**SIR microbial biomass**

Soil respiration directly relates to microbial biomass (typically a positive correlation). The quantity of microbial biomass in a soil sample is inherently difficult. To some degree, any measurement of microbial biomass is relative – different methodologies or variations in methodologies will yield microbial biomass estimates that are not directly comparable. The method we will use today is called **substrate induced respiration** (SIR) (West and Sparling, 1986). SIR estimates microbial biomass based on the short-term respiration rates (CO2 production) when soil is given an excess supply of labile C. The higher the respiration rate, the more microbial biomass in a soil. The SIR method is straightforward, consistent, and reasonably quick; however, the biomass estimates are relative – the method will not give direct estimates of microbial biomass C (or N).

**Microbial biomass (substrate induced respiration (SIR))**

* Label ball jars with corresponding Soil ID\_SIR and record weight. Make it clear which jars are SIR jars and which are unamended respiration samples!
* Place ~10 g homogenized soil into the corresponding jar and record the weight.
* Place the lid loosely over the top to prevent soil from drying.
* Add 10mls of the yeast extract (12 g/ L) to the jars.

Cap the jars and place on the shaker table at 120rpm. **After** **15 minutes, take a T0 CO2** **measurement on each jar**.

1. Check the back of the needles are well sealed in the clear tubing connected to the  LI-COR 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.
4. 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.
5. 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.
6. Replace each jar on the shaker table when you have completed a batch in order to keep shaking.
7. Allow to flow for a few seconds until the CO2 returns to room air levels
8. Start on the next jar, remember to resume logging on the LI-COR software

1.5 hour and 3 hours after the initial CO2 reading, take a second (T2) and third (T3) CO2 measurement.

Calculate the slope of the line relating CO2 concentrations to time. The average respiration rate (µmol CO2 g-1 dry soil dry weight hr-1) over the incubation period is an index of the SIR-responsive microbial biomass. Calculate an R2 value for the line describing CO2 concentration change over time to make sure the relationship is linear.

Microbial biomass C (µg g-1 soil) is determined using eq. 1:

Eq 1:

This technique is modified from West and Sparling. 1986. *Journal of Microbiological Methods*. 5: 177-189.