**Microbial respiration**

**Objective**: Basal respiration is the amount of CO2 produced by the biological activity of soil at field conditions(Phillips and Nickerson, 2015). These measurements can be used to determine the efficiency of microbial communities (when used in conjunction with microbial biomass as the ratio of respiration rate/microbial biomass).

**Equipment:**

* Ball jars (1 per sample for respiration and 1 per sample for SIR)
* Ball jar lids with silicone “septa”, and corresponding rings
* Ball jar boxes
* Benchtop LI-COR 850 (measures CO2 concentration through absorbance in the infrared).
* Computer to run benchtop LI-COR (+ software)
* Fresh soil (directly from field or fridge, record how long stored and nature of storage, at least 20 g/sample, to be split into duplicate pseudoreplicates)
* fan

**Microbial respiration (incubation study)**

* Label ball jars with corresponding Soil ID (\*IN DUPLICATE, either ‘A’ or ‘B’\*) and record weights in your lab notebook as shown in template in ‘biogeochem\_example.xlsx.
* Place ~10 g homogenized soil into the corresponding jar and record the weights in your lab notebook and the project data template (equivalent to ‘biogeochem\_example.xlsx’)
* Place the lid loosely over the top to prevent soil from drying.
* Directly before measuring for the 1st time, fan the samples then tightly cap.

Check that the LiCOR is reading a stable temperature and that the CO2 is reasonable (concentrations can get high indoors but should not be <400 or >600ppm).

When the LI-COR is stable, take a **Time 0 (T0)** CO2 measurement on each jar.

1. Check the back of the needles are well sealed in the clear tubing connected to the LiCor and that there are no leaks.
2. Hit the START button to begin recording in the LI-COR software.
3. Insert the “in” needle into one port, rapidly place the “out” needle into the other port.  Watch for the CO2 level to stabilize and then write down the CO2 concentration (ppm) and the exact time from the software. This is T0 for the SIR data.
4. Remove the out needle and then the in needle. Be careful that the needle base is still inserted well into the tubing and seals well.
5. Allow to flow for a few seconds until the CO2 returns to room air levels
6. Start on the next jar, remember to ensure the LI-COR software is logging
7. Once you finish your samples, you will take another measurement **(T1) 2 hours** after T0, (**T2) 4** hours after T0
8. If necessary, more measurements will be taken at 6 hours (T3) and 24 hours (T4)

**Data to record (example)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Soil ID/duplicate (A or B) | fresh weight (g) | Time (hours) | exact measurement time | CO2 measured (μmol CO2/mol) |
|  |  | 0 |  |  |
| 2 |  |  |
| 4 |  |  |
|  |  | 0 |  |  |
| 2 |  |  |
| 4 |  |  |

Respiration rate (µg C-CO2 g-1 soil dry weight hr-1) is calculated as the change in CO2 concentration over time divided by the dry soil mass \* time incubated (hour). Run this data in R using the lab script to assess respiration rate.

Convert from µM (ppm) to µg C-CO2 as follows:

Text, letter

Description automatically generated

Text

Description automatically generated

Assume 236.6 mls of volume (8fl oz jar) and the room was 20 ˚C

This yields: [ (9.84\*0.001\*12) \* ppm CO2]/ (dry weight soil \* hr)

You will need to calculate the amount of soil (dry weight) based on the field-moist amount added to each sample jar.

**Notes:**

* If there is very rapid CO2production your readings might creep up during the measurement. If the jar or needles are leaking, there will be a continuous slow decline. The LI-COR will always fluctuate a bit. Record the number when it begins to stabilize and fluctuate within a range of +/- 5ppm.
* If there is evidence of leaking at T0 and the hoses and needles are well connected, replace the lid. Wait another 5-10 minutes and take a new T0 measurement.
* If you have leaks in a few jars in a row, it is probably the IRGA leaking at where the needles meet the tubing rather than the jars leaking.