**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. Big cylinder must be closed. Connect the flasks in a line of flushing, two needles, inlet from tank, outlet to the atmosphere.
2. Open the valves of all the pipes that are connected to the inlet needles of the flasks.
3. Open the valves of the cylinder. **black valve slowly and then the gray.**
4. Let the flow in and out for 2 minutes.
5. When finished, close the inlet valve, take the needle out and after 15 seconds, take the outlet needle out as well, to avoid overpressure.
6. Close the cylinder valves. First close the valve of the cylinder and then the one of the manometer.
7. Note down the time, that is the incubation starting point.

**Measurement of gas concentration**

**Calibration of the LICOR (with small tank 3000 or 30000 ppm)**

1. Start the system 1 hour before the first reading.
2. Open the N valve by turning it counterclockwise. Open the big tank as well (synthetic air). Keep the valve of N entering the LICOR open.
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 and the **water trap** is blue, not purple and the **soda lime** (not older than three months)
5. After one hour. Use the calibration gas of 3000 ppm (small tank). Open the **black valve slowly and then the gray**. Let the pipes get flushed.
6. Fill the syringe with 5mm. Fill the syringe putting in contact the two pipes openings obliquely in order to expulse the air volume with the air coming out of the tank.
7. Disconnect the syringe from the gas supply. Adjust the amount of ml when the stopcock is open, then close it. Take the first time 5 ml (this first record is not noted, it is to purge)
8. Adjust the setup by the first time:
   1. select the integrate-function: „*Function*“ 🡪 „*9*“ 🡪 *Enter*
   2. select the channel code for CO2 in µmol/mol abs.: „*22*“ 🡪 *Enter*
   3. select the starting point of your measurement : „On Exit“ (integration will start immediately) or „Thrsh“ (integration starts when selected channel rises above the threshold value) 🡪 select „*Thrsh*“ 🡪 *Enter*
   4. select the starting threshold value: „0.25“ 🡪 *Enter Press Thrs 2 times*
   5. select the ending point of your measurement : „Manuel“, „elaps tm“ or „Thrsh“ 🡪 select „*Thrsh*“ (integration stops at threshold value of 0.25µm/m) 🡪 don`t press Enter yet!... Inject 5 ml and ignore (that first measurement). Press enter and start to inject 5 ml for first values.
9. Connect needle and inject the gas inside the lower hole at a consistent speed.
10. Note down the value Peak area (int) when it stops.
11. Press #2 for integral maximal, note down.
12. Press #1 to restart and then function – 9 – enter - enter – enter -enter - enter
13. Repeat measurements with the **amount of gas** according to the table.

**Measure samples for the rubber glasses**

1. Connect the syringe with valve and needle to the flask through the rubber lid (can be the same needle and syringe of the calibration).
2. Push the piston upwards to 1 ml to extract the dead volume of the pipes. To create a vacuum, open the valve fast while sucking a bit of air. Close it fast and then unscrew it to expulse the dead air. Screw the syringe again.
3. Open the valve. Mix it several times and keep 5 ml. Close the valves.
4. Adjust to 5 ml of air and inject it into the Licor.
5. Note down the peak area and the Int. max.
6. When finished, turn off the LICOR, close the tanks (does not matter which valve first) and then close the N turning the valve clockwise.