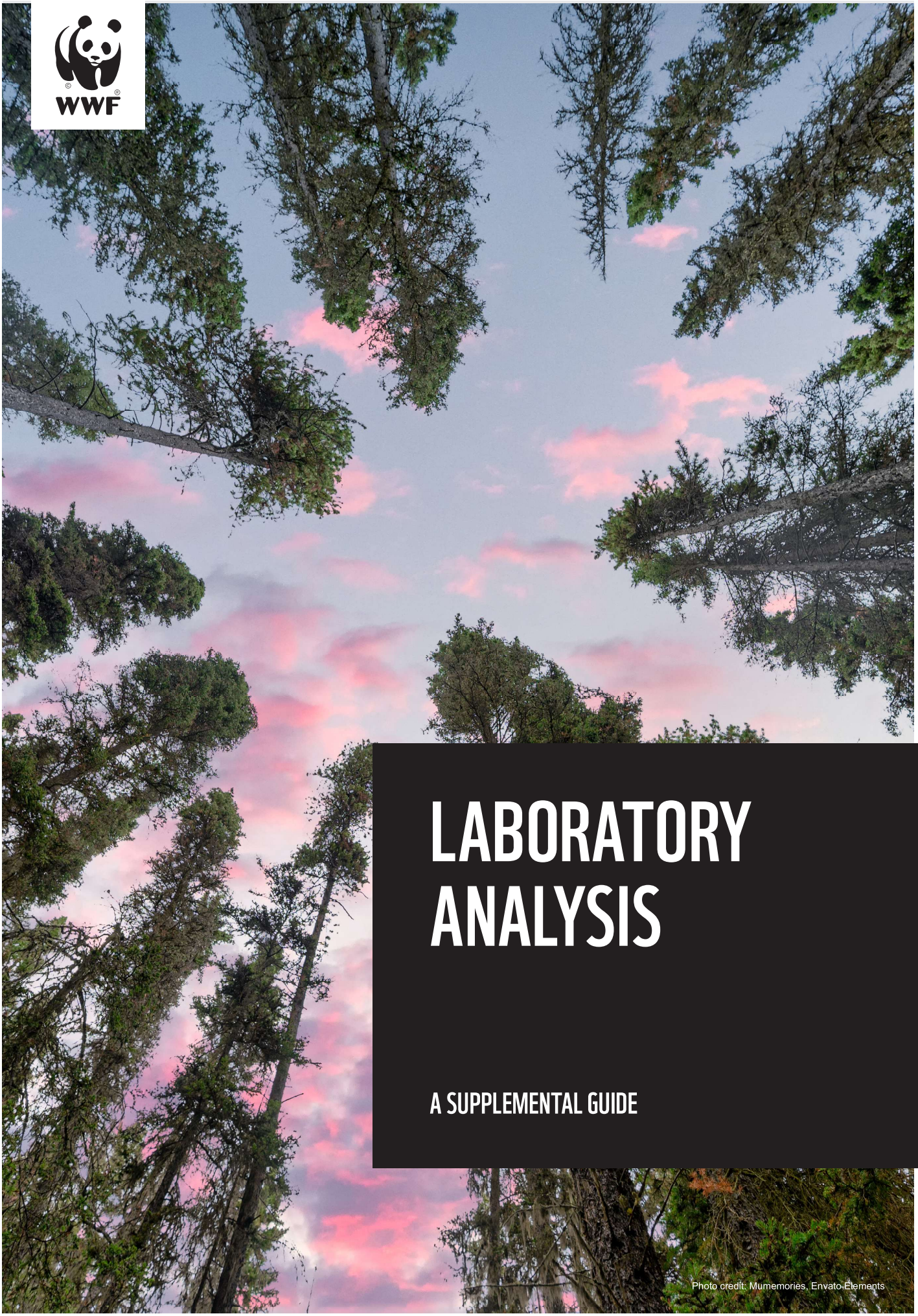

LABORATORY ANALYSIS

A SUPPLEMENTAL GUIDE

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LABORATORY ANALYSIS

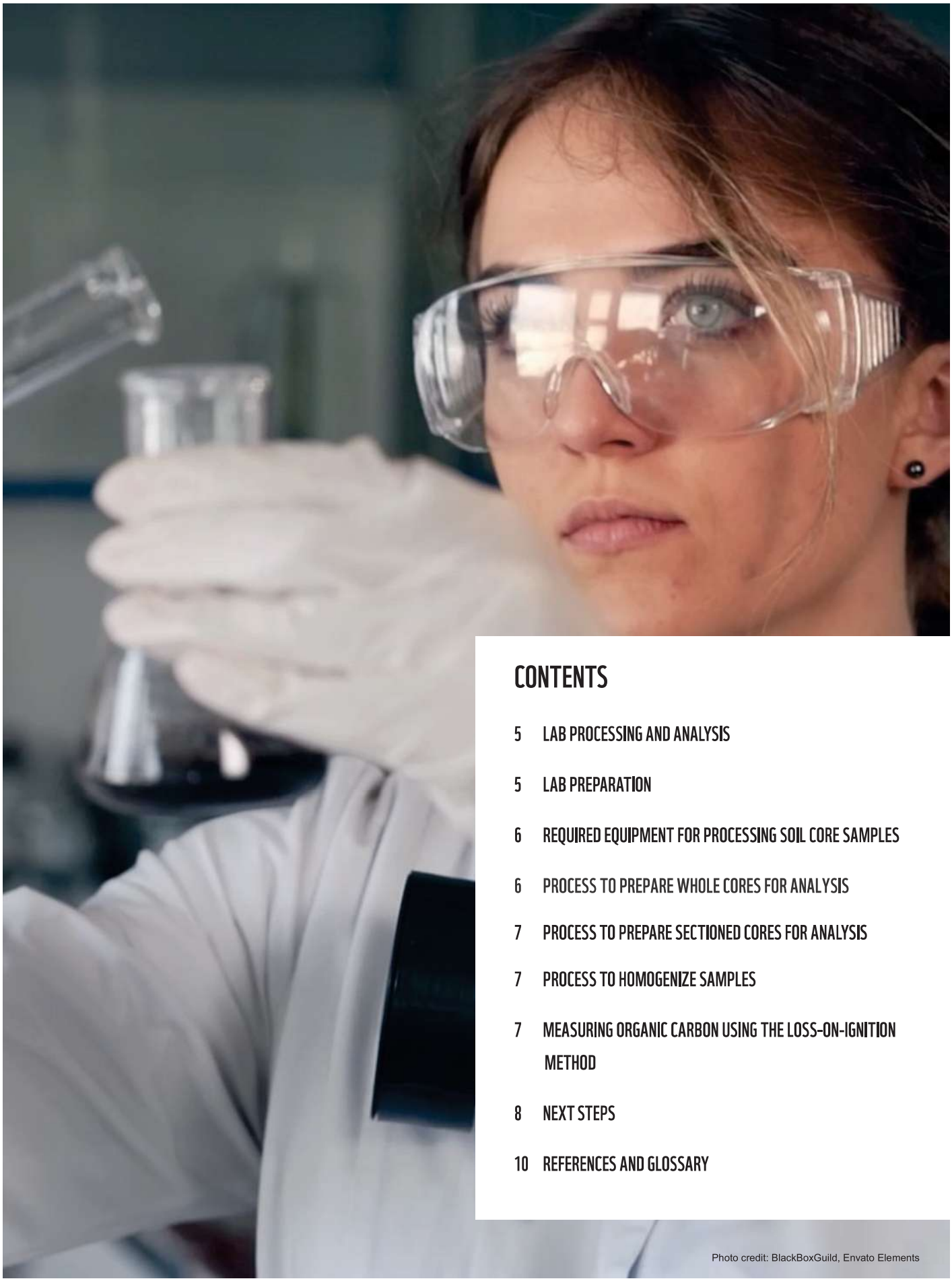

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Photo credit: BlackBoxGuild, Envato Elements

LAB PROCESSING AND ANALYSIS

BACKGROUND INFORMATION

This guide outlines the lab analysis procedures for mineral soil, organic soil, peat, and coastal sediment samples; for brevity, all sample types will be referred to as "soil" throughout the document. In general, there are three types of carbon you can choose to analyze in your samples; these are organic carbon (%Corg), which is the carbon that is derived from biotic materials, inorganic carbon (%Cinorg), which is carbon that comes from the erosion of rocks and the breakdown of soils into minerals from decomposition, and total carbon (%Ctotal), which combines both organic and inorganic carbon.

For all soil samples collected, obtaining the soil organic carbon content is the most relevant measure, as this is the carbon responsible for soil sequestration processes that are derived from photosynthesis, as well as the release of carbon from the soil due to decomposition. Common methods for determining soil organic carbon include **loss-on-ignition** (LOI_{550}; Chambers et al. (2011)) and infrared spectroscopy (Semella et al. (2023)). The most cost-effective method is the LOI_{550} technique, where the samples are dried and then burned in a muffle furnace at approximately 550°C for four hours. This combustion technique burns off the organic matter of the sample, leaving the inorganic sample as the mass of ash remaining.

In soils that are expected to have higher inorganic carbon, such as mineral wetlands or deeper forest soils, organic carbon is typically paired with total carbon measurements for more accurate results. A common method for determining total carbon is using a CHN elemental analyzer. The equipment required for this analysis is expensive and difficult to upkeep, so samples will have to be sent to one of the many labs that provide this service. Unlike the LOI method, elemental analyzers directly measure total C content (inorganic + organic), with reliable results and the ability to analyze many samples in a short time following sample preparation. These instruments also simultaneously measure nitrogen (N) content, as well as other elements.
For more details on using loss-on-ignition in conjunction with CHN analysis, refer to Bansal et al. (2023) and the Blue Carbon Handbook.

The following lab procedures outline the necessary steps to obtain bulk density, moisture content and soil organic carbon (LOI_{550}) of your samples. For more information regarding these processes, please refer to Chambers et al. (2011), Bansal et al. (2023) and the Blue Carbon Handbook.

LAB PROCESSING AND ANALYSIS

All soil samples should be properly packaged and stored in a refrigerator between 2-8°C until they are ready for lab analysis. If necessary, samples can be stored for longer periods (1-month or more) in a freezer, in which case they should be thawed in a fridge within a week of being processed. Follow the procedures according to the specific lab's sample and shipping instructions. In the lab, the samples will be weighed, dried, ground-up and analyzed for physical, chemical and biological properties. Once analyzed in the lab, it will be possible to assign a measure of the amount of carbon stored in the soil for each sample, core, site and study area.

LAB PREPARATION

Consider that the soils collected for carbon assessments can also be used for a variety of other analyses, and that these analyses may have their own sampling criteria. Sub-sampling of sections may also be necessary for these types of analyses, so make sure to consider such requirements in advance.

Examples of these analyses:

- Sediment dry bulk density, which is needed to obtain carbon stocks
- Grain size
- Nitrogen content
- Carbon and nitrogen isotopes
- Radiocarbon and lead-210 dating, which is used to obtain carbon accumulation rates
- Trace metals
- Pollutants

If a suitable drying oven and a scale are available, the samples can be prepared for carbon content analysis. To analyze the samples for carbon content, a muffle furnace and crucibles are required. If suitable lab equipment is not available, the samples can be sent to an external lab.

REQUIRED EQUIPMENT FOR PROCESSING SOIL SAMPLES

- Scale
- Drying oven
- Metal trays
- Gloves
- Measuring tape/ruler
- Notebook
- Glass vials, paper bags, or resealable bags
- Lab coat
- Closed-toe shoes
- Permanent marker
- Coffee grinder, a mortar and pestle, or a sediment-homogenizing device
- Desiccator (optional)
- For organic matter composition:
    - Crucibles
    - Muffle furnace

PROCESS TO PREPARE WHOLE CORES FOR ANALYSIS

If soil core sectioning does not occur in the field, it must be done in the lab.

1) Wear gloves, a lab coat and close-toed shoes
2) Prepare metal trays labelled with unique IDs and weigh the trays using a scale. Record the tray ID and tray weight in a notebook.
3) Unwrap the soil core and measure the length. Record this value in a notebook.
4) Section the sample starting from the top (soil closer to the surface) and working down the core to the bottom.
    a. Each section should be cut at a distinct layer of soil profile, so that each is a homogenous soil mass.
    b. Sections can be divided between layers, as well, to achieve higher precision at 1 cm to 5 cm in length depending on the core diameter, aiming for a dry sample weight of at least 15-30 g per sample.
5) Working one section at a time, use a serrated knife to cut the section. Use a trowel to remove the section and place it in a metal tray.
6) Remove any large objects, roots, rocks, etc., from the section and place them in a separate metal tray.
7) For each section and for any removed objects, record:
    a. Sample site
    b. Core ID
    c. Section ID
    d. Top depth (cm)
    e. Bottom depth (cm)
    f. Length (cm)
    g. Volume (cm^3)
    h. Weigh the section. Record the sample and tray weight (g) value in a notebook next to the respective tray ID.
8) Repeat steps three to seven for the rest of the core.
9) When all samples are sectioned, weighed and in metal trays, place them in a drying oven set to 65°C for 48–72 hours.
10) After the allotted time, stable weights can be identified by following these steps:
    a. Remove the samples from the oven and let them rest at room temperature for 10 minutes (optionally, store in a desiccator)
    b. Weigh the samples, record the weight, and put them back into the oven at 65°C for 1 hour
    c. After 1 hour, remove the samples from the oven and let them rest at room temperature for 10 minutes, then weigh them again.
    d. Subtract the second measured weight by the first. If the resulting difference is greater than negative 0.1g, the sample has reached its stable weight. If not, dry for 2–24 more hours and try again.
    e. Once stable weight is achieved, record the first weight in a notebook.

PROCESS TO PREPARE SECTIONED CORES FOR ANALYSIS

If the soil cores were sectioned in the field:

1) Wear gloves, a lab coat and closed-toe shoes.
2) All frozen sections should be thawed for 24–48 hours before processing in the lab.
3) Prepare metal trays labelled with unique IDs and weigh the trays using a scale. Record the tray ID and tray weight in a notebook.
4) Remove any large objects, roots, rocks, etc., from the section and place them in a separate metal tray.
5) For each section and for any removed objects, record:
    a. Sample site
    b. CoreID
    c. Section
    d. Top depth (cm)
    e. Bottom depth (cm)
    f. Length (cm)
    g. Volume (cm^3)
    h. Weigh the section. Record the sample and tray weight (g) value in a notebook next to the respective tray ID.
6) Repeat steps three to five for the rest of the core.
7) When all samples are sectioned, weighed and in metal trays, place them in a drying oven set to 65°C for 48–72 hours.
8) After the allotted time, stable weights can be identified by following these steps:
    a. Remove the samples from the oven and let them rest at room temperature for 10 minutes (optionally, store in a desiccator)
    b. Weigh the samples, record the weight, and put them back into the oven at 65°C for 1 hour
    c. After 1 hour, remove the samples from the oven and let them rest at room temperature for 10 minutes, then weigh them again.
    d. Subtract the second measured weight by the first. If the resulting difference is greater than negative 0.1g, the sample has reached its stable weight. If not, dry for 2–24 more hours and try again.
    e. Once stable weight is achieved, record the first weight in a notebook

PROCESS TO HOMOGENIZE SAMPLES

1) Using tape and permanent marker, prepare storage containers for the dried samples with proper labels containing:
    a. Site
    b. Core ID
    c. Sample ID
    d. Depth interval = top depth (cm) - bottom depth (cm)
    e. Date
2) Grind the samples until the soil is homogenous and contains no distinguishable bits. This can be done using a coffee grinder, a mortar and pestle, or another sediment-homogenizing device.
3) Once homogenized, place the sample in a labelled storage container.
4) Repeat for each sample. Ensure the homogenization device is properly cleaned and dried between each sample to avoid contamination.

The samples are now ready for analysis or to be shipped to an external lab for further analysis. Follow the procedures according to the specific lab's sample and shipping instructions.

STEPS FOR MEASURING ORGANIC CARBON USING THE LOSS-ON-IGNITION METHOD

1) Prepare crucibles with unique labels marked in pencil.
2) Record the crucible ID and weight of the crucibles.
3) Place 1 g of each sample into a crucible and record the sample name with its respective crucible ID.
4) Place samples in the muffle furnace set to 550°C for four hours.
5) It will take some time for the furnace to preheat; ensure the samples are already in the furnace while it's preheating.
6) It is very important not to open the furnace when the temperature exceeds 100°C.
    a. After four hours, turn off the furnace.
    b. Wait until the furnace has cooled; this may take a long time (e.g., minimum six hours).

Note: *The high temperatures of the muffle furnace can do personal harm. Ensure that the furnace is attended when in use. Carefully read and follow the manufacturer's instructions before use.*

7) Remove the crucibles and let them rest until they reach room temperature (typically about 20 minutes).
8) Weigh the samples and record the following in a notebook:
    a. Core ID
    b. Sample ID
    c. Weight (g)

NEXT STEPS

Following the above steps, we can now calculate the dry bulk density (g/ cm^3) and soil organic carbon content (%Corg) of the samples. Dry bulk density is determined by dividing the dry weight of your samples (g) by the measured volume of your samples (cm^3; Example: volume = area of corer face (cm^2) * depth interval (cm)).

Next, we can estimate the organic carbon content of your samples. Using the results from the loss-on-ignition, subtract the pre-burned weight of the crucibles by the weight of the burned samples. This value is the organic matter content of your samples. Multiply this value the carbon conversion factor of 0.5 to obtain the organic carbon content of your samples; further multiply this value by 100 to obtain the percent organic carbon content (%Corg).

Note*: The carbon conversion factor of 0.5 is an approximation. If possible, send a subset of samples for CHN elemental analysis to derive specific LOI-to-%Corg relationships, as published ratios can vary considerably between ecosystems, soil types and soil depths.*

The next steps are also available in the supplemental guides for peat and non-peat soils. Calculating the total soil carbon for the study area requires the following information:

- Dry bulk density (g/cm^3)
- Total carbon content (%C) or organic carbon content (%Corg)
- Subsection depth interval (cm) = bottom depth (cm) - top depth (cm)

The average carbon stock of a core can be determined as follows:

1) For each core subsection, calculate the soil **organic** carbon density:
    - **Eq 1: Soil organic carbon density (g/cm^3) = dry bulk density (g/cm^3) * (% Corg/100)**

Note*: Organic carbon content (%Corg) can be substituted with total carbon content (%Ctotal) to determine the total soil carbon density (g Ctotal/cm^3), and average total carbon stock (g Ctotal/cm^2).*

2) To calculate the average amount of carbon in a subsection, multiply each soil carbon density value obtained in Eq 1 by the subsection depth interval (cm):
    - **Eq 2: Average carbon stock of subsection (g/cm^2) = soil carbon density (g/cm^3) * subsection depth interval (cm)**

3) To obtain the average carbon stock (g/cm^2) of the core, add up the values for each subsection calculated above:
    - **Eq 3: Average carbon stock of core (g/cm^2) = sum of average carbon stocks of subsections**

Note: *Subsections must add up to 100 per cent of the core to obtain the average carbon stock.*

4) Convert the average carbon stock of the core (g/cm^2) from Eq 3 into units of kg/m^2 by multiplying by 10, or more formally:
    - **Eq 4: Average carbon stock of core (kg/m^2) = average carbon stock of core (g/cm^2) * (1 kg/1,000 g) * (10,000 cm^2/ 1 m^2)**

5) Repeat steps one to four for each core.

The carbon stock of a study area can be determined as follows:

1) To obtain the average carbon stock of each study site (kg/m^2), add up the average carbon values from Eq 4 (kg/m^2) for each core obtained and divide it by the number of cores taken in each site.

   - **Eq 5: Average carbon stock of site (kg/m^2) = sum of average carbon stocks of cores (kg/m^2)/ number of cores**

2) Multiply the average carbon stock of the site by the size of the site (in metres squared) to obtain the total carbon stock of each site (kg C).

   - **Eq 6: Total carbon stock of study site (kg C) = average carbon stock of study site (kg C/m^2) * study site size (m^2)**

3) Add up the total carbon stocks for the sites and divide by the sum of the site sizes (m^2). This gives the average carbon stock of the study area (kg C/m^2).

   - **Eq 7: Average carbon stock of study area (kg C/m^2) = sum of total carbon stocks of sites (kg C) / sum of site sizes (m^2)**

4) Finally, to calculate the total carbon stock of the study area (kg C), multiply the average carbon stock of the study area by the size of the study area (in metres squared).

   - **Eq 8: Total carbon stock of study area (kg C) = average carbon stock of study area (kg C/m^2) * study area size (m^2)**

NOTE: *The carbon values calculated here are in units of "C", If interested in units of "CO_2 equivalents," multiply by 3.67.*

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GLOSSARY

Bulk density: The amount of mass per unit volume of a material, usually expressed in g/cm^3 or kg/m^3. An example of high bulk density would be highly compacted sandy soils. An example of low bulk density would be peaty soils with lots of air and water present.

Carbon content: The proportion of carbon in a sample, typically expressed as a percentage or decimal.

Carbon stock: A measure of the amount of carbon in a carbon pool, typically presented in kilograms or tonnes.

Crucible: A ceramic or metal container in which soil or other substances may be melted or subjected to very high temperatures.

CHN elemental analyzer: A device used for rapid, quantitative determination of carbon, hydrogen, nitrogen and sulfur in organic samples. Through a combustion process, these elements are converted to CO_2, H_2O, N_2 and SO_2 gases and sent to the thermal conductivity detector to record the electrical signal proportional to the amount of each gas. This electrical signal then gives, for example, the percentage of elemental composition in proportion to the curve areas obtained in the spectrum.

Dry unit weight: Weight of soil solids (i.e., without any water content).

Dry bulk density: Dry mass of a sample divided by its volume, usually expressed in g/cm^3 or kg/m^3.

Isotope: Atoms with the same number of protons but different numbers of neutrons; can be used to infer the date of an object through the isotope's distinct half-life (i.e., rate of decay).

Lead-210 dating: A method for determining the rate of sediment accumulation within a 100- to 200-year time span using the decay of excess 210Pb activity.

Loss-on-ignition: Scientific method for determining the fraction of organic matter in a sample of soil by burning the sample between 450–950·C for several hours.

Muffle furnace: A laboratory instrument used to heat materials to extremely high temperatures while isolating them from fuel and the byproducts of combustion from the heat source.

Organic carbon: The amount of carbon found within the biomass of a thing (living or dead).

Peat: The surface organic layer of a soil that consists of partially decomposed organic matter, derived mostly from plant material, which has accumulated under conditions of waterlogging, oxygen deficiency, high acidity and nutrient deficiency.

Radio-carbon dating: A method for estimating the age of carbon-based materials that originated from living organisms through analyzing the ratio of carbon-13 to carbon-14 in a sample. While radio-carbon dating can be carried out in many ecosystems, it is challenging to accurately estimate soil age of grasslands and forest soils types due to high root turnover and downward movement of soil in these ecosystems.

Soil organic carbon: The carbon that remains in the soil after partial decomposition of any material produced by living organisms.

Soil organic matter: The fraction of the soil that consists of plant or animal tissue in various stages of breakdown (decomposition).

Wet weight: Weight of soil sample before drying or losing any water content.

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