**How to care for your beloved microcosms**

*Gas Sampling*

Materials:

1. 37 of the 2mL gas vials (I pre-crimped them with grey butyl stoppers)
2. Tank of He gas and gassing station
3. 27 2/3% gauge needles (you’ll inevitably bend lots of them during the gassing process)
4. 0.5mL and 10mL gas tight syringes (with the green-red stop cock)
5. Tank of the gas standard (attached to ring stand at gassing station)
6. Microcosms!

Methods:

1. Turn on He tank while you prep things – allows time to clear the tubing of ambient air.
2. Stick a needle into the edge of the stopper of a 2mL vial. (repeat until you have 6 vials with needles sticking out of them)
   1. NOTE: Goal for puncturing the septa is to keep it to a minimal. More punctures = more chances for gas to escape :)
3. Put vial on a needle on the gassing manifold, set timer for 5 min.
4. When removing vial after flushing, make sure both needles are pulled out at the exact same time.
5. Repeat until you have 37 vials (Can only flush 6 at a time on the manifold)
6. Label vials with date, treatment, and sample number.

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| --- | --- |
| Treatment | Sample numbers |
| H2O | 1,2,3,4,5 |
| 12C only (control) | 1,2 |
| 13C only (control) | 1,2,3,4,5 |
| 12C 700 | 1,2 |
| 13C 700 | 1,2,3,4,5 |
| 12C 100 | 1,2 |
| 13C 100 | 1,2,3,4,5 |

* 1. Remaining vials will be: 2 vials of He blank, 1 air, and 8 for the std curve

1. Pull tabs off of six 10mL vials and label 0, 0.5, 1, 2, 5, 10 (these will be the vials the gas stds are made up in)
2. Flush these vials the same way the 2mL vials are flushed (except flush them for 20min instead of 5min)
   1. \*\*\*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!
3. Sampling from microcosms: Use 0.5mL gas tight syringe (with green/red stop cock)
4. Push needle through 18gauge sampling port and visually check to make sure the needle is all the way in. It’s a side port needle, so just check for a dark hole on the needle. If you can see it, it’s in all the way!
5. Pump the plunger several times to mix the gas
6. Then pull plunger up to 0.25mL and push the red side of the stop cock

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| --- | --- |
| Treatment | Tape color |
| H2O | White |
| 12C only (control) | Orange |
| 13C only (control) | Yellow |
| 12C 700 | Blue |
| 13C 700 | Green |
| 12C 100 | Pink |
| 13C 100 | Red |

1. Pull syringe out of sampling port and puncture into respective 2mL vial
   1. CHECK TO MAKE SURE THE VIAL YOU’RE SAMPLING INTO MATCHES THE FLASK YOU SAMPLED FROM!
2. Repeat for all flasks
3. 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.
4. Next step is to air out flasks – so 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.
5. At around 2 min til the end of the airing flush each flask with house air for ~5sec. \*\*\*Make sure the air has a very slow flow, we wouldn’t want to blow the soil out of the flasks!
6. Restopper all the flasks after 10 min. Make sure they’re pushed in air tight.
7. Take a sample of air with the syringe (250uL) and inject it into the “air” 2mL vial.
8. Note the time you ended flushing the flasks because this will serve as the starting time for the next gas sampling.
9. By now, your stds vials should have finished their He gassing. So now it’s time to build a std curve!
10. Using gas standard tank (to left in gassing station, on a ring stand), turn it counterclock wise to open it.
11. Use 10mL stopcock gastight syringe to make *most* of the stds. Insert syringe into regulator through sampling port, fill beyond your desired volume, press in red button and pull out.
12. 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.
13. Stds to make with 10mL syringe:
    1. 1mL of std gas into one of your 10mL He flushed vials
    2. 2mL of std gas into another 10mL He flushed vials
    3. 5mL
    4. 10mL
14. Make the 0.5mL std with the 0.5mL syringe. (same as you make the others)
15. Should have 6 stds each in a 10mL vial now: 0 (no injection), 0.5mL, 1mL, 2mL, 5mL, and 10mL
16. Inject 250uL of each of the stds (from the 10mL vials) into the 2mL vials. Some will be over pressured. Just make sure you pull the plunger up to 250uL, close the stop cock, and then release the plunger (it will likely appear as more than 250uL at this point as the pressure has equalized…THIS IS OK!!!)
17. For the 2 final stds: Use the highest std you made (10mL) and inject 500uL of it into a 2 mL vial and 1mL of it into a separate 2mL vial.
18. So for the 2mL vials of stds, you have 8 total! 0, 0.5mL, 1mL, 2mL, 5mL, 10.25mL, 10.5mL, 10\_1mL
19. Make sure you turn off all the gas tanks and you’re done!

*Thursday Additions*

Materials:

1. Scale
2. Weigh sheet (to note weight of each flask before and after additions)
3. 1000uL pipette and tips
4. Four 15mL falcon tubes
5. Sterile H2O
6. Sterile 100ug C solution
7. Timer
8. Microcosms!

Methods:

1. Sample gases from flasks first (see section 1: gas sampling for how to do that)
2. Once gas sampling has been completed move your whole production to the weigh station
   1. Pipette/tips
   2. Microcosms – move them via the cart
   3. Solutions – H2O and 100ug C.
      1. Fill 2 falcons tubes about half full with H2O and 2 half full with 100ug C. LABEL THEM!!!! Important not to mix them up, if you’re not sure, make up new falcon tubes.
      2. The reason there are two of each is so there is no potential of transferring 13C carbon into 12C flasks or vice versa, so you have a 12C H2O and a 13C H2O. (Same for 100ug)
   4. Timer and weigh sheets
3. Preweigh flasks and note them on the sheet. I usually do them in groups based on the addition they get:
   1. Group 1: H2O, 12C only, 12C 700
   2. Group 2: 13C only, 13C 700
   3. Group 3: 12C 100 and 13C 100
4. After you have weighed a group, remove their stoppers and turn timer on for 10 minutes.
5. Add 250uL to each flask of their respective addition. When adding the addition try to deliver it as evenly over the soil as possible. 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. But do what works for you, just make it even!
   1. Additions:

|  |  |
| --- | --- |
| Treatment | Addition |
| H2O | 12C H2O |
| 12C only (control) | 12C H2O |
| 13C only (control) | **13C H2O** |
| 12C 700 | 12C H2O |
| 13C 700 | **13C H2O** |
| 12C 100 | 12C 100ug |
| 13C 100 | **13C 100ug** |

* 1. \*\*\*NOTE: When doing the last group (group 3) remember that those treatments within that group get TWO DIFFERENT additions. (technically they’re the same, but different to minimize potential 12C/13C cross contamination)

1. After additions, use house air to air out each flask for 5 secs.
2. Reposition the sampling ports in the stoppers.
   1. Make sure you don’t puncture through the stopper all the way.
3. If the timer is done (10 min has passed) then stopper the flasks again. (make sure they’re tight). If 10mins isn’t up, then wait til the timer alarms. You can start weighing the next group of flasks while you wait.
4. Repeat with groups 2 and 3.
5. Post-weigh all flasks and note on weigh sheet.
6. Place flasks back in their happy cardboard villages.
7. Note what time you finished this, it will be the starting time for subsequent gas sampling.
8. Go have a beer! You’re done!

SUPER DOOPER CHECKER NOTES:

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. Are you sure the stoppers of the flask are tight?
5. Did you do all the clone libraries I needed done?!