# Notes on conducting the 2nd Full C-cycle microcosm experiment (ag vs meadow vs forest soils)

## Authorship

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## Treatments:

* 13C-Cellulose
* 13C-Xylose
* 13C-Vanillin
* 13C-Palmitic acid
* 13C-Amino acids
* 12C-Control
* H2O (to see general effect of C addition)

### Notes:

* Number of microcosm replicates: 3
* Total number of microcosms to sample: 63
* For each 13C treatment, other C substrates are added, but as 12C.
  + 13C per treatment: 0.4 mg (C) / g (soil)

## Sample collection

* 5x5 meter grid (flagged)
* 20 soil cores (non-SIP)
* 80 soil cores (SIP)

### Soil sieving:

* Sieved soil back in the lab (2 mm sieve).
* Homoginized soils by mixing in a plastic bag.
* Soils stored at 4oC until use.
* 15 g of soil dry weight added to each microcosm.

### Soil processing workflow

* Water holding capacity
* Based on water holding capcity:
  + weigh out enough soil for SIP microcosms and add to each microcosm
  + weigh out enough soil for each enrichmnet and add to each microcosm
  + weigh out enough soil for soil geochem analyses
    - dry soil in drying oven
    - store in ziplock bags at room temp
* Fill two 15 mL conicals with wet soil and place in -80oC

### Preincubation:

* ~2-3 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-Vanillin : 13C-Van
* 13C-Palmitic acid : 13C-Pal
* 13C-Amino acids : 13C-Ami
* 12C-Control : 12C-Con
* H20-Control : H2O-Con

## Gas Sampling

### Materials:

1. 2 mL gas vials (pre-crimped with grey butyl stoppers)

* Enough for all standards and microcosms (+ and 'air' control)

1. 6 of the 10 mL gas vials (pre-crimped with blue butyl rubber stoppers)
2. 0.5 mL and 10 mL gas tight syringes (with the green-red stop cock)
3. He gas tank at gassing station
4. Gas standard tanks
5. Microcosms

* **Note:** ~10 ml vials actually have an interval volume of ~11.5 ml (crimped with shallow septa).

### Standards

* Using 10 ml vials to make CO2 mixtures
* ml CO2 gas standard in each vial:
  + Tank (%CO2) : volume (ml)
  1. NA : 0
  2. 1% : 1
  3. 1% : 2
  4. 1% : 4
  5. 5% : 2
  6. 5% : 4
  7. 5% : 8
  8. 100% : 1 (if respiration rates are high)

### Methods:

#### Flushing vials:

1. Label, seal, and crimp all needed 2 ml and ~10 ml vials.
   * CO2 standard vial labeling:
     + "X% CO2" "x mL"
   * Microsom vial labeling:
     + Microcosm\_ID
2. All valves at the gassing station should initially be closed.
3. Turn on He tank (tank valve).
   * The PSI should be low (8-10 PSI) in order to not overpressurize the vials.
4. Stick a needle into each leur lock in the gassing manifolds.
   * Plug up any leur locks that will not be used.
   * Each manifold can be shut off using the valve just upstream.
5. Check to make sure gas is flowing out of each needle and each needle is tightened fully.
6. Place safey shield beteen you and vials.
7. Turn on venturi vacuum (sink).
8. For a cycle of vacuuming and gassing:
   * **NOTE:** Make sure the vacuum and gas valves are not open simultaneously. The He gas will just be vacuumed out.
   * Open the valve for the vacuum.
   * Vacuum for: >=10 sec (if 2 ml vials) or >=20 sec (if 10 ml vials)
   * Close vacuum valve.
   * Open gas valve.
   * Gas for: >=10 sec (if 2 ml vials) or >=20 sec (if 10 ml vials)
   * Repeat vacuum-gas cycle for >=5 times.
   * Remove the vials while the He gas is still flowing.
9. Repeat the entire process until all vials are flushed.
10. Turn of He tank, purge the lines, and turn of the venturi vacuum.

#### Making standards:

* Use the gas standard tanks (to left in gassing station, on a ring stand)
* Use 1 mL and 10 mL stopcock gastight syringes to make the stds.

1. Turn the tank valve counter clockwise to open it.
   * The tank regulator should show an increase in pressure.
2. 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
3. Insert syringe into regulator through sampling port, fill beyond your desired volume, press in red button and pull out.
4. **VERY FAST:** open stop cock and push the syringe plunger to your desired volume.
   * This allows the gas to come to 1 atm which is very important for knowing exactly how much gas is in each std.
5. Add gas volume to pre-evacuated 10 ml vial.
6. 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 stopcock).
2. Push needle through 18 gauge sampling port and visually check to make sure the needle is all the way through the stopper.
3. Pump the plunger 5 times to mix the gas.
4. Pull plunger up to **0.25 mL** and push the red side of the stop cock to shut it.
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!
6. Repeat for all microcosms (flasks).
7. 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.
8. Air out *all* flasks. For each flask:
   * Unstopper and flush for 10 sec with filtered air.
     + **IMPORTANT:** Make sure the air has a very slow flow. We wouldn't want to blow the soil out of the flasks!
     + **MAKE SURE:** to time the 10 sec and keep it consistent between microcosms.
   * Re-stopper the flask.
     + **MAKE SURE:** the stopper is pushed in airtight.
   * *Don't forget the flasks that don't have gas sampling ports.*
   * **IMPORTANT:** Make sure all stoppers are pushed in airtight.
9. Take a sample of air with the syringe (250 uL) and inject it into the "air" 2 mL vial.
10. **MAKE SURE:** to note the time you ended flushing the flasks because this will serve as the starting time for the next gas sampling.

# Substrate additions

## Preparation

* This can be done 2-3 days in advance.
  + One of the soluble substrates (amino acids?) does undergo a color change over longer timeframes, so it is best to not make the substrates any earlier.

### Soluble substrates

#### Materials

* MilliQ H2O
* Murashige and Skoog base salts mixture (Sigma M5524)
* 12C substrates
* 13C substrates
* 15 ml conicals
* 10 ml syringes
* 0.2 um syringe filters (23 mm)

#### Method

* **MAKE SURE:** keep 12C and 13C substrates separate!

1. Label a conical for each treatment.
2. Add sterile H2O to each.
   * The volume of liquid added to each microcosm should bring the soil to 50% water holding capacity.
   * 50% water holding capacity for Penn Yan soils: 0.3 g (H2O)/ g (soil)
3. Add base salt mixture to each
   * Volume determined by C:N ratio (10:1)
4. Add 12C substrate to each
   * Leave out if 13C substrate for treatment
   * Vortex and heat to ~50oC to solubalize if needed
     + Heat needed to full solubilize vanillin
5. Add 13C substrate to each corresponding treatment
   * Make sure substrates are solubalized
6. Syringe filter each treatment solution into a new 15 ml conical
7. Store in fridge until use
   * The vanillin should not recrystalize in the fridge.

### Insoluble substrates

#### Materials

* 12C substrates
* 13C substrates
* aluminum foil
* weigh paper

#### Method

1. Weigh out 12C and 13C substrates in amounts for individual microcosm additions.
   * Pre-fold the weigh paper to make a wrapper.
   * **MAKE SURE:** keep 12C and 13C substrates separate!
2. Fold up weigh paper with substrate and fold that in aluminum foil
   * Label

## Application

### Materials

* Mucosal Atomization Device (MAD)
* Insoluble Substrate Addition Device (ISAD)
  + 50 ml pipette cut to 6 in
  + 250 um mesh
  + rubber band
  + parafilm
* Soluble substrate solutions
* Foil packets of insoluble substrate
* Sterile reagent troughs
* Sterile MilliQ water
* Metal stand with quick release tube clamp
* Long metal spatual

### Method

#### Soluble substrate

1. Flush MAD with 5 ml sterile purified water 3 times
   * Do this between each soluble substrate treatment
2. Pipette the amount of soluble substrate solution needed for 1 microcosm into trough
3. Suck up solution with 10 ml syringe (MAD not attached).
   * Suck in ~2 ml atmosphere (used for flushing all liquid out of MAD)
4. Attach MAD.
5. Apply substrate treatment to soil.
   * MAD tip should be ~1/2 to 3/4 inch from soil.
   * Move quickly around soil and soak it all.
6. Repeat for other microcosms.
   * Be sure to flush MAD in between substrate treatments.

#### Insoluble substrate

1. Clamp ISAD to metal stand
2. Place unstoppered microcosm under ISAD
   * This catches any substrate that falls through ISAD during loading.
3. Unwrap foil packet(s) of substrate needed for microcosm and dump onto weigh paper
4. Dump substrate into ISAD.
5. Move microcosm off stand.
   * Vibrations will cause loss of substate!
6. Place ISAD into microcosm (~1/2 - 1 inch from soil)
7. Repeatedly tap on ISAD to dispense substrate.
   * The metal spatual may need to be inserted into the ISAD to help with dispensing.
   * Make sure all of the substrate has been dispensed.

## 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 (and vacuum)?
   * Purge lines as necessary.
4. Did you turn of all of the gases (except for the GCMS)?
5. Are you sure the stoppers of the flask are tight?