# Notes on conducting the full cycle pilot microcosm experiment

## Goals:

1. Determine the time point to which each 13C treatment will be destructively sampled.
   * For the full experiment, only 1 time point will be taken per 13C treatment.
   * Different substrates will require different incubation times.
   * Determining time point to best process each treatment.
     + Time point based on 13-CO2 production rates.

## Treatments:

* 13C-Cellulose
* 13C-Xylose
* 13C-Glucose
* 13C-Glycerol
* 13C-Vanillin
* 13C-Palmitic acid
* 13C-Amino acids
* 13C-Na-Lactate
* 13C-Na-Oxalate
* 12C
* H2O (see general effect of C addition)

### Notes:

* Number of replicates: 4
* Total number of microcosms: 44
* For each 13C treatment, other C substrates are added, but as 12C.
  + Desired fraction C per treatment: 0.4 mg (C) / g (soil)

## Sample collection

### Soil sieving

* Sieved soil stored over night while soil moisture levels were determined.
* 15g of soil dry weight added to each microcosm.

### Measuring soil % moisture

* See 'Determine soil dry weight' of 'soil\_sampling\_protcol'

### Preincubation

* ~2-week incubation in microcosms.
  + Microcosms stoppered.
  + Waiting for CO2 respiration (measured by GCMS) to level off.
  + Measuring CO2 respiration:
    - Headspace collection from 6? of the microcosms every 3 days
  + Every 3 days: stoppers removed and gas flushed
    - Gas flushing for 2 min with 0.2um-filtered house air

## Microcosm labeling

* Treatment : Label
* 13C-Cellulose : 13C-cel
* 13C-Xylose : 13C-xyl
* 13C-Glucose : 13C-glu
* 13C-Glycerol : 13C-gly
* 13C-Vanillin : 13C-van
* 13C-Palmitic acid : 13C-pal
* 13C-Amino acids : 13C-ami
* 13C-Na-Lactate : 13C-lac
* 13C-Na-Oxalate : 13C-oxa
* 12C-control : 12C-con
* H20 : H2O

**MicrocosmID = [treatment]\_[rep#]**

## Carbon treatment additions

### Materials:

1. 12C and 13C substrates
2. Base salts mixture (thawed and filter-sterilized)
3. Sterile water
4. Microcosms!

### Methods:

* Additions (including water) should bring soil to 50% water holding capacity.
* Cellulose added by spinkling onto each microcosm.
* The other substrates weighed by mg needed per microcosm \* number\_replicates
  + Added to 1.5? ml Eppendorf tube
  + Glycerol added first (semi-solid)
    - Tare eppendorf, then add required amount
* Base salt mixture added to each eppendorf
* Water (to get to 50% holding capacity) added to each eppendorf.
* Vortex eppendorf to disolve substrates.
* Flush each microcosm prior to adding substrates.
* From eppendorf:
  + Pipette evenly onto soil of each microcosm
  + Ashley's method: I usually start dripping the addition around the wall of the flask and move into a circle/spiral formation towards the middle of the soil.
* Stopper flasks
* Seal with parafilm
* Note time (time point 0)

## Gas Sampling

### Materials:

1. 48 of the 2 mL gas vials (pre-crimped with grey butyl stoppers)
2. 7 of the 10 mL gas vials (pre-crimped with blue butyl rubber stoppers)
3. Tank of He gas and gassing station
4. 44 2/3% gauge needles (you'll inevitably bend lots of them during the gassing process)
5. 0.5 mL and 10 mL gas tight syringes (with the green-red stop cock)
6. Tank of the gas standard (attached to ring stand at gassing station)
7. Microcosms

### Gas vial labeling:

**2ml-vial-ID = [sample#]\_[microcosmID]\_[day]\_[time]**

* day = yymmdd
* time = military

### Standards

* Using 10 ml vials to make CO2 mixtures
* ml CO2 gas standard in each vial:
  + 0
  + 0.25
  + 0.5
  + 1
  + 2
  + 5
  + 7.5

### Methods:

#### Flushing vials

1. Turn on He tank while you prep things. This allows time to clear the tubing of ambient air.
2. Stick a needle into the edge of the stopper of a 2 mL vial.
   * Repeat until you have 6 vials with needles sticking out of them.
   * **NOTE:** Goal for puncturing the septa is to keep it minimumal. More punctures = more chances for gas to escape.

* Put vial on a needle on the gassing manifold, set timer for 5 min.
* When removing vial after flushing, make sure both needles are pulled out at the exact same time.
* Repeat until all vials are flushed (Can only flush 6 at a time on the manifold)
  + Remaining vials will be: 2 vials of He blank, 2 of air, and 7 for the std curve
* Pull tabs off of 10 mL vials and label (gas std vials).
* Flush these vials the same way the 2 mL vials are flushed (except flush them for 20 min instead of 5 min)
  + **NOTE:** I usually let these flush while I'm sampling gases from the microcosm flasks. Then they're finished and rippin', roarin', ready to go when you're done sampling!

#### Making standards

1. Using gas standard tank (to left in gassing station, on a ring stand), turn it counterclock wise to open it.
2. Use 10 mL stopcock gastight syringe to make most of the stds.
3. Pre-evacuate std vials to volume that you plan on filling
   * eg., remove 0.5 ml from vial if adding 0.5 ml CO2 gas mixture

* Insert syringe into regulator through sampling port, fill beyond your desired volume, press in red button and pull out.
* **VERY FAST:** open stop cock and push the syringe plunger to your desired volume.
  + This allows the gas to come to 1atm which is very important for knowing exactly how much gas is in each std.
* Add gas volume to pre-evacuated 10 ml vial.
* Inject 250 uL of each of the stds (from the 10 mL vials) into the 2 mL vials.

#### Sampling microcosms

1. Use 0.5 mL gas tight syringe (with green/red stop cock)
2. Push needle through 18 gauge sampling port and visually check to make sure the needle is all the way in.
3. Pump the plunger 5 times to mix the gas.
4. Then pull plunger up to **0.25 mL** and push the red side of the stop cock.
5. Pull syringe out of sampling port and puncture into respective 2 mL vial.
   * **IMPORTANT:** CHECK TO MAKE SURE THE VIAL YOU'RE SAMPLING INTO MATCHES THE FLASK YOU SAMPLED FROM!

* Repeat for all flasks.
* Note what time you finished sampling. This is very important because data is based on hourly rates. The sampling is for nothing if we don't know how much time has passed.
* Next step is to air out flasks. Take stoppers out of all flasks (don't forget the ones that don't have gas sampling ports) and set a timer for 10 min.
  + At around 2 min until the end of the airing, flush each flask with house air for ~5 sec.
  + **IMPORTANT:** Make sure the air has a very slow flow, we wouldn't want to blow the soil out of the flasks!
* Restopper all the flasks after 10 min. Make sure they're pushed in air tight.
* Take a sample of air with the syringe (250 uL) and inject it into the "air" 2 mL vial.
* Note the time you ended flushing the flasks because this will serve as the starting time for the next gas sampling.

#### Finishing up

* Make sure you turn off all the gas tanks and you're done!

## Clean up checklist:

1. Did you note the time you sampled the gas?
2. Did you note when you finished additions?
3. Did you turn off the gases?
4. Did you turn of all of the gases (except the GCMS)?
5. Are you sure the stoppers of the flask are tight?