HoliSoils
Working together for forest soils

D4.1 Protocol of site, soil property & GHG measurements at management test sites

Holistic management practices, modelling and monitoring for European forest soils, HoliSoils

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Deliverable D4.1: Protocol of site, soil property & GHG measurements		
D4.1 describes how HoliSoils' WP4 test sites have measured for soil respiration, soil microbiota, soil organic matter quality and for other properties.		
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Nature of the Deliverable		
R	Document, report	X
DEM	Demonstration, pilot, prototype, plan design	
DEC	Websites, patents filing, market studies, press & media actions, videos etc.	
OTHER	Software, technical diagram etc.	
Ethics	Ethics deliverables	

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Contents

1. Introduction

In the HoliSoils H2020 project, WP4 test sites soils are measured to estimate various properties from soil functioning, soil health, soil respiration, organic matter quality and soil microbiological diversity. Here, we document the measurement protocol for these test sites. Also, additional information and description of measurements from selected test site have been provided at the end of the document.

2. Actual measurements from test sites

2.1 Trenching and soil respiration measurements

2.1.1 Establishment of the trenching to measure heterotrophic respiration

Equipment requirement for microbiology:

- Notebook and pencil(s)
- Precision GPS device (e.g. in mountains) or a mobile phone with GPS
- Camera for taking photos before measurements
- Compass
- Measuring tape (20 m)
- Root prevention fabric
- Spades
- Plastic 1 * 2 m, to collect soil
- Sticks to mark soil respiration chamber locations
- Saw, to cut roots
- GHG analyser (Licor-7810, EGM-5, or similar) and non-transparent chamber
- Collars for soil GHG measurements (if soil requires that)
- Soil temperature, air temperature and moisture sensors

Selection a location for trenching

The purpose of trenching is to remove the influence of autotrophic respiration from total soil respiration which allows to estimate heterotrophic respiration component, which is useful e.g. for soil carbon model testing and for ecosystem GHG flux quantification (Díaz-Pinés et al. 2008).

The area for trenching should be large enough to avoid boundary effects of root prevention fabric and to allow multiple destructive soil sampling for microbiology, soil physical and chemical properties. In the illustration below (Figure 1) the trenching area is 2 m^2 to ensure possibility of additional sampling (indicated with red circles) and assuming that chamber diameter for CO_2 and CH_4 measurements would be around 30 cm (if chamber diameter is less than that, consider increasing number of measurement points). In the trenching area

there are 4 chamber locations, of which 2 are with and 2 without understorey vegetation. The understorey has been clipped always after measurements from 2 locations without understorey vegetation to quantify the role of understorey vegetation to soil GHG flux (clipping just before measurements may affect flux estimates). Soil temperature and moisture is measured from inside and from outside of the trenching fabric. The minimum requirements for measurements are to measure from 5-10 cm depth, but additional depths are also encouraged.

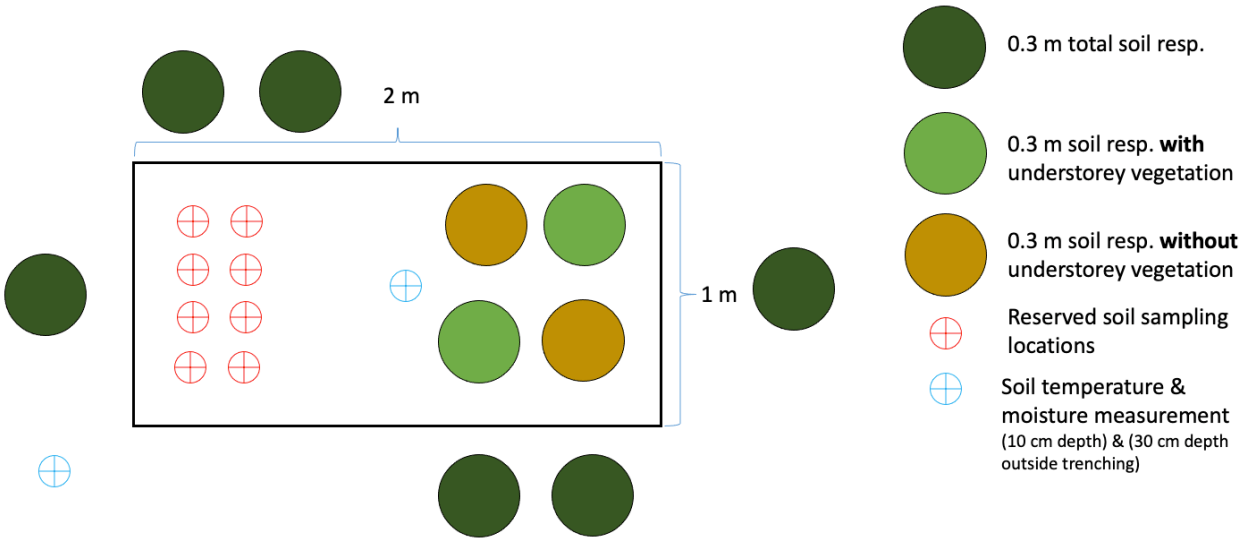

Figure 1. Illustration of trenching experiment

When establishing trenching, root prevention fabric is to be located around the area that is to be measured for heterotrophic soil respiration (Figure 2). Here 2 m * 1 m area has been ditched and soils have been removed by layers and woody roots have been cut. The diameter of woody roots has been recorded, to estimate amount of root biomass to be left to decompose. The depth of ditching should be approximately 30-40 cm depending on rooting depth of tree species.

Root prevention fabric with 50 µm mesh size is commonly available from hardware stores. This kind of root prevention fabric blocks roots but allow mycorrhizae to penetrate trenched area (Ryhti et al. 2021) and allows water to penetrate (Fenn et al. 2010).

After establishment of root prevention fabric soil layers will be reallocated into ditched pits according to their original order.

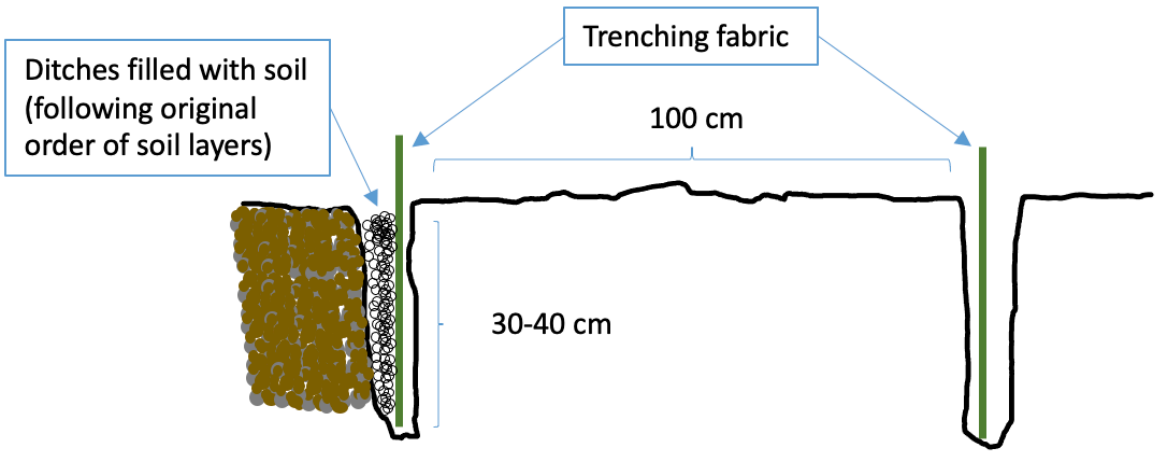

Figure 2. Illustration of establishment of trenching experiment

2.1.2 Installing soil moisture and temperature sensor

Please install sensors of your choice to forest soils in 5-10 cm from the surface (1 in control, 1 in trench) and 1 in 30 cm control (optional). Note, here control refers to area outside of trenching. Some candidate sensors have been listed below (Table 1). It is important to have sensors to measure soil temperature and moisture from 5-10 cm of the top.

Manufacturer	Website	Comments
Tomst TMS-4	https://tomst.com/web/en/	80 eur each, measures soil moisture from the depth of 10-15 cm and temperature from air, soil surface & 10-15 cm depth. Reading with Bluetooth
Soil Scout	https://soilscout.com	More expensive, good for locations where you need online data with wireless setup. Measure T and soil moisture from depth that sensor has been installed. Wireless, soil tillage/burning can be done while sensors measure deeper layers constantly.
EMS brno	http://www.emsbrno.cz/	A more expensive system (around €400) that includes data logger and measurements sensors attached by a cable. One can chose from sensors placed in gypsy blocks to measure soil water potential or/and sensors to measure soil moisture. It is possible to attach as much as 3 sensors to one datalogger.

HOBO	https://www.onsetcomp.com/	Bluetooth logger for water temperature (to be tested)
iButton	https://www.ibuttonlink.com/	For temperature logging (cheap). Perhaps as a backup system

Table 1. Potential soil temperature and moisture measurement instruments

2.1.3 Measuring soil respiration

Soil CO_2 flux from control and trenching locations should be measured optimally every second week (note that measurement intervals can be modified according to resources available). During long drought periods / or during episodes when soil activity is marginal (e.g. winter period), measurement frequency can be longer than 2 weeks, depending on the soil activity. If the measurement frequency during drought period or during winter was been reduced, measurements should be re-started shortly after precipitation event occurs and shortly after soil thaw.

It is a good practice to vary the order of sites and individual chamber locations across different measurement days to avoid that same locations would always be measured during the morning and others during the afternoon as fluxes may have daily patterns.

When establishing measurement points for chambers, one can use metal / rubber collars or to install chamber deeper into soil (organic layer) in a way that chamber is tightly against soils to avoid air flow between chamber and atmosphere. Chambers can be applied also without collars if soil surface allows and air tight measurements can be made without collars. But note, if having mosses / other soft vegetation pressing chamber hard against soil may create artificial gas movement from soil to atmosphere.

Measurement time of soil flux depends on chamber size and flux quantity. The time should be long enough to allow estimation of linear increase of concentration-based series of measurements and on the linear regression to be fitted later with that data.

In case of any collar damages caused by disturbances like wild boars, place collars (or new collar) in previously established spot and improve anchorage of those collars. Since CO_2 flux will be disrupted do not measure trench/collar immediately after re-establishment of measurement location.

2.1.4 Data analysis and storage

The centralised storage and analysis of raw data of soil GHG flux measurements from various devices (e.g. Licor-7810, EGM-5, Licor-870, Licor-7820) has been made available by Luke cloud service for HoliSoils project (under development, 5/2022). This cloud service supports EU open data policies and ensures that raw data has been treated in a uniform manner to obtain soil respiration flux estimates.

Users can upload raw data files and complementary excel file into cloud service, where information from those files is merged. On the “submit new measurement files” box you can enter a data file (from the measurement device), a field form file (an Excel sheet you fill), a comment (currently maximum size is 256 characters), and the date when these files were measured. If the files contain measurements from several days, the measurement date should be the first date. There is a maximum file size of 5 MiB for both files. Clicking “UPLOAD” should transfer the files and they should appear below on the “Your submitted files” box. The uploaded files on the “Your submitted files” box appear in the order you made the submissions. Each submission shows a “submission date” which is UTC. The server attempts to infer the device used in measuring by reading the contents of the submitted data file.

The purpose of the excel file is to add exact identification information for each soil flux measurement points by providing data on: “Monitoring site”, “Sub-site ID” and “Monitoring point”. Merging of the raw flux data with excel file (having meta data) is done by using the time of the flux measurement and time indicated in that excel file.

The web service facilitates the flux estimation by graphical interface that plots GHG concentration development over time, and it also supports flagging on suspicious observations.

A regular user can only see their own submitted files as they are listed on the “Your submitted files” box. You should also be able to view the main columns of the field form by clicking on “details” so that you can check which records were contained in the file. In this view one can also make changes to the “measurement date” and “comment” fields of the submission. The “details” view of the data file should allow you to draw the full time-series in the file. This feature works only on EGM5 data right now, but the rest will be added later.

2.2 Microbiology

2.2.1 Field sampling for microbiology

Equipment requirement for microbiology:

- Notebook and pencil(s)
- GPS device or a mobile phone with GPS
- Compass
- Measuring tape (20 m)
- Plastic soil cores, 4-cm diameter, 25 cm length, 6-7 per plot. Such cores can be easily cut from plastic tubes used by plumbers and available in hobby markets. One end of the core is straight, the other end has an angle of approx. 30° (photo).
- Rubber hammer(s)
- Spray for tree labelling
- Permanent marker(s)
- Plastic bags that accommodate 5 or more soil cores

- Adhesive tape
- Optional: Camera

Within each treatment replicate (treated area), locate an appropriate sample plot (a 5 m radius circle). The appropriate plot is generally representative of the treatment in terms of the composition of surrounding trees, ground vegetation and other properties and is located INSIDE the treatment area, ideally more than 20 m from the edge of the treatment area. Sometimes more than one sample plot per treatment replicate is required.

Record the date and time of sampling and the sample plot identification code. Define the plot center on the ground. Hammer the first soil core (center core) into the plot center to a depth of 15 cm.

Determine the GPS coordinates of the center core.

Find the nearest healthy living tree and tag it with a spray. We use two horizontal lines visible from all sides of the tree.

Using a measuring tape, measure the distance between the labelled tree and the central core. Using a compass, measure the angle between north and the direction from the tree to the central core. Please note that compass applications in mobile phones are not very precise. Steps above are essential if re-sampling of the same plot in time is needed.

For WP4 sites: Hammer in four additional cores, located 2 m south, 2 m north, 2 m east and 2 m west from the central core. In stony places, locate cores into less stony patches. If stones are underground at <15 cm depth, hammer the cores up to the stone layer (see Figure 3).

For WP5 sites: Hammer the five soil cores as a transect. The direction of the transect and the angle and distance from the labelled tree and the first soil core should be recorded. Soil cores are spaced of 50 cm, making a 2 m long transect of soil cores (see Figure 4).

Optional: If possible, take defined photos of the site: (a) forest stand, (b) forest ground (optimally showing the area between and around all five cores, (c) canopy.

Record tree species of trees in the circle defined by the soil cores and all closest trees around the perimeter. If possible, record also distance of each tree to central core and its breast height diameter (DBH).

Record numbers and species of juvenile trees / seedlings in the circle defined by the soil cores.

Optional: If possible, record ground vegetation (% of area barren litter/soil, % grasses and herbs, % ericoid shrubs), and plant species of dominant plants, if possible.

Pull out soil cores by twisting them gently left and right to prevent soil from falling out.

Place all five cores from the plot into a single plastic bag. Write the sampling plot identification code on a piece of paper and insert it into the bag. Wrap the bag tightly and fix it wrapped around the cores using adhesive tape. During handling and transport, keep the cores in a horizontal position as much as possible.

Write the sampling plot identification code onto the bag using a permanent marker.

Transport the samples into the labs for processing the same day and refrigerate them upon arrival to the laboratory (4°C). The soil cores should be processes in the laboratory the first day following sampling or at latest the second day after sampling.

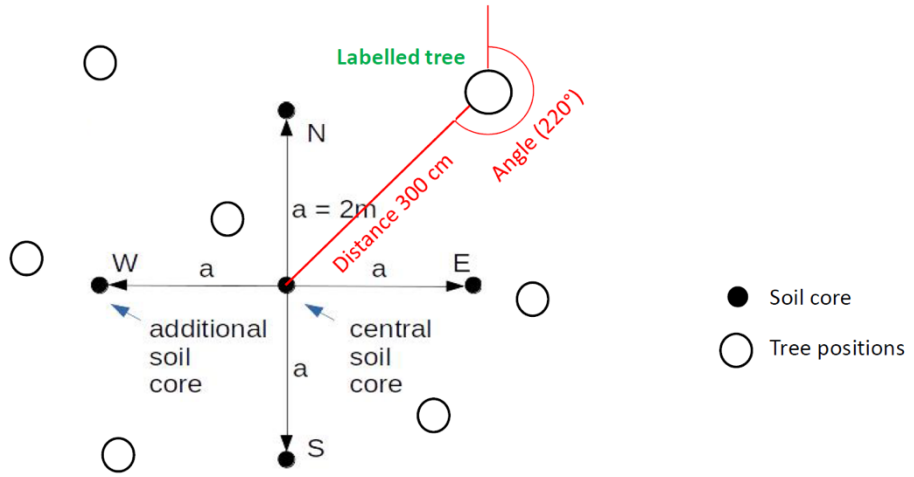

Figure 3: Illustration of soil sampling for biodiversity analysis for WP4 sites

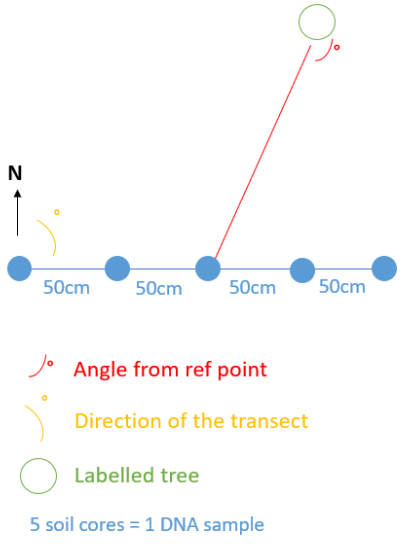

Figure 4: Illustration of soil sampling for biodiversity analysis for WP5 sites

2.2.2 Lab work for microbiology

Equipment requirement:

- Notebook and pencil(s)
- Balance
- Sheets of plastics (large plastic bags, transparent or light color, if possible)
- Sieve for soil with 0.5-mm mesh size (photo, catalogue ID)
- Plastic 50-ml Falcon tubes (photo, catalogue ID) – two for each sample of soil, one for each sample of litter (if collected), one for each sample of roots (if collected). Alternatively, plastic ziplock bags with 50 ml volume.
- Wooden rod, approx. 3 cm diameter
- Sharp knife
- Laboratory spoon
- Sheet of plastic
- Ruler, 20 cm
- Permanent marker(s)
- Latex gloves
- Scissors
- Cellulose tissues
- Optional: Tweezers
- Optional: Ethanol

Prepare weighted sterile 50 mL Falcon tubes for all samples and describe them with sampling site code and material code (S – soil, L – litter, R – roots) both on a cap and on the tube. Two tubes are needed for S, one for L (if collected), one for R (if collected). Litter can be collected if it is important to analyse its chemistry and/or microbial biomass. Roots can be used to assess the relative share of individual trees and plants in the root pool – if required.

Measure the mass of each empty tube+lid (resolution 0.01 g) and record it.

Prepare a sheet of plastic where to collect the content of soil cores. Wipe it free of soil and wash it with ethanol, if available. Wait until ethanol evaporates.

Wear Latex gloves when working with soil cores, if possible.

Push the content of the first soil cores out of the tubes with a wooden stick such as to cause minimal disturbance of the soil profile(s). From the core surface, collect all litter and put it aside if you plan to collect it, otherwise, discard it. To collect litter, start from leaves on the surface of the soil and continue to collect the material as long as you can recognize it is plant origin (fragments of leaves, needles, etc.).

After litter removal, measure 10 cm of the soil profile with a ruler, cut the extra soil from the bottom of the core aside with a knife and throw it away.

2.3 Characterisation of soil organic matter (SOM)

2.3.1 Sampling for organic matter

Equipment requirement:

- Notebook and pencil(s)
- Balance
- Sheets of plastics (large plastic bags, transparent or light color, if possible)
- Plastic bags (ziplock bags)
- Soil corer
- 2 mm sieve
- Laboratory spoon
- Ruler, 20 cm
- Permanent marker(s)
- Latex gloves
- Scissors
- Cellulose tissues
- Optional: Tweezers
- Ethanol
- Falcon tubes 15 ml
- Cool box (to keep samples at field and during transport, before putting into -20℃)

Actual field sampling

Using soil corer take at least 3 soil cores per plot. Divide into litter, organic layer, and upper mineral layer of the soil. Put it on plastic sheet, cleaned before with ethanol. Wait until ethanol evaporates. Record the volume of the sample (for bulk density estimation).

To plastic bags collect litter. Store samples in the field in cool box.

From organic layer and mineral soil remove roots wash the roots in a plastic beaker with water to remove soil and dry them with cellulose tissues. Collect roots to separate plastic bags of 15 ml Falcon tubes (depending on the root size). Mark the samples.

Also remove stones (if present) from soil samples, measure their weight and throw them away (no need to collect them).

Optionally for mineral soils (not possible for peat soils): using the 2 mm sieve, sieve the soil, and collect into separate plastic bags, record the mass. Collect all roots that remained on the sieve and add them to the root pool (see above).

Put 5 g of fresh sieved soil to Falcon tubes (for inorganic N measurement).

Before processing next sample, clean all tools and the plastic sheet used to collect soil. Remember to mark the samples with permanent marker.

Keep plastic bags in cool box and transport the samples to the laboratory in a cool box immediately after sampling. Use soil from 15 ml Falcons with 5 g of soil to measure inorganic N, the rest of the samples you can freeze at -20°C before analyses. For other than inorganic N analyses soil and root material should be freeze-dried and grounded.

2.3.2 Measurements for soil organic matter

Total C, N, SOM and pH,

Total soil C and N contents is determined with elemental CN analyser. SOM content is measured as loss on ignition at 550℃ and soil pH in a soil-water suspension of 1:2.5.

Inorganic nitrogen

Fresh soil was extracted with 1 M KCl and IN (NO_3-N and NH_4-N) was measured according to Hood-Nowotny et al (2010). The assay for ammonium-N is based on a modified indophenol method and for NO_3-N on the reduction of nitrate by vanadium (III).

Concentration of condensed tannins

After extraction of tannins from soil with 70% aqueous acetone and drying, redissolved tannins were estimated with acid-butanol assay (Smolander et al. 2005, Hagerman 2012). The method involves the HCl-catalysed depolymerization of CT in butanol to yield a pink-red anthocyanidin product, which was measured spectrophotometrically.

Concentration of amino sugars

Amino sugars are measured with high-pressure liquid chromatography (HPLC) as in Adamczyk et al. (2020). Briefly, soil was at first treated with NaOH to remove proteins and amino acids, and later hydrolyzed with 6M HCl. Amino sugars were derivatized with 9-flourenylmethylchloroformate (FMOC), and measured with HPLC with fluorescence detector.

SOM characterization with Fourier transform infrared spectroscopy (FTIR)

Optionally, SOM may be also characterized with FTIR (as in Adamczyk et al. 2019), which measures absorbance of infrared light at frequencies specific to bond types in compounds. Briefly, 3 mg of dry and finely ground material is mixed with 300 mg of KBr and pressed into discs, and analysed with FTIR.

Chemically stable SOM-C pool

One gram of soil is hydrolyzed with 6 M HCl at 100℃ for 18h (Kallenbach et al 2016). Non-hydrolyzable residue is rinsed with deionized water and chemically stable C is estimated on the difference in C remaining in the non-acid hydrolysable pool and the initial C content of the sample.

Pyrogenic carbon

Pyrogenic C (black C) is estimated as in Wiedemeier et al. (2013). Pyrogenic C in soil samples is digested with nitric acid to produce benzene polycarboxylic acids measured with HPLC.

MAOM (mineral associated organic matter) and POM (particulate organic matter)

These two fractions is separated physically using wet sieving (53 µm sieve) as in Cotrufo et al. (2019). Carbon content of separated fractions is estimated with TOC.

Lignin concentration

Lignin concentration is measured with acetyl bromide method (Danise et al. 2020). Briefly, samples are extracted with acetone and dried residue is exposed to acetyl bromide in glacial acetic acid at 50°C (2h) and glacial acetic acid with NaOH and later hydroxylamine is added. Absorbance is read at spectrometer.

2.3.3 Litter decomposition measurements

Optional litter decomposition measurement campaign supports soil food web analysis and also provides testing material for soil C models.

Freshly abscised needles are collected over the whole period of maximum litter fall in June and July using litter traps suspended under the tree canopy. Needles are dried at ambient temperature and stored until the beginning of the experiment. Needle litter decomposition is studied by using the litterbag method (Swift 1989) during 24 months. 20 × 20 cm litterbags with 2 distinct mesh sizes (4 mm or 0.125 mm to exclude soil fauna) are filled with 10 g of dry needle litter and placed on the field at the end of July. Four replicates per plot are collected after 3, 6, 12 and 24 months for a total of 320 litterbags (4 litterbags × 4 sampling times × 20 plots). After collection, litterbags are immediately sealed in plastic bags to prevent litter material lost and transported to the laboratory. In order to prevent contamination of litter by soil, we wiped each needle thoroughly prior to laboratory analyses: litter mass, C, N and P loss during the decomposition process.

2.3.4 Soil fauna analysis

Optional soil fauna measurement campaign supports soil food web analysis and also provides testing material for soil C models (e.g. Keylink model).

Macrofauna (i.e. large invertebrates >3 mm in body length including earthworms) is collected up by hand sorting directly in the field from an excavated soil monolith per plot (25 cm × 25 cm to a 15 cm depth) following the Tropical Soil Biology and Fertility method (TSBF, Anderson and Ingram 1993). Mesofauna is collected in the field using three pooled soil cores per plot and extracted at laboratory using the Berlese-Tullgren funnel method (dry extraction, Berlese 1905). Individuals of meso- and macrofauna are stored in 96% ethanol prior to counting, identification and classification into a trophic group (microbivore, detritivore, predator, omnivore or herbivore). Microfauna is collected in the field using three pooled soil

cores per plot and extracted at laboratory using a modified Baermann funnel method (Cesarz et al. 2019). The collected nematodes are counted under a binocular loupe and then fixed in a mixture of formaldehyde and glycerol. A representative subsample is mounted on mass slides to nematode identification and classification into a trophic group (bacterivore, fungivore, herbivore, omnivore or predator).

2.4 Site specific additional measurements

Quantification of the number of soil respiration measurements, soil samples for soil chemistry and microbiology analysis has been estimated for individual sites and is shown along with the schedule of the measurements in Fig. 5. The difficulties with carrying out the measurements and soil sampling were related to protecting the locations from vandalism, and from disturbances of soil and sensors by animals.

2.4.1 Karstula

Karstula test site is a part of fertilisation experiments, established by the Finnish Forest Research Institute by late 1950s. The experimental data on fertilisation and soil properties has been analysed by Saarsalmi et al. (2014).

For HoliSoils project following additional measurements have been planned:

(i) needle litter fall has been quantified in the since summer 2021. And those measurements continue during 2022.
(ii) understorey biomass measurements from all treatments from sites (75, 76 & 77) have been planned
(iii) increment core measurements for ring widths and for $\delta^{13}C$ isotopes from stem wood from N and control (75, 76) would take place during 2022
(iv) Also, a weather station will be installed into the Karstula site during 2022

2.4.2 Ränskälänkorpi

Ränskälänkorpi test site is a Norway spruce on drained peatland that has control, clear-cut and selection harvest areas. This site includes 2 eddy covariance towers, automatic chambers for soil GHG fluxes and has various other measurements. Additional measurements for Ränskälänkorpi has been described by Laurila et al. (2021).

2.4.3 Buchenhorst

Additional measurements of greenhouse gases will be performed on five plots at this site. Fully automated chambers will be used for the analysis of CO_2-, CH_4- and N_2O-emissions. Each plot is equipped with an intransparent (1100x1100x1040 mm) and a transparent chamber (1060x1060x1090 mm). They are used successively to measure ecosystem respiration and net ecosystem exchange directly after on each plot. Mixing ratios and fluxes are measured and calculated onsite and transferred via remote data transmission. Trenching is aspired on two plots. Further, soil temperature and soil moisture three depth levels),

radiation values (PAR, NDVI) and air temperature are measured on each plot. The site is equipped with a weather sensor.

2.4.4 Eisenstraßenmoor

Additional measurements of greenhouse gases will be performed on five plots at this site. Fully automated chambers will be used for the analysis of CO_2-, CH_4- and N_2O-emissions. Each plot is equipped with an intransparent (1100x1100x1040 mm) and a transparent chamber (1060x1060x1090 mm). They are used successively to measure ecosystem respiration and net ecosystem exchange directly after on each plot. Mixing ratios and fluxes are measured and calculated onsite and transferred via remote data transmission. Trenching is aspired on two plots. Further, soil temperature and soil moisture three depth levels), radiation values (PAR, NDVI) and air temperature are measured on each plot. The site is equipped with a weather sensor.

2.4.5 Kranzberg

Additional measurements for all sub-sites within HoliSoils test site E (TUM) encompass stand growth parameters as described in Pretzsch et al. (2014) and litter fall (2022-04 – 2024-04). Within the Kranzberg sub-site, extensive sets of physiological and plant/root/soil system parameters similar as described in Grams et al. (2021) are measured.

2.4.6 Sites in Netherlands

The heterotrophic soil respiration – CO_2 is measured on 3 (Kroondomein, NP Hoge Veluwe and Zwolse Bos) out of 5 test sites in the Netherlands. Each measured site is located in Scots pine (*Pinus sylvestris)* stand under 4 different treatments (control, clear-cut, shelterwood and high-thinning). All treatments were established in 2018 for PhD research which focused on nutrient budgets under different harvest regimes, stand productivity (C dynamics aboveground), soil (carbon cycling and biodiversity) as well as above- and belowground response to drought (Sterck et al. 2022).

2.4.7 Dobroc

The experimental site Dobroc was established in 2021 for the purpose of the HoliSoil project. It includes trenching plots placed in approx. 70-year-old spruce monoculture (a stand that was planted and managed by negative thinning from bellow to keep the forest structure homogeneous) as well as in a neighbouring mixed-species forest stand (silver fir, European beech, sycamore maple, Norway spruce) with no management for a couple of decades. Two trenching plots per stand were established in May 2021. The measurement of CO_2 and CH_4 has started in August 2021 and measured every 2 weeks (once per month during winter periods). In 2021, Minikins to measure forest microclimate (temperature and humidity) were established in both stands. In addition, a close-by open place was equipped with a climate station to measure air temperature, humidity and precipitation. In 2022, the plan is to do soil sampling to quantify soil microbial diversity and soil properties. Collectors to collect litter fall are also planned to be established in 2022. In May 2022, automatic continual chamber of 30 cm diameter was installed on the spruce site with continual data being sent online. In total, 12 band dendrometers were installed on both sites to continually measure tree growth and

tree water deficit. In addition, 4 trees were equipped with sapflow sensors. In the soil, temperature, soil water potential and soil moisture are continually measured as well.

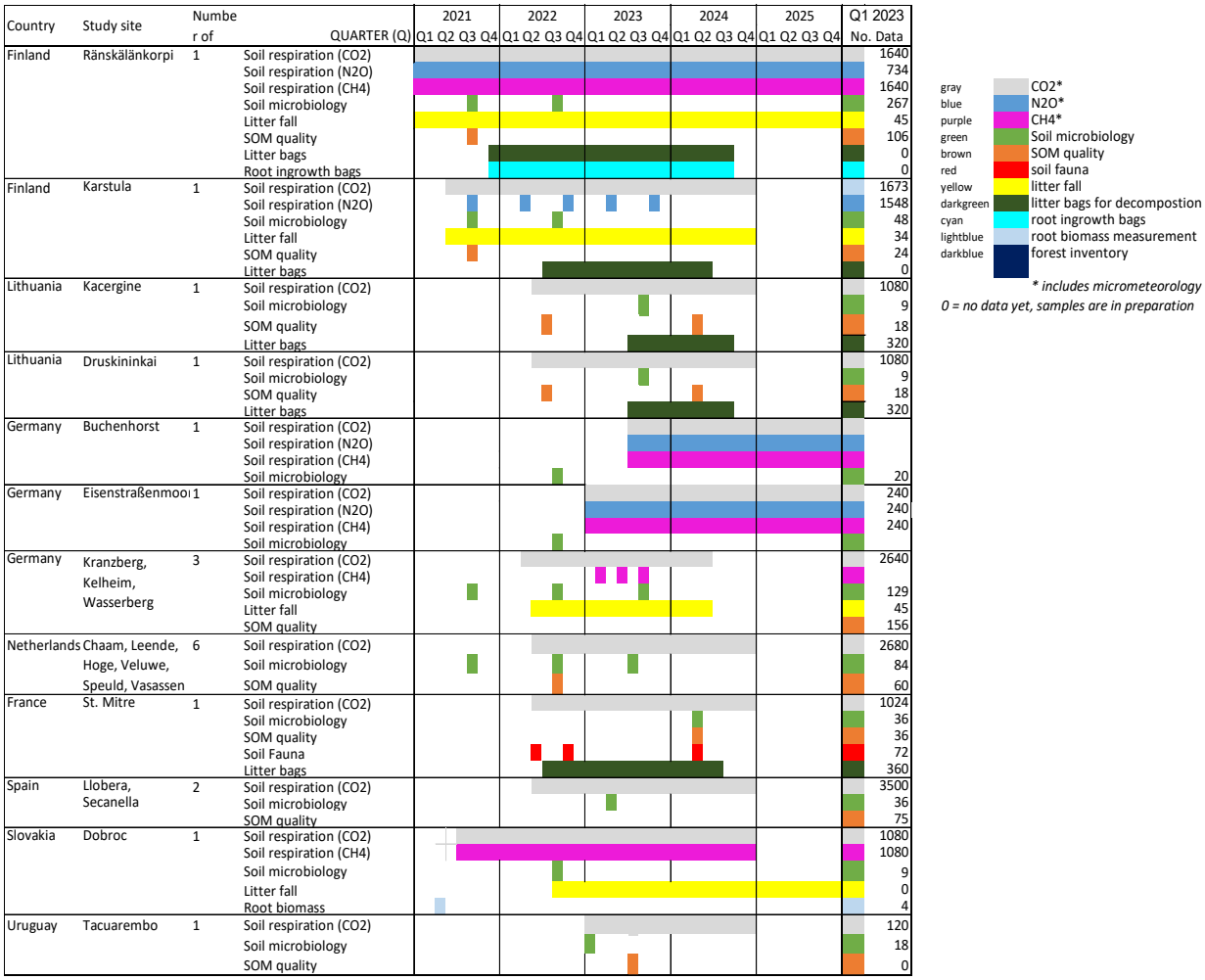

Figure 5: Gantt chart for WP4 test site measurements

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