**Soil preparation for incubation initiation**

1. Collect topsoils using cores of known volume if bulk density must be determined. Dry soils at 40 ⁰C to preserve organic carbon. The bulk density of the soils may be evaluated by weighing dry soil before and after sieving (2 mm diameter mesh) to account for coarse fragment content (Poeplau et al., 2017).
2. The maximum water holding capacity (WHCmax) of each soil sample (Nelson et al., 2024; Schinner et al., 1996) is determined as follows. Soil sub-samples (ca. 5 g) are weighed before and after drying at 105°C for at least 12 hours to determine the current gravimetric water content. Another set of soil sub-samples (ca. 5 g) is saturated with water in filter funnels and placed on an absorbent membrane for at least 12 hours. The funnels are then placed in a sand layer to allow excess water to drain by gravity for 30 minutes and weighed to determine the water content at WHCmax. The soil water content equivalent to 60% of the sample’s WHCmax (typical target water content for incubation studies) was calculated.
3. Sieved soil samples are incubated in sealed jars. Incubations are initiated by moistening soils with the volume of water required to bring soils to 60% WHC.

**Flush with synthetic air (CO2 free) for soil samples with big tank**

1. Connect the flasks to manifold with inlet needle to large gas tank (synthetic air) and outlet needle open to the atmosphere.
2. Open the stopcock valves to the inlets of all the flasks.
3. Open the main black valve of the gas cylinder slowly then adjust flow with gray valve.
4. Flush incubation flasks for 2 minutes.
5. When finished, close the inlet valve, remove the needle and after 15 seconds, remove outlet needle out to avoid overpressure.
6. Close the valve of the cylinder and then the manometer.
7. Note the time (incubation starting point).
8. Incubate for desired number of days. Measure gas concentration at least once a week until theoretically 1 mg C in the jar (minimum for 14C measurement) if radiocarbon measurements are planned.

**Calibration of LICOR and measurement of CO2 concentration**

1. Start the system 1 hour before the first reading.
2. Open the N2 valve by turning it counterclockwise. Open the large gas tank (synthetic air).
3. Adjust the CO2 baseline between 0.020 and -0.020 with the knob “CO2 zero”
4. Check that the pressure is at 600 mbar, water trap is blue (not purple), the soda limenot older than three month and septum is not too punctured.
5. Slowly open main black valve of the small gas cylinder (3000 ppm CO2 for calibration) then adjust flow with gray valve.
6. Choose a range of 5 to 10 gas volumes suitable for a calibration curve for your samples’ expected concentrations (typically 0.4-5 mL but may need up to 12 mL for highly concentrated samples).
7. Fill the syringe with calibration gas slightly more than the desired volume by screwing the syringe into suitable fitting on the gas outlet, opening stopcock of the fitting and allowing the syringe to expand or pulling back of the syringe if it does not expand by itself (pulling a vacuum means the stopcock is closed or the gas cylinder is not open).
8. Remove the syringe from the calibration gas connection and adjust the volume to desired amount by expelling excess gas.
9. Select LICOR settings
   1. Select “Function” 🡪 “9” (to calculate integral) 🡪 Enter
   2. Select channel: “22” (to show units in µmol/mol) 🡪 Enter
   3. Select starting point: “Thrsh” (starts measurement when channel rises above threshold value) 🡪 Enter
   4. Select starting and end point thresholds: “0.25” 🡪 Enter
   5. Continue selecting Enter until “Stop”.
   6. Insert syringe needle into septum, press Enter and inject at fast but consistent speed.
10. After measurement reaches threshold and stabilizes, record peak area (int) value and press #2 to view and record peak height (max) value.
11. Press #1 and start next sample by repeating from step 7.
12. For samples collected from flasks, pierce rubber lid with syringe needle, pull incubation gas into syringe and expel back into flask, repeating several times to mix. Pull more than desired volume, remove needle and adjust to volume by expelling excess gas.
13. When finished, turn off the LICOR, close the valves of all tanks and N2.