N Cycle Experiment Protocol

Soil Sampling

- Materials:
  - 2” Gouge agar
  - Soil knife
  - Plastic bags
  - Gloves
  - Sharpies
  - 70% ethanol
  - Ice in cooler
- Locations & Replicates
  - Coring at each CB Synoptic site: GCrew, MSM, GWI, SWH
  - 3 replicate cores from each zone (Upland – UP, Transition – TR, Wetland – WC)
  - Total cores per site: 9
  - Total cores for experiment: 36
- Field sampling
  - Push gouge agar into the ground, twist, and pull out
  - With gloved hands measure the top 10cm of the core, cut off any extra and push the core into the labeled plastic bag
  - Place the sample on ice in the cooler
  - Clean hands, agar, and knife with paper towel and ethanol
  - Prepare for next sample
  - Use Stevens probe to get temperature and conductivity near coring sites
- Transport
  - Keep samples on ice until you reach the lab
  - Once in the lab, keep in fridge or on ice until ready to weight out the samples

Lab Incubation Protocol:

1. Remove any large roots and homogenize the 10cm core sample
2. Weigh out sediment samples into labeled exetainer tubes (we will do this on the lab bench – not in the anaerobic chamber because the cores are not kept in tubes and these are potential rate measurements)
   - Use Styrofoam holder for tube - be sure to zero/tare the scale with tube to ensure correct amt.
   - Use spatula to scoop sediment - ethanol and flame prior to use and in between samples (between different cores or depth sections)
   - 1g of sediment (within 0.95-1.05g)
   - Cap samples
     - need to be snug but don’t over tighten because exetainers can leave if they are really scrunched up
3. Spin down samples to get all sediment at the bottom of the exetainer
   - Roughly 2000 rpm for about 2 minutes
4. Vacuum the headspace out of the exetainers (2 mins)
5. Flush Headspace in tubes with He 5mins

- Turn on He - turn big knob a few ½ turns; small black knob next to tube loosen slightly
- Be sure manifold is set correctly so that pasture pipette is not going if it is not needed
- Change needles
- Insert He needle into tube 1st then the output needle
- Flush 5 mins
- Take out output needle then He needle
- Adjust He flow so that it is not bubbling too hard
- Turn off He when finished - make sure needles are in black stopper

6. Incubate 24 hours at in situ temperature
    - To get rid of background NO2- and NO3-
    - Re-flush with He halfway through the pre-incubation
7. After incubation for background reflush tubes with He again
    - 5 mins
8. Clear headspace in substrate tubes (15NO3 and ZnCl)
    - use 1mM 15NO3- (10uL of 1M solution in 10mL)
    - Use 50% ZnCl to kill (50g ZnCl in 100mL water)
    - Change inflow needles to long ones and adjust manifold
    - Be careful that the outflow needle is not in the liquid and that the air is not causing too much bubbling
    - He needle in first and out last
    - Flush 3-5 mins
9. Kill T0 with ZnCl
    - Get a 3mL syringe and a needle
    - Switch manifold to have He flow through pasteur pipette
    - Turn on He
    - Flush syringe 2-3x and fill with He
    - Turn off He at the manifold
    - Put needle into ZnCl bottle and purge He
    - Invert bottle and slowly extract ZnCl needed (I fill the syring)
    - Flick needle to get rid of any air bubbles
    - Remove needle
    - Spike needle into sample tube *0.5mL of ZnCl into each sample* - keep pressure on plunger because positive pressure in sample tube may force air into syringe if not careful
    - Dispense needed amount, pull out needle and continue
    - When used all flush out any residual ZnCl in the sink and reflush needle with He before refilling - or throw out needle and syringe
    - VORTEX Sample + ZnCl
10. Spike substrate (15NO3) into ALL sample tubes
    - Confirm sample tubes and substrate match
    - New 1 mL syringe and needle
    - Flush needle/syringe with He in pasteur pipette
    - Stick needle in substrate tube; dispense He
    - Pull needed amount

- Take time; flick out bubbles
- Spike needle into sample tube *typically spike 0.1mL of 1mM 15NO3-*; keep pressure on tube so as to counteract any positive pressure in the sample tube
- Dispense amount needed and continue
- In between refills flush out any residual in the sink and flush with He before refilling
- Change syringe/needle with each substrate
- VORTEX sample + 15NO3

11. Record time
12. Incubate for necessary time (T1 = 2 hours) at in-situ temperature
    - Incubator set to in situ temp
    - Incubation time points:
        - 0, 2, 4, 8, 12, 24, 36 hours roughly – record times
13. Kill T1/TF samples with ZnCl at time point needed - use spike technique
    - Vortex after ZnCl is added
14. Spin down again 2000 rpm for 2 mins

N_2 isotope analysis:

1. In IRMS lab, run daily zeros
    - Check the standard deviation
2. Prepare standards
    - Get 12-15 Helium flushed exetainers
    - Add air to the tubes at the following amounts 0, 5, 10, 20, and 30 uL
    - Place them in the first row of samples in the autosampler
    - Put blanks intermittently throughout the run
3. Prepare the sample table for the IRMS
4. Load samples in the same order as listed in the sample table
5. Start run

NH_4^+ extraction for DNRA run on MIMS from incubation sample:

- We will use all A samples of duplicate incubations
- Add 5mL of 2M KCl to incubation exetainer
    - Shake @ 200rpm for 1 hour
    - Spin down (200 rpm 3-5 mins)
- Pipette off 5mL from exetainer use long skinny pipette
- Dilute 4mL of KCl extract with 22mL MiliQ
- Pore diluted extract into an exetainer and close
    - Freeze remaining extract
- Spike 200 uL of hypobromite solution into exetainer
- Let sit 15mins prior to running on the MIMS

N_2O production from Incubation:

- We will use the B samples of duplicate incubations
- We will extract a small gas volume from the incubation exetainer
- Inject the N2O sample onto the new GC column to get N2O concentration in headspace

Nutrient extractions from incubation sample:

- We will use the remaining extract that was not used for the MIMS
- Prepare standards in 2M KCl for the SEAL
- Run protocol for porewater nutrients but with 2M KCl

Soil Characteristics:

- Soil pH
    - Combine soil and DI water
    - Vortex to mix
    - Read pH with pH meter
- % water, % organics
    - Weight sample into a weigh boat
    - Record boat weight and wet sample weight
    - Put sample in incubator @ 60C for two days
    - Reweigh sample after drying
    - Place samples in combustion oven
        - Combust at 500 C for 4 hours
    - Reweight sample after combustion
    - Calculate %water and % organics
- Bulk density
    - Weight how much sample fills 1mL in a 15mL falcon tube
    - Tare tube, fill to 1mL, weigh
- Extractable nutrients
    - Use 2M KCl extraction
    - 2g of soil 10mL of 2M KCl in a falcon tube
    - Shake at 200 rpm for 1 hour
    - Spin down samples
    - Pour off sample into a syringe and filter into new falcon tube
    - Run these extracts on the SEAL autoanalyzer
- Sediment CHN
    - Weight samples into tins
    - Run on Elemental analyzer

VIMS: Gas Bench II and Delta V IRMS - N2 analysis protocol

Sample prep:

- Samples cannot exceed 1 atm% 15/14N
- Samples should be at room temperature during analysis and tightly sealed (in exetainers)
- Septum surface must be free of moisture and salts use a swab or kimwipe to remove this before coming to the IRMS lab
- Minimize mixing of contents in the exetainer tube to avoid material adhering to the underside of the septum
- Include 10 extra He purged tubes for standards and analysis blanks, plus at least 1 additional purged tube to secure the needle after the run. If running more than 30 samples add a few more purged tubes for check standards

Instrument:

- Check gas tanks: UPC He (5.0), Zero Air and UHP Nitrogen
    - None should be below 200 psi and output at tank regulators should be set to 60 psi
- Check the He regulator on the front of the GB II
    - Should be set between 0.9 mBar
    - Reference 3: Nitrogen, should be at 2 mBar

Software – Isodat Acquisition (Acqu) page:

- 'MS' window – check that the source in on: HV is ~3.0 kV and 28,29,30 amu <300
    - Vac ~ 1.2 e-006 (should no vary by more than 0.1)
    - When baseline equilibrium is reached 28 and 29 amu will be less than 10mV and 30 amu <300.
    - Check Box and Trap voltage – values add up to 1.5 and should be almost the same (~0.70) but okay if either is less than 0.82
- Place sample needle in a fresh blank He-evacuated tube
    - Ensure that the top fitting of the needle (where the capillary and SS He line emerge) is hand tight onto the top of the knurled nut assembly
    - Then Start 5 N2+ Zero runs form the N2+Zeros_5 "Sequence" file in the "File Browser" window (lower left of Acqu page). When the s.d. for del 15N is 0.06, stop and autofocus.

Autofocus (after at least 3 N2+ Zeros have run)

- Open Ref 3 valve – click on the bar next to "Ref 3" in 'GasBench' window
- After 28 and 29 have reached steady state (about 5s) open the 'Focus Delta' window then right click anywhere in it and press "Autofocus"
- At the bottom of the main Acqu window is a status bar showing the elapsed % of the autofocus

    - When complete right click in the blank area of the ‘Focus Delta’ window and choose “Pass to Gas Configuration”; close this window ******close reference 3*********
- Run at least 1 more N2+Zeros:
- Left click on the d15/14N column from the Acqu graph generated for the last N2+Zero run to highlight the entire column, then right click and choose calculate
    - A stat window pops up and includes S.D. – if 0.06 or less, okay to run samples
    - IF NOT – see Michele Cochran – first double check that the needle is in a fresh tube and gas pressures are correct. If not, repeat the above
- Record the appropriate data in the Excel file: Gas Bench IRMS Log Sheet – located on the desktop

Sample Run:

- Set up a Sequence table with the appropriate AS #, sample i.d. (identifier1 and 2) and method fields – save the file. Include your initials in the file name and use this Sequence file as a template for future runs. A sequence file can also be made in Excel and copy and pasted into an isodat sequence file.
- Runs should include duplicates of blanks and 5, 10, 20 and 30 uL air standards with one set run at the beginning and one at the end with a blank and check standard included for every 30 samples
- It may be necessary to add an additional blank between every 6 to 10 samples (variable depending on carryover)
- Place samples in the Auto-sampler tray, SECURE the top plate and tighten the 2 nuts – DO NOT leave loose; do not use positions 96 thru 100
- Once N2+ Zeros are complete and the SD is <0.06 and not trending, remove the needle from the blank tube and secure in the AS carriage arm and hand tighten the knurled lock-down nuts.
    - Check that the SS needle is not bent
    - Loop the capillary and SS He lines from the needle through the top plastic guide and snap the guide shut one click
- Start the run in the Sequ – changing anything necessary in the isodat object window that pops up – see following paragraph. Immediately return to the AS and observe that the needle is penetrating close to the middle of the septum of the first blank and not near the plastic cap

Isodat Object Window:

- There are many options but typically the fields to fill in are:
- Folder Name – Post – Date – fill in name field (usually your initials and project name)
- File Name – Post – Identifier 1

- Export – Format – choose <None>
- Data will be re-processed after a run ends

Data Retrieval:

- Data files can be viewed on the Isodat Workspace page under the Results tab at any time

Post Sample Run:

- Remove the GB needle tubing from the autosampler clip. Loosen the bottom knurled nut at the top of the needle. Lift out the needle form the housing and place in a clean He-evacuated tube
- Remove all sample tubes/racks and wipe up area around the autosampler between runs