses. Area-based emission factors (EFs), describing the net annual soil GHG emissions/removals, have been developed to reflect the impacts of ecosystem type, land management, and environmental conditions. Countries may opt for different methodological levels in their GHG reporting, so-called Tier-1 to -3, where Tier-1 is the most straightforward approach with default EFs of the IPCC. The accuracy of EFs can be improved as more peer-reviewed data become available and quantify a wider set of specific management options and ecological conditions for a given country or region. In LIFE OrgBalt, we are working to form Tier-2 level EFs for soil CO₂, CH₄, and N_2 O balances in monitoring included site types.

Quantifying the soil GHG balance, especially for carbon dioxide (CO₂), in forests and other ecosystems on organic soils is technically challenging. Monitoring needs to take into account that:

- C-sequestration into plant biomass takes place in a potentially voluminous and diverse vegetation community with uneven spatial distribution,
- C transfer from biomass into dead organic matter takes place both in the aboveground and belowground parts,
- physical and biochemical characteristics in organic soils change over time,
- CO₂ release through heterotrophic processes takes place both in recently deposited litter and in a soil composed of previously accumulated dead organic matter,
- CO₂ formed in the heterotrophic processes in the soil must be separated from similarly large CO₂ emissions formed in autotrophic root respiration in flux measurements,
- rates of biological processes change over the year and differ between years depending on weather conditions, stand development and management.

In this document, "soil CO₂ balance" includes C transfer fluxes to the soil as above- and belowground litter and losses by decomposition of litter and soil organic matter (Figure 1).

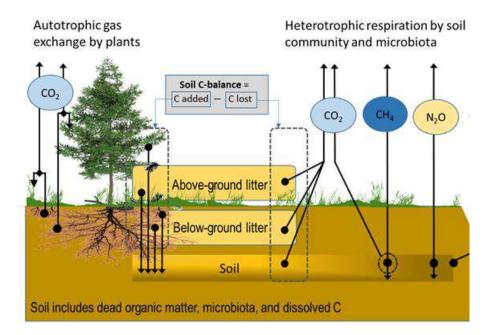


Figure 1. CO₂, CH₄, and N₂O fluxes and mass transfer components (arrows indicate flux/transfer direction) contribute to soil C-stock changes in a forest ecosystem on drained organic soil (as in IPCC, 2014), modified from Jauhiainen et al. (2019).

Soil CO₂ balance is estimated using the chambers-based measurement technique, which typically includes CO₂ exchange monitoring at the soil surface without ground vegetation and roots. Trenching (explained in subsequent paragraphs) prevents live root presence and regular sprout cutting prevents vegetation growth on the soil surface. Annual soil CO₂ balance is formed by using (1) summarised CO₂ flux data over the year in monitoring and (2) data on mass-based C stock changes, such as C inputs and decomposition as litter aboveground and belowground. Removal/inclusion of above ground litter in CO₂ flux monitoring needs to be considered in soil CO₂ balance equation, i.e., if the litter is removed from the measurement plots, the rates of both the input and decomposition of above ground litter need to be estimated.

For forming the Efs for methane (CH₄) and nitrous oxide (N₂O), there is no guidance on how living vegetation presence or litter dynamics should be taken into account in flux measurements, except that vegetation presence can be reported for CH₄ monitoring locations (IPCC, 2014). Wetland plants that have roots with aerenchymatous tissue are known to pipe out CH₄ from waterlogged peat layers (Askaer et al., 2011; Kokkonen et al., 2019) or attenuate the emissions in drained sites (Strack et al., 2006). Furthermore, belowground biomass disturbance, e.g., rhizosphere and mycorrhizal mycelia removal by trenching, has resulted in increased N₂O flux in drained organic forest soils (Ernfors et al., 2011). However, it seems clear that vegetation should be kept intact in studies of CH₄ and N₂O fluxes if possible. Annual soil CH₄ and N₂O balance are based on modelled fluxes over the year in monitoring.

The LIFE OrgBalt project aims to implement a wide range of innovative organic soil management measures to demonstrate how these areas can be managed sustainably, considering economic, social and climate aspects. 17 project demonstration sites have been established in Latvia and Finland. In the project, GHG fluxes are monitored in 53 sites, including all project demonstration sites and reference sites. Table 1 shows the list of all implemented demonstration sites with a short description of the potential benefits of the applied climate change mitigation measures.

Table 1. LIFE OrgBalt demonstration sites

#	Country	Code	CCM measure	Potential CCM benefits
1	Latvia	LVC303	Paludiculture	Potential benefits of the establishment of forest
			afforestation of	paludiculture in rewetted grassland:
			grassland with black	• Reduced GHG emissions from the soil due to
			alder and birch	the improvement of the water regime by
				mounding and establishment of the network of
				shallow furrows to drain exceeding surface
				water
				Reduction of risks associated with natural
				disturbances in forests with wet organic soils
				• Accumulation of CO2 in living and dead
				biomass, soil and litter and replacement effect
	T	THEOLOG		of forest biofuel and harvested wood products
2	Latvia	LVC302	Conventional	Potential benefits of afforestation:
			afforestation	• Reduced GHG emissions from soil
			considering shorter	• Accumulation of CO2 in living and dead
			rotation	biomass, soil and litter and replacement effect
				of forest biofuel and harvested wood products • Shorter rotation and more intensified
				management ensure higher yield and replacement effect, as well as reduces carbon
				losses due to root rot and other disturbances
3	Latvia	LVC308	Continuous forest	Potential benefits of continuous forest cover:
	200710	2.000	cover as a forest	• Reduced CH ₄ and N ₂ O emissions from soil due
			regeneration method	to avoiding of increase in the groundwater level
			in spruce stands	after harvesting
4	Latvia	LVC307	Application of wood	Potential benefits of wood ash application in the
			ash after commercial	forest on organic soils:
			thinning in spruce	• Increased CO2 removals in living biomass,
		stands		deadwood, soil, litter and harvested wood
				products due to improved growing conditions
				and additional increment in living biomass
5	Latvia	LVC311	Riparian buffer zone	Potential benefits of improved planting of black
			in forest land planted	alder in riparian buffer zone:
			with black alder	• Reduced GHG emissions from soil due to the
				improvement of the water regime by mounding
				and establishment of network of shallow
				furrows to drain exceeding surface water

#	Country	Code	CCM measure	Potential CCM benefits
	J			• Reduction of risks associated with natural
				disturbances in forests with wet organic soils
				• Accumulation of CO2 in living and dead
				biomass, soil and litter and replacement effect
				of forest biofuel and harvested wood products
6	Latvia	LVC309	Semi-natural	Potential benefits of forest stand regeneration
			regeneration of clear-	without reconstruction of drainage systems (from
			felling sites with	naturally wet or rewetted organic soils):
			grey alder without	• Reduced GHG emissions from the soil due to
			reconstruction of	the improvement of the water regime by
			drainage systems	mounding and establishment of the network of
			aramage systems	shallow furrows to drain exceeding surface
				water
				• Reduction of risks associated with natural
				disturbances in forests with wet organic soils
				• Accumulation of CO2 in living and dead
				biomass, soil and litter and replacement effect
				of forest biofuel and harvested wood products
7	Latvia	LVC306	Agroforestry— fast	Potential benefits of agroforestry:
			growing trees and	• Increased CO2 removals in living biomass and
			grass	soil
				• Reduced GHG emissions from soil and
				replacement effect of woody and herbaceous
				biofuel and harvested wood products
8	Latvia	LVC310	Fast growing species	Potential benefits of fast-growing species in
			in riparian buffer	riparian buffer zones:
			zones	• Increased CO2 removals in living biomass and
				soil
				• Replacement effect of woody and herbaceous
				biofuel and harvested wood products
				Avoided nutrients leakage from farmlands
9	Latvia	LVC301	Conversion of	Potential benefits of cropland conversion to
			cropland used for	grassland:
			cereal production	• Reduced GHG emissions from soil
			into grassland	• Increased carbon stock in soil and belowground
			considering periodic	biomass
			ploughing	• Reduced risks of nutrient leaching and soil
				erosion
10	Latvia	LVC305	Controlled drainage	Potential benefits of controlled drainage:
			of grassland	• Reduced GHG emissions from organic soils
			considering even	due to reduced fluctuations of groundwater
			groundwater level	level

#	Country	Code	CCM measure	Potential CCM benefits
			during the whole	• Reduced leaching of nutrients to surface water
			vegetation period	bodies
				• In summer drought additional water is available
				to meet crop demand ensuring higher carbon
				inputs into soil
11	Latvia	LVC304a	Introduction of	Potential benefits of legumes in conventional
			legumes in	crop rotation:
			conventional farm	• Reduced N2O emissions from soil reported in
			crop rotation	agriculture sector because of avoided mineral
				fertiliser application and gradual nitrogen input
				by symbiotic organisms
				• Increased carbon input with plants ensuring
				increased soil carbon stock
12	Latvia	LVC304b	Introduction of	Potential benefits of legumes in conventional
			legumes in	crop rotation:
			conventional farm	• Reduced N2O emissions from soil reported in
			crop rotation	agriculture sector because of avoided mineral
				fertiliser application and gradual nitrogen input
				by symbiotic organisms
				Increased carbon input with plants ensuring
				increased soil carbon stock
13	Latvia	LVC313	Strip harvesting in	Potential benefits of strip harvesting:
			pine stands	• Reduced CH4 and N2O emissions from soil due
				to avoiding of increase of the groundwater level
				after harvesting in comparison to clear-felling
14	Latvia	LVC312	Forest regeneration	Potential benefits of forest regeneration with
			(coniferous trees)	coniferous trees without reconstruction of
			without	drainage systems:
			reconstruction of	• Reduced GHG emissions from soil due to
			drainage systems	improvement of water regime by mounding and
				establishment of network of shallow furrows to
				drain exceeding surface water
				• Reduction of risks associated with natural
				disturbances in forests with wet organic soils
				• Accumulation of CO2 in living and dead
				biomass, soil and litter and replacement effect
				of forest biofuel and harvested wood products
15	Finland	FIC301	Continuous cover	Potential benefits of continuous forest cover
			forestry on	forestry practices:
			peatland. Selective	• Lower impact to environment conditions in
			felling without full	forest stand
			ditch network	• Remaining tree stand evapotranspiration
			maintenance.	controls soil water-table

#	Country	Code	CCM measure	Potential CCM benefits
			Conventional clear	• Reduced/no need for ditch network
			cut and uncut plots	maintenance
			are used as	• Reduced change in soil CO2 emission after
			comparison. Three	harvesting
			sites in monitoring in	• Reduced inputs of water and plant nutrients to
			South Finland.	surface water bodies
16	Finland	FIC302	Shifting to	Potential benefits of continuous forest cover
			continuous cover	forestry practices:
			forestry on	• Lower impact on environmental conditions in
			peatland. Forest	the forest stand
			regeneration	• Remaining tree stand evapotranspiration
			following harvesting	controls soil water-table
			of overstorey.	■ Reduced/no need for ditch network
			Conventional	maintenance
			clearcut + ditch	• Reduced change in soil CO2 emission after
			mounding + planting	harvesting
			and uncut forest are	• Reduced inputs of water and plant nutrients to
			used for comparison.	surface water bodies
			Three sites in	
			monitoring in South	
			Finland.	
17	Finland	FIC303	Shifting to	Potential benefits of continuous forest cover
			continuous cover	forestry practices:
			forestry on	• Lower impact on environmental conditions in
			peatland. Forest	the forest stand
			regeneration	• Remaining tree stand evapotranspiration
			following small gap	controls soil water-table
			harvesting and	• Reduced/no need for ditch network
			natural regeneration.	maintenance
			A spruce shelter tree	• Reduced change in soil CO2 emission after
			stand with natural	harvesting
			regeneration is used	• Reduced inputs of water and plant nutrients to
			as a comparison.	surface water bodies
			Two sites in	
			monitoring in North	
			Finland.	

1.1.1 Greenhouse gas flux monitoring

Two dark closed chamber methods were used to monitor GHG fluxes between soil and the atmosphere in field conditions. In both chamber methods, a known area and volume of airspace on top of the monitored soil surface are closed by a chamber headspace. GHG concentration increases inside the chamber over the time of the deployment period, and the GHG flux rate is determined by combining information on the closed soil surface area, the volume of the closed airspace, and the GHG

concentrations over the deployment period. The practical difference between the methods is the timing between the air sampling event at the field and the GHG gas concentration analysis that provides the final GHG flux reading. The first method involves a series of individual air samples collected during deployment time from the closed chamber at the field, storing the samples for transportation, subsequent GHG concentration analysis in the laboratory and calculation of the GHG fluxes (hereafter referred also as method-1). The second method involves closing the monitored airspace by closed chamber and circulation of air between the closed chamber and GHG analyser, and instant GHG concentration analysis and flux readout provided at the field (hereafter referred also as method-2). The first method is often referred to as the "static chamber method" and the latter as the "dynamic chamber method".

Traditionally the static chamber method has been more practical because (1) the GHG concentration analysis is based on common laboratory equipment and the analytical method by gas chromatography, and (2) several important GHG species, including CO₂, CH₄ and N₂O, can be analysed from the same gas sample, which usually makes the cost per sample affordable. The downside of the method is general slowness and labour intensiveness (e.g., long deployment time at air sample collection, especially for CH₄ and N₂O, potentially long time in sample transport/storage before the analysis by gas chromatography) before the actual GHG fluxes can be calculated.

The first portable gas analysers suitable for use in field conditions during vegetation season and using the dynamic chambers were for CO₂ data collection (trademarks such as ADC, EGM, Licor, etc.). Monitoring multiple GHG species (CO₂ and/or CH₄ and/or N₂O) has become possible in field conditions only recently due to technical development in instrumentation, and the price of analysers (e.g., Licor, Picarro, Gasmet, etc.) have gradually become more affordable. The key benefit of this method (in comparison to static chambers) is speed due to short deployment time and instantly available flux readout(s) for GHG(s). At the monitoring location, it allows renewed flux monitoring in the event of technical failures (e.g., chamber leakage) or any other unexpected pattern. Further, shorter deployment times makes it possible to collect GHG data from a higher number of monitoring points/conditions compared to the static chamber method. The downside of the approach includes the high price of the analyser, still developing techniques for use in demanding weather/climate conditions and sites, and analyser-specific limitations in GHG species included. The "Method" onsite gas sampling using dark closed static chambers (e.g., Hutchinson and Livingston, 1993; Ojanen et al., 2010) is used to measure total ecosystem respiration (Rtotal CO2) of the soil, CH4 and N2O. Collars (Ø 50 cm) in five replicates are pre-installed in the soil to form permanent bases for chambers and to keep the vegetation within the collar enclosed soil surfaces undisturbed. In the event of planned field management operations, collars in cropland and grassland sites are temporarily removed. During a 30-60 minute (depending on the volume of the chambers) long deployment period, four air samples are drawn from the cylindrical chamber headspace into pre-evacuated glass bottles. CH₄ and N₂O concentrations are analysed in the laboratory using gas chromatography to analyse the soil net gas exchange determination for these gases. This method was used on sites during winter as this method is not so demanding for weather conditions, but still limited in Nothern Finland due to high snow depth of up to 1.5 meters.

In grasslands, the transparent closed dynamic chamber is also used to assess the net ecosystem exchange of CO₂ during the growing period. 'Method-' is used for in-situ CO₂ flux monitoring by using a closed dynamic chamber (Järveoja et al., 2016; Ojanen et al., 2012). Concentration change and flux are determined using a portable gas analyser (e.g. EGM-4, EGM-5, Licor). On each site, 3 permanent flux monitoring point groups (i.e. sub-plots) are established for heterotrophic soil CO₂ emissions monitoring. Each flux monitoring point group includes three monitoring points (Ø 30 cm), i.e. a total of 9 monitoring points at each site. To prevent autotrophic root respiration contributions into CO₂ fluxes, flux monitoring enclosed surfaces were trenched and root-ingrowth preventing cloth is installed beforehand (belowground litter deposition and carbon loss as CO₂ will be determined separately). All monitoring surfaces were kept free from litter during monitoring (litter deposition and emissions from litter decomposition will be determined separately). In the method, the soil respiration chamber is set gas-tightly on the soil surface. During each flux measurement, CO₂ concentration and temperature inside the chamber are recorded over a deployment period of up to 3 min. A higher number of monitoring points was reserved for CO₂ monitoring based on the high importance of this specific greenhouse gas from drained organic soils (IPCC, 2014). This approach yields a sufficient amount of observed data of CO₂ emissions, keeping in mind that several different processes, both spatially and temporarily, contribute to the emission (Hiraishi et al., 2013), and monitoring by IRGA allows relatively fast CO₂ flux data collection. After each monitoring round at the field, GHG flux data was uploaded to the server operated and maintained in Luke. Data quality was automatically pre-screened based on agreed criteria and stored on the server. Fluxes stored on the server can be accessed at any time. Still, annual flux calculation can be performed after a complete one-year-long dataset becomes available.

Fluxes of CO₂, CH₄ and N₂O are calculated from the change in gas concentration in the chamber headspace over time, adjusted by the ground area enclosed by the collar, volume of chamber headspace, air density and molar mass of gas at the measured chamber. Flux monitoring at each site was continued at least monthly for 24 months. The same sampling and flux calculation methods were applied both for reference and demo sites but also the same time period is used for sampling to guarantee comparability of data between the sites and countries.

As the final outcome, gaseous flux monitoring data provides the soil net balance for CH₄ and N₂O fluxes over the monitoring period ''method-''). For estimating soil net CO₂ flux at all monitoring sites, heterotrophic CO₂ fluxes estimated by the ''method-'' needs to be combined with relevant mass-based C-flux flows in above- and belowground litter for providing complete soil net CO₂ flux. In addition, soil net CO₂ balance in non-forested sites can be estimated from modelled net ecosystem CO₂ exchange based on in-situ collected data.

1.1.2 Tree stand biomass measurements

Carbon fluxes mediated by vegetation are estimated by measurements of plant biomass and production (Ojanen et al., 2013; Uri et al., 2017). Tree stand aboveground and belowground biomass (coarse root) estimation are based on measuring the tree stand diameter distribution (breast height diameter) of all trees on the sample plot, and further parameters (e.g., tree height and length of the live crown) for sample trees. Sample tree data forms a complementary set of variables for all trees.

Biomass of different stand components (stems, branches, foliage, stump and coarse root systems) are estimated with allometric functions that use breast height diameter, either alone or together with the complementary variables, as explanatory variables (see Figure 2, Figure 3). Such functions are available for all our common forest tree species (e.g., Zianis et al., 2005; Liepiņš et al., 2017). Biomass production estimations are based on the annual diameter growth of measured sample trees. The growth data are used to construct diameter distributions and the complementary set of variables for the stand in consecutive years. The allometric functions are fitted into these data sets, and the annual biomass production are estimated as the difference between biomass values of consecutive years. Values are transformed per square meter using a sample plot area.

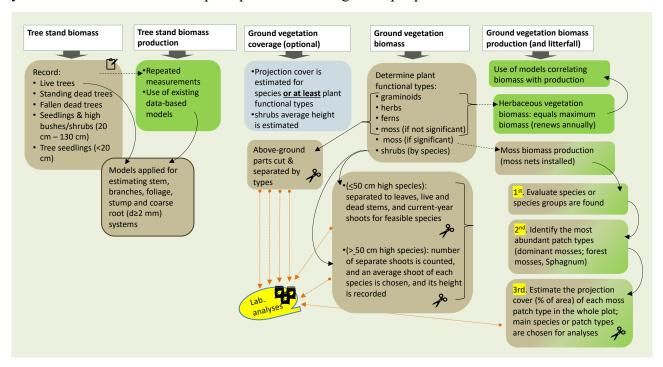


Figure 2. Outline of planned aboveground biomass and biomass production determination in tree-, understory- and ground vegetation layers in LIFE OrgBalt.

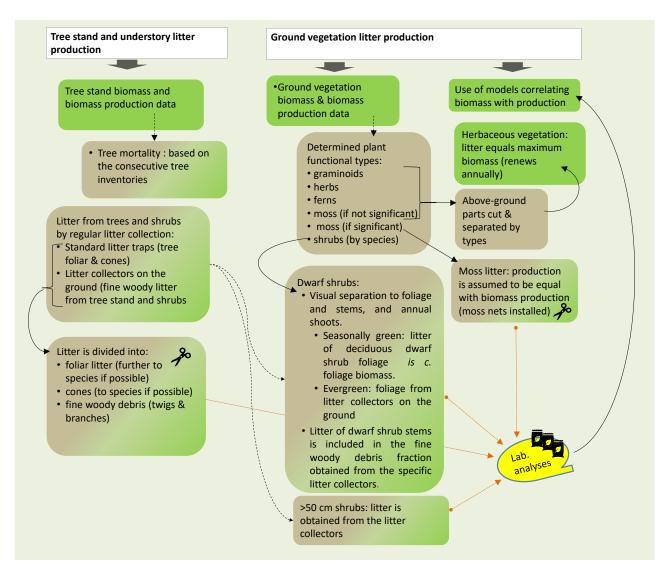


Figure 3. Outline of planned aboveground litter production measurements in tree-, understory-and ground vegetation layers in LIFE OrgBalt.

1.1.3 Ground vegetation measurements

The aboveground biomass of the ground vegetation was measured by harvesting, drying and weighing the aboveground vegetation of small plots at the time of peak biomass in summer 2021 in Finland and 2022 in the Baltic states (see Figure 2). In the method, the samples are separated into plant functional types (shrubs, graminoids, forbs, and mosses, as applicable). For deciduous shrubs, the biomass is separated into leaves and stems. For all shrubs, current-year shoots are separated. Shrub stem radial growth is estimated using literature data for plots with substantial shrub layer. Otherwise, deciduous leaves and current-year shoots will be considered as annual biomass production. For herbaceous plants, total biomass is regarded as annual aboveground production. Values are transformed per square meter using a sample plot area. Existing data on correlations between biomass and annual production rates in different species are applied where possible and further developed in forest sites to ease laborious harvesting, separation, and drying work.

<u>Fine root biomass</u> (<2mm) is estimated from volume-exact soil cores, analysed down to the rooting zone lower limit in 10-cm sections (see Figure 4). The end of live-root occurrence is confirmed from the samples. Roots are separated from soil by hand, washed free of soil, dried and weighted, and soil bulk density will be used to generalise root mass per sample volume to values per square meter.

<u>Fine-root production</u> is estimated by the ingrowth-core method modified for peat soils (Laiho et al., 2014; Bhuiyan et al., 2017), or the root mesh method (Uri et al., 2017) for annual plants. The amount of ingrown roots represents fine-root production over the 1-2 years-long incubation period, which will be generalised into annual production per square meter. Pilot studies suggest that two years of incubation time is needed for sites with perennial vegetation (Bhuiyan et al., 2017 and unpublished data). In the root mesh method, roots grown through the strips during the incubation period and thereafter measured for a known volume on 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) is estimated as production per biomass. Roots in biomass and ingrowth core samples are separated into tree and ground vegetation roots to the extent possible; this task is labour intensive and requires expertise.

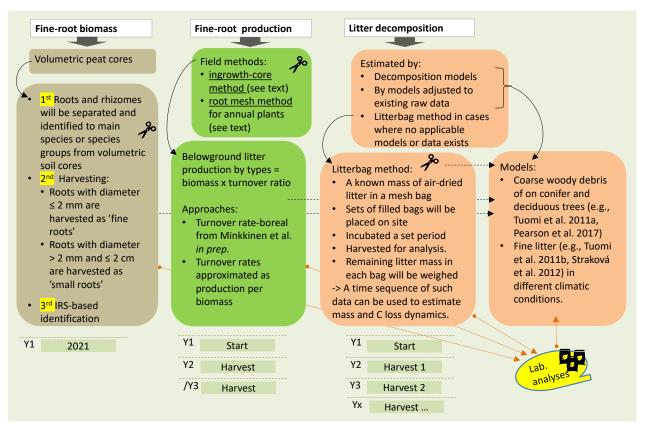


Figure 4. Outline of belowground fine-root biomass determination, biomass production determination, and belowground decomposition determination in LIFE OrgBalt.

1.1.4 Carbon inputs with dead biomass and carbon loss rates

Estimates of current carbon stock in litter and deadwood are obtained by the area-based sampling in each site. For forested sites, annual tree mortality estimates are based on monitoring data from other

projects or tree mortality models (e.g., Jutras et al., 2003), where applicable. Estimates on the <u>amount</u> <u>of deadwood</u> can be made from tree stand biomass.

<u>Carbon input with the annual aboveground litter from perennial plants</u> are based on a recurrent collection of litter from litter traps of known area (e.g., Ojanen et al., 2013; Uri et al., 2017), following the litter classification and analysis by methodology defined for ICP forest monitoring (see Figure 3). Litter traps were set at the sites at the time of site establishment and the start of gaseous flux monitoring. For annual plants, the annual biomass production equals also the amount of litter input (i.e. annual plant litter estimates are based on ground vegetation biomass monitoring). Annual fine-root litter input rates are based on the production/biomass ratio as described in previous chapters.

<u>Decomposition of aboveground litter C pools</u> is estimated using decomposition models, separately for the coarse woody debris of conifer and deciduous trees (e.g., Pearson et al., 2017; Tuomi, Rasinmaki, et al., 2011; Tuomi et al. 2011a; Pearson et al., 2017), and fine litter (e.g., Strakova et al., 2012; Tuomi et al., 2011a) in different climatic conditions. The litterbag method (Strakova et al., 2012) is used for estimating litter decomposition rates in cases where no applicable models exist (see Figure 5).



Figure 5. Examples of decomposition experiment litterbags containing a known amount of tree twig litter with two diameter classes (left) and different litter types harvested from litter collector (right).

Typical litter types on the chosen experiment sites were used to collect new litter decomposition data. The listed materials include deciduous leaves (alder or birch), needles (spruce or pine), dead shrubs (*Filipendula* sp. or *Rubus chamaemorus*), small twigs (diam. <5 mm), thicker twigs (diam. 10 mm < x <20 mm), *Sphagnum* moss (if abundant on-site), forest mosses (if abundant on-site), unsorted (twig-free) litter from litter collectors. The suggested litter types for the study in the Baltic states are based on conditions at the suggested/selected sites (

Table 2) for this experiment.

Table 2. Suggested forest sites for the decomposition study in the Baltic states based durin the planning process in September 2021

Country	Black alder	Birch	Pine	Spruce
Lithuania	LTC109	LTC108	-	LTC104
Estonia	EEC108	EEC106	EEC105	EEC104
Latvia	LVC109	LVC108	LVC110 (LVC107) ⁽¹⁾	LVC106

 $^{^{(1)}}$ Optional addition to include (old stand) for adding number of sites to 3 sites studied

<u>Litter traps</u> collecting litterfall from trees and ground level were set at forest sites during autumn 2020 in all partner countries. The traps were emptied for deposited litter materials monthly during the warm season and after snow melts. Existing litter collections in Finland were upgraded according to LIFE OrgBalt standards.

<u>Litter decomposition</u> study materials were collected in selected sites starting coherent to the site establishment and the monitoring from 2020 onwards. Litterbags were prepared and set to the chosen sites in Finland and in the Baltic states. Harvesting of the litter bags is done after 1, 2, 3 (and 4) years after the experiment start, and processed in the laboratories for their weight and carbon content.

1.1.5 Characterising soil microbial communities

Within this project, the focus lies on the whole microbiota: fungi, archaea and bacteria, in particular on the forested organic sites LIFE OrgBalt offers. This is because the main GHG in drained organic sites is CO₂. The microbiome is analysed by amplicon sequencing using ITS and 16S primers. 30 sites are included in the analysis (8 birch-dominated, 4 black alder-dominated, 7 pine-dominated/mixed, 9 spruce-dominated, and 2 spruce/birch mixed forests), totalling 180 separate soil samples. Selected forest sites can be grouped to include differences in tree composition (deciduous, conifer, and mixed tree stands), tree stand age, and typical water table levels in soil (high and low water table sites). In each chosen site, soil sampling (performed in August /September 2021) was made at each of the three subplots with two treatments; trenched and un-trenched. Sampling made in un-trenched conditions (points established for N₂O, CH₄ and total CO₂ monitoring) includes soil environment with semi-decomposed organic soil, recent belowground litter, living roots, mycorrhizae, soil animals etc.. In contrast, living roots are excluded from the trenched conditions (points established for heterotrophic CO₂ monitoring). This chosen main strategy is adjusted according to the data on CO₂ emissions on these drained soils.

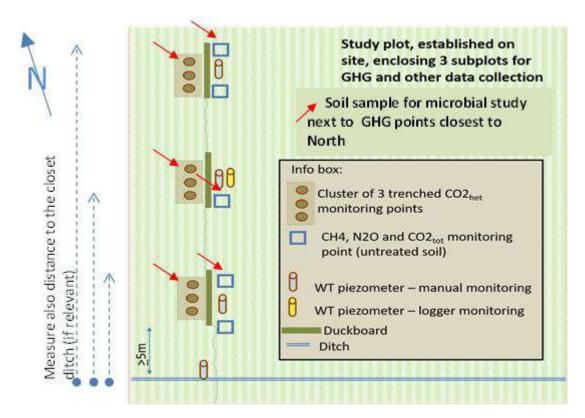


Figure 6. Soil sampling strategy in included forest sites

As described for the whole procedure in Kosunen et al. (2020) the DNA is extracted from the samples using a NucleoSpin soil kit (Macherey Nagel, Germany). Nanodrop One (Thermo Scientific) is used to measure DNA concentrations. The bacterial and fungal community structure was assessed with amplicon sequencing targeting the 16S and ITS regions, respectively. As a deviation from the proposal plan, the main focus was on the microbial decomposer community involved in in the CO₂ emissions because (1) it is likely to be the main GHG gas species emitted from these primarily drained nutrient-rich organic soils, and (2) it would be challenging to sample soil profile depths to below the ground water level necessary for studying methanogens and methanotrophs.

ITS2 region for fungi and V4 region of 16S SSU rRNA for bacteria are amplified by polymerase chain reaction (PCR). The fragments are then sequenced with the MiSeq platform (Illumina) by utilising the MiSeq v3 kit. PipeCraft 1.0 pipeline software is used for quality filtering as well as the removal of artefacts, primer-dimers and primers from the raw 16S rRNA and ITS sequence reads. After assembling paired-end reads and a two-step quality filtering, an OTU table is created from the sequence reads. OTUs are then annotated taxonomically using BLAST and a reference ITS2 database (sh_genral_release_dynamic_01.12.2018.fasta) from UNITE and a 16S rRNA database (SILVA_123_SSURef_Nr99_tax_silva.fasta) from SILVA to find representative fungal and bacterial sequences, respectively. After quality filtering, functional information of fungal guilds of OTUs is derived from FUNGuild. Sequence annotation took place at Luke in 2022/2023.

1.1.6 Soil screening with infrared spectroscopy (IRS, FTIR)

Information on soil nutrient concentrations and other soil properties, e.g., soil organic matter characteristics, are needed for many purposes. The rates of many soil processes and, consequently, soil greenhouse gas emissions depend at least to some extent on the nutrient regime of the site (IPCC 2014). Infrared spectroscopy (IRS) is a rapid, cost-effective and relatively easy-to-use technique that has long been used for the characterisation of different sample materials, including the determination of several chemical and biological characteristics of soils (e.g., Holmgren and Nordén, 1988; Confalonieri et al. 2001; Terhoeven-Urselmans et al., 2008; Cécillon et al. 2009; Bellon-Maurel and McBratney 2011; Krumins et al., 2012; Hayes et al., 2015; Straková and Laiho, 2016). IRS has long been applied in characterising samples with complex chemical compositions, including peat. Infrared radiation is the region of electromagnetic radiation where wavelengths range from ca. 780 nm to ca. 1 mm. Infrared waves are thus longer than those of visible light. Infrared spectroscopy is based on each chemical bond absorbing infrared radiation in a specific manner that depends on the nature of the bond. Thus, an infrared absorbance spectrum, showing for each wavelength or wavenumber the proportion of radiation absorbed by the sample, shows the relative abundance of different chemical bonds in the sample, that is, a summary of the chemical composition of the sample (e.g., Coates, 2000). The power of IRS is based on each chemical bond present in a sample absorbing IR radiation in a specific manner that depends on the nature of the bond. Thus, an IR absorbance spectrum, showing for each wavelength or wavenumber the proportion of radiation absorbed by the sample, shows the relative abundance of different chemical bonds in the sample. IR spectra thus summarise the whole chemical composition of the sample. The spectra can either be used for direct interpretation of the absorbance intensities at different wavelengths or be reduced into a smaller number of variables that contain summarised information on the systematic variation in the spectra by, e.g., Principal Component Analysis (PCA) or other multivariate methods (Adamczyk et al., 2016). Such summary variables may then be used as predictive variables (e.g., Vávrová et al., 2008), in our case, for GHG emissions. These approaches can be combined by first seeking the characteristics of the spectra that have the best predictive power and then interpreting them (Adamczyk et al., 2016).

The LIFE OrgBalt project tested IRS as such solution for peat and soil samples collected in cool temperate moist climate zone in forest land, cropland and grassland. In parallel, peat samples collected previously in the LIFE REstore project (from 42 GHG measurement and demo sites) were used to cover the full spectrum of peat properties – from nutrient-poor Sphagnum peat to fertile peat of mesotrophic bogs. Soil samples from peatlands with various land uses and samples from naturally wet and drained forest stands with different forest site type classification were selected. Sample set dominated by organic soils with some exceptions of mineral soil from deeper soil layers. The project ensures comparability with the GLOSOLAN network by utilising the GLOSOLAN specifications-based equipment and procedures. The spectroscopic analyses on the LIFE REstore project samples were run in Silava laboratories in 2021.

This study aimed to start building a spectral library for organic soils (including peat) and to create initial calibration models to evaluate the method's potential to predict pH value and C, N, P, K, Ca and Mg concentration in peat samples. The results are reported in LIFE OrgBalt Mid Term reporting

(Annex C2_03 Harmonized methodology for characterising peat properties using infrared screening method). In the scope of this study, the residual prediction deviation value (RPD) was considerably lower than the 2 signals - a possible difficulty in applying the current methodological approach for quantitative analyte prediction in unknown samples. The highest potential of prediction performance was observed for pH, Ca, and Mg, but the lowest perspective for P and K. C, N and humic acid as well as other parameter prediction performance, may be improved by primary increasing count and variety of calibration samples (spectra) and secondary by increasing count of measurement replicates for the same sample to discard replicates that increases relative standard deviation of prediction replicates above the threshold, e.g. 10 %. It was observed that mostly the highest performance of analyte prediction in peat samples was for prediction models elaborated by the peat soil calibration data set only; the addition of forest soil sample spectra to the calibration data set did not improve model performance.

Nevertheless, also for such calibration data sets with peat soils only, PCA often indicated significant spectral differences that could have added uncertainty to values predicted by the model. In the scope of the study separation of spectra by PCA did not improve model quality as model robustness may have decreased to the insufficient number of spectra. The higher number of spectra would allow for making separate calibration models by focusing more on PCA results. Afterwards, these models could be applied to unknown samples by the guidance of values of spectral residues and Mahalanobis distance to match appropriate models and unknown spectra. Another potential solution for improving model prediction capabilities may be improving sample preparation procedures, e.g., ensuring more homogenous samples.

1.1.7 Soil and water analyses

A comprehensive evaluation of soil properties down to 100 cm depth was done in all gas fluxes measurement plots while establishing the reference and demonstration sites. Soil properties were implemented once during the project implementation, in 2021-2022. Soil sampling and analyses were performed according to ICP Forest guidelines (Cools and de Vos, 2010; Konig et al., 2010), methodology providing comparable results. Sampling was done in 3 repetitions in every reference and demo site or using a method providing comparable results. A good procedure is sampling at north and south from gas measurement sites, as close as possible to gas sampling & measurement sites. Sampling sites were located in a flat area representing average conditions in a reference or demo site. 100 cm3 undisturbed soil samples were collected at 0-10, 10-20, 20-30, 30-40, 40-50 cm depth and disturbed samples at 50-75 and 75-100 cm depth. After collection, samples were transferred to plastic bags with labels containing information on the project, sampling plot, repetition, depth and date.

Additionally, litter samples ($10 \times 10 \text{ cm}$ to the whole depth) were collected nearby soil sampling sites in forest land. Small pits can be dug to collect samples if sampling with an auger is impossible. Litter samples in the field or in the laboratory should be cleaned from plants' green (living) parts.

Soil and litter samples were collected in the spring and summer of 2021 or 2022.

After collection, samples were transported to LSFRI Silava laboratory of Forest environment and airdried. Then all samples were dried at 105°C degrees, weighted to determine bulk density, milled and

screened through a 1 mm sieve, and samples for elemental analyses were milled and sieved through a 0.25 mm sieve. After the preparation of samples following parameters were determined: bulk density, pH, N, P, K, Ca, Mg, C and ash content. Parameters determined in soil and reference methods are provided in Table 2.

Table 3. Parameters and reference methods of soil analyses

No.	Parameter	Reference method	Application1
1.	Sample pre-treatment	ISO 11464	IR
2.	Soil Moisture Content	ISO 11465	IR
3.	Bulk Density	ISO 11272 (adopted to	Ι
		organic material)	
4.	рН	ISO 10390	IR
5.	Organic Carbon (C)	ISO 10694	Ι
6.	Total nitrogen (N)	ISO 13878	IR
7.	Aqua regia extractable phosphorus	ISO 11466	IR2
	(P), potassium (K), calcium (Ca) and		
	magnesium (Mg)		
8.	Ash content	ISO 1171	I

Water samples (0.5 L per piezometer per time) were collected from piezometers during every site visit (monthly base on average), simultaneously with gas sampling. Sampling was done from one of the piezometers. The other should be used for continuous water level measurement, and additional 2 piezometers were used for manual water level measurement during site visits if the sample plot is split into subplots. Water samples after collection are transported in a cooling boxes and stored in a fridge at a low temperature (4°C). Once per month, all samples were transported in cooling boxes to Latvia for laboratory analysis. This was done simultaneously with the transportation of gas bottles for gas analyses, or by courier services. At LSFRI Silava following parameters were determined in water N total, NO₃-, P, K, Ca, Mg, DOC). Additional parameters, e.g., Hg may be considered in case of additional funding to determine the linkage between environmental conditions and Hg outputs into water. Parameters determined in water samples and reference methods are provided in Table 3.

Table 4. Parameters and reference methods of water analyses

No.	Parameter	Reference method
1.	Sample pre-treatment	ISO 10523, ISO 7888
2.	рН	ISO 10523
3.	Electrical conductivity	BS EN 27888
5.	Total N, NO ₃ -, 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

The results of the analyses are used to determine possible correlations and covariations with GHG fluxes, particularly, after the proposed actions are implemented in the project demo sites. Water properties are further used as additional parameters to increase the elaborate GHG emission models'

accuracy and improve the ability to predict GHG fluxes under different management scenarios and land uses.

1.2 Modelling

The SUSI peatland simulator is aimed for application in boreal and tropical climate zone to calculate growth response on the drainage of organic soils, including estimation of soil carbon losses. SUSI peatland simulator is based on the assumption that forest growth is limited by the accessibility of nutrients, which are released during the decomposition of organic matter. The increased groundwater level is slowing down the decomposition of organic matter and the availability of nutrients, reducing the growth of trees and carbon losses. Susi peatland simulator is aimed at the parametrisation of these variables. The main modelling aim is to upgrade the SUSI peatland simulation for use in projecting CC scenarios and make the software useable within the LIFE OrgBalt region. Furthermore, SUSI will be delivered as open-source software to be readily and widely adaptable for drained organic soil research and land use studies.

The SUSI peatland simulator is developed, but its improvement is an ongoing process. The effort placed on this task has been increased by the addition of postdoctoral researcher Jani Anttila to the project. Considerable effort is now made to improve the accessibility of the simulator. This includes writing documentation, and user instructions, improving the readability of model output, creating well-explained example use cases, as well as improving the actual user interface to the simulation code via Jupyter notebooks and from the command line. The model has also been made publicly available on Github at https://github.com/annamarilauren/susi so that researchers and developers can access the source code and suggest improvements directly to the maintainers.

The current challenge in applying the SUSI model in Baltic countries is generating the appropriate input data. These data need to contain specifics, such as tree biomass partitioning into branches, leaves, roots, etc., which need to be estimated with statistical models appropriate to the site and tree species. More effort and cooperation are currently directed toward achieving this task of creating suitable inputs for the model.

2 Overview of the monitored field and laboratory activities

In the LIFE OrgBalt project, the fieldwork has been carried out in all demonstration and reference sites, 53 sites in Latvia, Lithuania, Estonia, and Finland.

2.1 Greenhouse gas flux monitoring

The continuous sampling started twice per month in Estonia and monthly in Latvia by January 2021. In Lithuania, the sampling started on a monthly base in autumn 2021. Sampling in Finland started in spring 2020. In all countries, the sampling lasted at least 24 consecutive months.

GHG fluxes measurements and sampling have been done by using three different methods:

- 1) Static dark chamber method (see sub-chapter 1.1.1. 'Method -1') to measure N₂O, CH₄ and total ecosystem respiration (CO₂) throughout all seasons
- 2) Heterotrophic respiration soil CO₂ emissions monitoring during the warmer period (see subchapter 1.1.1., 'Method-2'),
- 3) NEE transparent chamber (in grasslands) during the vegetation period.

On regular bases, data quality check and flux calculations have been done:

- Raw data quality check of the measurement data with static dark chamber and the flux calculations of N₂O and CH₄
- Raw data quality check and heterotrophic respiration flux calculations (by using flux calculation platform created by Luke)
- Raw data quality check of NEE.

GHG measurements overview in the project partner countries:

- **Finland:** measurements were carried out every third week during the warm season. In wintertime, monitoring was approximately every five/six weeks because of typically low temperatures (challenge of operation temperature of mobile analyser) and snow conditions (challenges of having site access). Monitoring was carried out from May 2020 to April 2022 at the demo sites. In total, 1'470 readouts/samples for each CO₂ and CH₄, and 346 for N₂O measurements were taken. The lower number of N₂O samples were due to availability limitations of the used Licor mobile analyser.
- Estonia: all the measurements are carried out twice per month. During each measurement campaign, the gas samples were collected in 6 replicates (6 chambers) and from each chamber during the 1 h-long deployment time, 4 samples were collected. In total, 11'520 gas samples were collected and analysed.
- Latvia: measurements are carried out monthly. During each measurement campaign, the gas samples were collected in 5 replicates (5 chambers) and from each chamber during the deployment time, 4 samples were collected. In total, 13'380 gas samples were collected and analysed.

• **Lithuania**: measurements were carried out monthly from October 2021 to September 2023. During each measurement campaign, the gas samples were collected in 5 replicates (5 chambers) and from each chamber during the 1-hour long deployment time, 4 samples were collected. In total, 4'840 gas samples were collected and analysed.

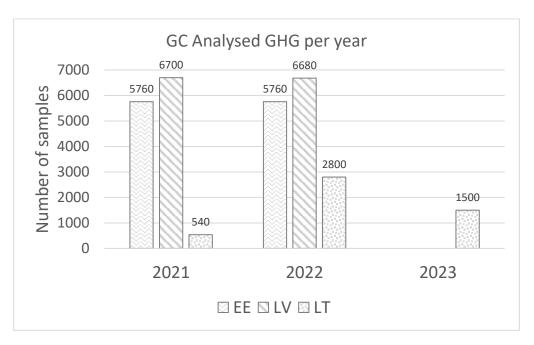


Figure 7: Number of analysed gas samples, analysed with GC in the laboratory at the University of Tartu

Soil heterotrophic respiration measurements ('Method-2') were done at the same frequency as the static dark chamber measurements ('Method-1'): in Estonia twice per month, in Latvia and Lithuania once per month during the vegetation period using the portable gas analysers (EGM-4 and EGM-5). In total, 2690 (Estonia), 787 (Lithuania), 3263 (Latvia), and 1289 (Finland) samples of heterotrophic respiration were taken, uploaded to the flux calculation platform created by Luke, and the data quality checked, the fluxes calculated, and data analysed.

NEE measurements with transparent chambers are carried out on the grasslands at the same frequency as other GHG flux measurements in Estonia and Latvia from April to November 2021 and 2022, respectively. Regarding the NEE flux calculations (transparent chamber method), there is a plan to create a platform/system, and integrate to the heterotrophic respiration data calculation platform, to further study the possibilities to automate annual flux calculations. However, these novel automation and machine learning developments are considered LIFE OrgBalt inspired spin-offs that need additional funding support as they were not part of the original project plan.

2.2 Biomass-related measurements quantifying annual production

Ground vegetation coverage measurements and ground vegetation biomass sampling (biomass and biomass production samples) were made earlier in Finland. Ground vegetation coverage

measurements and ground vegetation biomass sampling in the Baltic states started in 2021 and 2022 by utilising on-site harvested samples, as supplemented modelling-based approaches to ease the large workload involved. As a part of ground vegetation biomass monitoring, moss nets were installed on forest sites with abundant moss coverage during autumn 2020-2021. Sampling for fine root biomass determination was carried out in 2022. Root ingrowth cores were set in forest sites in 2020.

<u>Tree biomass</u> measurements in Finland were made in 2016 at all sites, repeated during LIFE OrgBalt 2020 at FIC301 and FIC302 sites, and in spring 2022 at FIC303. In Latvia, the measurements were done in 2021 on all forest sites, and data calculation and analyses for the tree stand biomass done. In Estonia and Lithuania, the tree stands aboveground and belowground (coarse root) biomass estimations took place in summer and autumn 2022.

Aboveground biomass sampling on the grassland and cropland has been carried out in all Baltic states at the time of maximum vegetation growth – at the end of July or the beginning of August 2021. Samples have been dried, weighted and analysed for chemical parameters (C and N content). Belowground biomass samples from the grasslands were collected in April 2021 and the beginning of August 2021. All the root samples have been washed out, sorted, dried, weighted, and analysed for C and N content.

Moss biomass and moss biomass production in forest sites. In Finland and Latvia, the installed moss nets from 2020 were harvested in 2021 and analysed, in Lithuania and Estonia in 2021 and 2022, respectively.

The ground vegetation cover and biomass collection were carried out in Finland in 2020 and 2021, the analyses finished in 2022. Collected biomass data has been used in the testing possibility to model shrubs biomass based on simplified shrubs data – the results from this method's development are further studied and evaluated. In Latvia, the samplings and measurements were done in summer 2021, and data calculation and analyses are done in 2022. In Estonia and Lithuania, estimations of annual biomass production and litter inputs from ground vegetation took place in summer and autumn 2022, calculations and analysis finished by 2023.

<u>Fine root biomass</u> samples (soil cores) were collected from the Finland sites in 2020 and analysed in 2021. In Latvia, the samples were collected, washed out, sorted, weighted and analysed in 2021. In Lithuania and Estonia, the fine root biomass sampling was carried out in autumn 2022 and analysis ended in 2023.

The fine root production measurements started in Finland by root sock incubation at 2 sites in 2020. samples from previously stated incubation at one site were harvested in autumn 2020 and analysed in 2021. In the Baltic states, the fine root production measurements started in 2022, analysis was finished by 2023.

Overall, biomass samples in severe fractions were analysed at Silava for their respective C and N content: 246 (Finland), 223 (Estonia), 1444 (Latvia), and 81 (Lithuania).

2.3 Carbon inputs with dead biomass and carbon loss rates

<u>Litter</u> materials for carbon input and decomposition was collected in selected sites starting coherent to the site establishment and the monitoring from 2020 onwards. Litterbags were prepared and set to the chosen sites in Finland and in the Baltic states. Harvesting of the litter bags is done after 1, 2, 3 (and 4) years after the experiment start and processed in the laboratories for their weight and carbon content.

<u>Litter traps</u> were set at the forest sites at the start of gaseous flux monitoring and litter material collection was followed simultaneously with the flux monitoring. The collected litter materials were separated in the fractions, dried, weighted, and analysed for C and N.

<u>Decomposition studies</u>. Litter decomposition study materials in Finland were collected in selected sites in 2020. Litterbags were prepared and set to the chosen sites in spring 2021, and the first bags got in spring 2022. The four-years decomposition experiment was set up in Estonia, Latvia and Lithuania in spring and autumn of 2022, i.e. the first- and second-year sampling batches were meanwhile harvested, weighed and analysed for their respective C and N content. Pre-existing materials and data (from former applicable studies in Finland), and the ongoing data collection in the Baltic states are surveyed for possibilities to use in extended decomposition modelling studies.

2.4 Characterising soil microbial communities

The soil samples for the microbial community study were collected in 2021, the DNA extracted in 2022 and sequencing finalised by the end of 2022/beginning of 2023.

Soil samples were collected in August – September 2021, starting from the Northmost sites. Each participating country was responsible for the national sampling and sample shipment in frozen condition to laboratories in Finland. Soil samples (10 x 10 x 10 cm sample) are taken from only one depth below the litter layer at c. 15 cm depth in the soil profile. The labour-intensive DNA extraction, the following sequencing, and the data analysis was carried out in 2022 onwards and finalised with the submission of a scientific manuscript by the end of 2023.

2.5 Soil screening with infrared spectroscopy (IRS, FTIR)

The first part of the activity (building the FTIR library) was implemented in 2021 and is reported in LIFE OrgBalt Midterm reporting 2021. The second part of FTIR analyses, based on LIFE OrgBalt soil samples, was conducted in 2022. The consequential analytics include IRS data comparison with GHG fluxes, as well as with soil properties - pH, N, P, K, Ca, Mg, C and ash content in parallel to the implementation of conventional methods.

2.6 Soil and water analyses

Physio-chemical analyses of soil and water samples were done in ISO 17025 accredited Laboratory of Forest Environment of Latvian State Forest Research Institute "Silava".

Soil samples collected in 2020 and 2021 were shipped to the laboratory of "Silava" for further processing. A total of 171, 640, 210, and 116 soil samples from various soil depths (0-10; 10-20; 20-30; 30-40; 40-50; 50-75, and 75-100) have been collected and analysed from sites in Estonia, Latvia, Lithuania, and Finland, respectively. Sample processing started in 2021 and the parameters like bulk density, total carbon, total nitrogen pH and ash content have been determined; analysis of HNO₃ extractable parameters K, Ca, Mg, P will continue in 2022. FTIR analyses were scheduled and carried out in 2022 (see 2.5). The soil samples were prepared for analysis according to the LVS ISO 11464 (2005) standard. Chemical parameters were determined from organic soil milled till fine powder and fine earth fraction (D < 2 mm) of mineral soil (prepared according to LVS ISO 11277), and analysed according to standard methods (Table 5). Organic carbon concentration (g kg⁻¹) in soil was calculated as the difference between total carbon concentration and inorganic carbon (carbonate) concentration. Analysis of ash content was used to calculate the content of organic matter.

Table 5. Soil sample analysis methods

Parameter	Unit	Method principle	Standard method
Bulk density	kg m ⁻³	Gravimetry	LVS ISO 11272:2017
pH _{CaCl2}	log unit	Potentiometry	LVS ISO 10390:2021
Total carbon	g kg ⁻¹	Elementary analysis (dry combustion)	LVS ISO 10694:2006
Total nitrogen	g kg ⁻¹	Elementary analysis (dry combustion)	LVS ISO 13878:1998
CaCO ₃	g kg ⁻¹	Volumetry	ISO 10693
HNO ₃ extractable K, Ca, Mg and P	g kg ⁻¹	ICP-OES	LVS EN ISO 11885:2009
Ash content	g kg ⁻¹	Combustion	LVS CEN/TS 14775:2004

<u>Water samples</u> have been collected once per month simultaneously with gas flux measurements. Samples from Latvia, Estonia and Lithuania were shipped and centrally analysed at "Silava". For all the samples, standard methods were applied (Table 6). In total, 239, 790, and 262 water samples have been collected in Estonia, Latvia, and Lithuania accordingly, and all parameters have been analysed. Water samples from Finish sites were investigated locally at LUKE, in total 68 water samples were analysed.

Table 6. Water sample analysis methods

Water samples			
pН	log unit	Potentiometry	LVS ISO 10523:2012

Conductivity (EC)	μS cm ⁻¹	Conductometry	LVS EN 27888:1993
Total nitrogen (N)	mg L ⁻¹	Catalytic oxidation	LVS EN 12260:2004
NO ₃ and PO ₄	mg L ⁻¹	Ion chromatography	ISO 10304-1:2007
NH ₄	mg L ⁻¹	Photometry	LVS ISO 7150-1:1984
DOC	mg L ⁻¹	Catalytical combustion	LVS EN 1484:2000

Water from the Finnish sites were analysed locally at LUKE, since there was no budgeting available for water analytical tools at the field (YSI meter), and, secondly, regular delivery of water samples to Silava was considered a risk regarding the water quality due to long transport time. Samples from the Finnish sites have been collected regularly at times of gaseous flux measurements since spring 2021, and water analyses have been made locally in LUKE laboratories.

3 Post LIFE OrgBalt impact assessment

A replicability and transferability strategy has been published under action A2 to multiply the impact of the project results during its implementation and to replicate and transfer its findings after its end, to reach a wider audience and implement its results in further sites and regions, other than the project demo sites.

A key role in this respect is represented by the elaboration of a Simulation model (SM) under action C5. The simulation model serves as a policy planning/decision support tool for the development of GHG emissions projections at a national level and the analysis of the socio-economic impact for 2 scenarios – with and without implementation of CCM measures - with dynamic background information on changes of technical conditions of drainage systems. The elaboration of these models is possible on the bases of the results of Activities C1 and C2, namely the elaboration of a catalogue of climate change mitigation measures, including a socio-economic impact assessment, the improvement of GHG emission factors and of the methodologies for GHG inventory reporting together with the related national reports, and finally the elaboration of mathematical equations and tools for GHG projections from organic soils. The simulation model is to be understood as an evaluation tool to determine the extent to which measures should be implemented in each evaluated country. This will support the development and the evaluation of climate change mitigation measures related projects in the context of the Common Agricultural Policy. The simulation model's main targets are policy and decision-makers, consultants, non-governmental organisations of farmers and foresters, and individual stakeholders (major foresters and farmers). The model includes data on organic soils at a national level and the potential for land-use change according to the 17 climate reduction scenarios identified in the LIFE OrgBalt project. Data on organic soils and their use in each evaluated country got integrated. Feedback from the involved stakeholders was collected during the dissemination, training and networking activities planned under actions E.2 and E.3, i.e., National workshops, Thematic Workgroup meetings, Networking workshops on the national level and Experience exchange visits. This feedback was gathered to improve the developed models as well as to evaluate the results obtained through them in terms of GHG emissions reductions and the socioeconomic impacts under different management scenarios. In addition, the project envisaged a total of 10 training seminars -2 for each country - planned to be organised at two levels - one for consultants and the other for individual stakeholders, i.e., landowners and managers. During the training workshops, the simulation tool (online available at https://bioekonomika.lbtu.lv/orgbalt/) was presented to give a national perspective of the implemented climate change mitigation measures.

All the collated field and laboratory data will be further elaborated, to continuously improve the existing knowledge and impact assessment strategies. Several scientific and public articles were already published or are close to be submitted to adequate scientific journals. Furthermore, since selected experiments and field activities are by far exceeding the LIFE OrgBalt lifetime, those will be made public in due time.

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