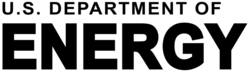
**Molecular Observation Network**

*Standardized Laboratory Protocols*

Environmental Molecular Sciences Laboratory

****United States Department of Energy



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# Core Receiving and Processing

## Summary:

This protocol details the proper procedure for receiving and processing fresh soil cores for the Molecular Observation Network (MONet). All samples are collected using the standardized field sampling protocol and sampling kit provided by MONet. Samples are shipped overnight, and core processing begins immediately upon arrival.

**Materials:**

* Site Cores (Cores A, B, C1-4)
* Core Liner Cutter
* Soil Core Holder
* Aluminum Foil
* 70% ethanol
* Kimwipes
* Gloves
* Bench Scraper
* Ruler/Measuring tape in cm
* Sharpies/writing utensils
* 4 mm sieves and base
* Scoopulas
* 50 mL conical tubes (replicates for both top and bottom for each core labelled: siteID\_core#\_depth\_replicate\_analysis, e.g., 60881\_1\_TOP\_1\_PEXT)
  + **pH** – 1 replicate
  + **Phosphorous Extraction (PEXT)** – 3 replicates for both top & bottom
  + **Nitrogen Extraction (NEXT)** – 3 replicates for both top & bottom
  + **Microbial Biomass (MicB)** – 3 replicates for both top & bottom (additionally MicB\_BACK and MicB\_FUM)
  + **Water-Extractable Organic Matter (WEOM)**– 3 replicates for both top & bottom
* 15 mL conical tubes (replicates for both top and bottom for each core labelled: siteID\_core#\_depth\_replicate\_analysis, e.g., 60881\_1\_TOP\_1\_MAOM)
  + **Mineral Associated Organic Matter (MAOM)** – 3 replicates for both top & bottom
* Whirlpak bags
  + **DNA**- 2 per core (top and bottom)
* Large aluminum pie tins – 3 per core (top, middle, bottom)
* Small aluminum weigh boats
  + **GWC** – 6 per core (3 replicates for both top and bottom)
* Large weigh boats
  + **Respiration** – 2 per core (top and bottom)
  + **Removed mass** – 2 per core (top and bottom)
* Balance

## Protocol:

1. Check there are cores labeled Core A, Core B, and Cores C1-4 included in the sample cooler. Make sure all cores are labeled with the proper site codes, core numbers, and top/bottom.
2. Place Core A back into the site bag and store in the MONet refrigerator in 1206 at 4C. All following steps pertain to Core B and C1-4.
3. Spray table with a 70% EtOH solution and wipe down using Kimwipes.
   1. Keep each core in its own dedicated area/table.
4. Wearing gloves, tear two large sections of foil (~2.5 ft in length) and place them overlapping by half onto the cleaned tabletop. Spray surface of foil using 70% EtOH solution.
5. Using a permanent marker write the site code, core number, and indicate the top and bottom of the core on the foil. Carefully remove the caps off the B core.
   1. If the soil is unstable (does not hold its shape) leave the bottom cap on.
6. Put a piece of aluminum foil into the soil core holder, sanitizing it with 70% EtOH before laying the B core down. Align the 2 small blades on the core liner cutter with the lip of the core liner. Pull down firmly, ensuring both blades are properly hooked on and cutting the liner.
7. Once halfway cut, wrap the Velcro strap around the previously cut top of the core to prevent the core from lifting as the bottom of the liner is cut.
   1. If bottom cap is left on, stop the core liner cutter before reaching the cap. Do not cut the core cap.
   2. Discard the removed piece of core liner.
8. After the liner is cut. Place the core on the foil horizontally ensuring the top and bottom are correctly labelled on the foil.
   1. If applicable, carefully remove bottom cap, use caution to minimize disturbance of the soil.
   2. Use a box cutter to carefully score and cut the remaining distance down the liner until the cut section can be removed and discarded.
9. Leaving the core in the remaining liner, record the length of the core in centimeters and write it on the foil. Take photographs of the soil core with a measuring tape making sure to include the site label, core number, top/bottom, and length in the photo.
10. **All the following data is to be recorded in the Processing Mastersheet on the MONet Google Drive.**
11. Slice and section the core 10 cm from the top and 10 cm from the bottom. Record the depth range for each section and the total length of the core. Record the depth of all four C cores and combine C1-C4 soil with the top 10 cm section of the core.
12. Take each section through the Bulk Density Protocol below.
13. Using 70% EtOH wipe out clean (rinsed with water) 4 mm sieve. Stacking the sieve on top of the lower pan scrape each section (top and bottom) into its own clean and labeled sieve.
    1. **The middle section does not get sieved. Place the middle section into a labelled pie tin to be weighed later.**
14. Gently shake and stir (with a clean sterilized gloved hand) the soil in the sieve being careful not to force soil through sieve.
    1. If the soils have a high clay content, you may need to use a sterilized paint brush to break up the particles using a stippling motion (vertical tapping).
    2. Break up large aggregates between fingers and continue sieving.
15. Once sieving is completed, collect all items (such as rocks and roots) remaining on the sieve into a large weigh boat. Record the mass removed from each section.
16. Dump each lower sieve pan into a corresponding labelled aluminum pie tin. Tare out a scale with the weight of a pie tin, then weigh each section before aliquoting, recording the mass.
17. To measure gravimetric water content (GWC) on the top and bottom core sections, record the mass of the empty tin and the mass of the tin + 10 g of soil. Place the tins in the 60 C degree oven for 48-72 hours or a 100 C degree oven for 24 hours. GWC is collected in triplicate, ensure the tins are properly labelled (SiteID\_Core#\_depth\_replicate)
    1. Make sure to record the wet mass and dry mass (after 24 or 48 hours).
18. Collect a minimum of 10 g of soil for DNA into a whirlpak bag for the top and bottom sections. These samples will be labelled with the site code, core number, depth, and “DNA”.
19. Collect 20g (within 0.1 g) of soil for pH into a 50 mL falcon tube for the top and bottom sections.
20. Collect ~12g of soil for phosphorus extraction into a 50 mL falcon tube for the top and bottom sections.
    1. Following pH measurement, phosphorous extraction will occur as follows:
       1. pH less than 7.4 will be extracted using the Bray Method
       2. pH greater than 7.4 will be extracted using the Olsen Method
21. To measure microbial biomass and N extractions, collect exactly 8 g (within 0.02 g) of soil into properly labelled 50mL falcon tubes.
22. Collect 6g of soil into 50 mL falcon tubes for WEOM extraction.
23. Collect 0.25g of soil into 15 mL falcon tubes for MAOM extraction.
24. Collect 50g of soil into a large weigh boat for Respiration analysis.
25. The remaining sieved soil will be air dried in the BSC hood.
26. Store samples in the appropriate location:
    1. Store the DNA samples in the plastic container in the –80C freezer.
    2. Refrigerate the pH, Phosphorous, WEOM, MAOM, and Microbial Biomass samples until extractions can be conducted.
    3. Place GWC tins in the drying oven.
    4. Respiration aliquots should be placed into the VELP flasks to begin analysis immediately.
    5. Dry the remaining soils in the BSC hood for 48 to 72 hours or until dry.

# Bulk Density

## Summary:

Bulk Density provides information on the mass of fresh soil occupying a known volume. This method requires measuring the height of the soil core, weighing fresh soil, and calculating bulk density.

## Materials:

* Fresh soil
* Measuring tape in cm
* Balance
* Large aluminum pie tins

## Protocol:

1. Record the mass of the empty labeled tins.
2. Take the combined soil from the top section and C1-4, and soil from the bottom section, and add them to their respective empty labeled tins.
3. Record the mass of both and subtract the empty tin weigh to get a total mass of the fresh soil.
4. Use the height measurements of each section and the radius of the core to calculate the core volume. Combine the calculated volume of the top section and the C1-4 cores.
5. After GWC data is collected, convert fresh mass to dry mass using GWC percentage.
6. Divide the total dry mass by the volume it occupied to get a final bulk density in g/cm3.
7. Record all values in the processing mastersheet.

# Gravimetric Water Content (GWC)

## Summary:

Gravimetric water content (GWC) provides insight into the amount of water in a soil sample. This method requires weighing fresh soil, oven drying it at 100 degrees Celsius, and then reweighing the dry soil.

## Materials:

* Small aluminum weigh boats (3 per site and depth)
* Soil (~30 g per site and depth)
* Balance
* Drying oven set to 100 degrees Celsius

## Protocol:

1. Record the mass of the empty labeled tin in the processing mastersheet in the MONet Google Drive. Without taring the weight of the tin, add 10g of soil and record the mass of the tin and soil.
2. Place the soils in the drying oven for 24 hours at 100C.
3. After 24 hours, remove samples from drying oven and allow to cool. Record the final mass of the soil and tin in the processing mastersheet.
4. Discard remaining sample and tin.

# Soil pH

**(Based on OSU Soil Health Lab methods)**

## Summary:

Soil pH can be measured at either a 1:1 or 1:2 soil to water ratio. Because of the use of the log scale, the two ratios typically provide similar results. A soil with high clay or organic matter may require a 1:2 or even 1:5 ratio to allow for enough fluid to make a measurement. With either soil to water ratio, the soil and water are placed in a tube and shaken for 15 minutes on a reciprocating shaker to achieve soil solution equilibrium. A calibrated probe accounting for temperature is used for the measurement. One CAL standard sample is included in each batch.

## Materials:

* pH and temperature probe
* 50 mL centrifuge tube
* Reciprocating shaker
* Balance
* pH calibration standards (4.01, 7.01, 10.01)
* DI water (or other solution as requested)
* Beaker for waste

## Protocol:

1. Measure 20 ± 0.1 g soil into 50 mL centrifuge tube (this should be done in the core processing step)
2. Add 20 mL of DI water
3. Place on reciprocating shaker for 15 minutes at 1000 RPM
4. Rinse the pH probe well with DI water and gently pat dry with a Kimwipe.
5. Check the calibration of the pH meter using the pH 4.01 and 7 standard. If either of the pH values differ from the known value calibrate the pH probe.
   1. Calibrate the pH meter with a minimum of two standards that bracket the potential sample results according to the instructions on the screen.
   2. The probe should be calibrated using standards in the same type of vessel as the samples will be measured in (50 mL centrifuge tubes).
6. Insert rinsed pH/temperature probe combo into the sample.
   1. Be careful to keep the probes still and not against the wall or bottom of the vessels.
   2. Make sure the diaphragm is submerged in suspension.
   3. Record results when the numbers stabilize onto the processing mastersheet in the MONet Google Drive.
7. Between measurements, rinse the probe well with DI water over the beaker and gently pat dry the probe with a Kimwipe.
8. When not measuring, make sure that the pH probe is rinsed and fully submerged in 3M KCl storage solution. After measuring the pH, discard the soil water slurry into autoclave waste.

## References:

OSU Soil Health Lab pH Protocol: <https://cropandsoil.oregonstate.edu/shl/methods-and-equipment>

# Extractable Phosphorus

## Summary:

Following soil pH, soils will be extracted for phosphorus using Bray extraction (soil pH less than 7.4) or Olsen extraction (soil pH greater than or equal to 7.4). Measurement of phosphorus concentrations are outsourced to KUO Testing Labs.

## Materials:

* 50 mL polypropylene centrifuge tube (3 per site and depth)
* ~12 g of fresh soil
* Bray extract solution **OR** Olsen extract solution
* Centrifuge
* Reciprocating shaker
* 0.45 μm pre-rinsed Whatman PES syringe filters (filter rinsing protocol in WEOM Sample Prep and Filtering Protocol)
* 20 mL syringe

## Protocol:

## Bray Solution:

1. 50mL of .5M HCl
   1. 20.85 mL of 12M (37%) HCl in 500mL of MilliQ (DI works too) for .5M HCl, 41.7 mL of 12M (37%) HCl in 1L of MilliQ for .5M HCl
2. 30mL of 1M NH4F
   1. 18.52 g NH4F, bring to 500 ml using MilliQ water or DI for 1M NH4F
3. 50 ml of 0.5M HCl and 30 ml of 1M NH4F to ~900 ml DI
4. Adjust pH to 2.60 with dilute HCl
5. Bring total solution volume to 1L

**Bray Extraction:**

1. Weigh out 2 grams of soil into labeled 50 mL centrifuge tubes.
   1. siteID\_core#\_depth\_replicate\_PEXT
2. Add 14 mL of Bray Extract solution using the dispensette.
3. Cap the samples and shake to mix. Add samples to foam block on shaker.
4. Shake samples at 1000 RPM for 5 minutes.
5. Centrifuge samples for 20 minutes at 3300 RCF.
6. Pour off liquid into a clean and labelled 50 mL centrifuge tube.
7. Follow sample filtering protocol below.
8. Freeze samples at -20 °C on their side to prevent tube breaking.

## Olsen Solution:

1. Prepare 1L extraction solution
   1. Dissolve 42.01 g NaHCO3 (sodium bicarbonate) in ~900 ml MilliQ or DI
   2. Adjust pH to 8.5 with 2M NaOH (39.997 g in 500mL MilliQ (or DI)) (difficult to do because sodium bicarb is a buffer, may need to add a lot of the base)
   3. Bring total solution volume to 1L

## Olsen Extraction:

1. Weigh out 3 grams of soil into labeled 50 mL centrifuge tubes.
   1. siteID\_core#\_depth\_replicate\_PEXT
2. Add 20 mL of Olsen Extract solution using the Dispensette.
3. Cap the samples and shake to mix. Add samples to foam block on shaker.
4. Shake samples at 1000 RPM for 5 minutes.
5. Centrifuge samples 20 minutes at 3300 RCF.
6. Pour off liquid into a clean and labelled 50 mL centrifuge tube.
7. Freeze samples at -20 °C on their side to prevent tube breaking.
8. Follow sample filtering protocol below when ready to filter extracts.

**Bray and Olsen Filtering Protocol:**

1. Using a new, clean 20 mL syringe for each triplicate, remove the plunger and attach a pre-rinsed 0.45 μm filter.
2. Pour sample from the 50 mL centrifuge tube into the syringe with the filter attached, insert the plunger just enough to invert the syringe without spilling sample, remove the filter (without touching the tip) and push excess air out of the syringe.
3. Replace the filter back on the syringe and press the sample through the filter into the labelled centrifuge tubes for analysis.
4. Store at -20 °C until ready to send for outsourced analysis.

## References:

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Watanabe, F.S., and S.R. Olsen. 1965. Test of an ascorbic acid method for determining phosphorus in water and NaHCO3 extracts from soils. Soil Sci. Soc. Am. Proc. 29:677-678.

# Water Extractable Organic Matter

## Summary:

Water extractable organic matter (WEOM) represents the fraction of organic carbon that is typically available to microbial communities. Fresh soils are shaken with MilliQ water (1:5 w/v ratio) and filtered, and WEOC is analyzed as non-purgeable organic carbon (NPOC).

## Materials:

* Genesee Scientific Centrifuge Tubes 50 mL (3 per sample)
* Genesee Scientific Centrifuge Tubes 15 mL (3 per sample)
* Field moist soil
* MilliQ water
* iBrandTech Dispensette
* Orbital shaker
* Centrifuge
* 0.45 μm polyether sulfone (PES) syringe filters
* 60 mL syringe
* 20 mL syringe

## Sample Extraction:

1. Weigh out 6 grams of field moist soil in triplicate into a labeled 50 mL falcon tube
2. Add 30 mL of Ultrapure DI (maintaining a 1:5 w/v ratio) using the Dispensette
3. Place samples onto orbital shaker for 2 hours at 200 rpm
4. Once completed, centrifuge samples at 3300 RCF for 20 minutes
5. Pour off 10mL into the 15 mL centrifuge tube for FT-ICR-MS
6. Filter the remaining supernatant using a 0.45 μm PES Syringe Filter for TOC/TN.
7. Freeze the FT-ICR-MS and TOC/TN sample on the side at –20 °C until analysis

## Sample Filtering (TOC/TN)

1. Using a new clean 60 mL syringe, pull up 40 mL up Ultrapure DI water.
2. Attach a new 0.45 um filter to the syringe and press the water through the filter to rinse the filter.
   1. Steps 1 & 2 can be done in batches, using the same clean syringe for all filters.
3. Using a new, clean 20 mL syringe for each triplicate, remove the plunger and attach a pre-rinsed 0.45 um filter.
4. Pour sample from the 50 mL centrifuge tube into the syringe with the filter attached, insert the plunger just enough to invert the syringe without spilling sample, remove the filter (without touching the tip) and push excess air out of the syringe.
5. Replace the filter back on the syringe and press the sample through the filter into the respective centrifuge tubes for analysis.
   1. The same 20 mL syringe and 0.45 um filter can be used within triplicate samples.
   2. If the filter becomes difficult to press sample through, attach a new pre-rinsed filter and continue filtering.
6. Store filtered samples in the freezer prior to analyses.
7. NPOC analysis is performed on the filtered samples.

# Mineral Associated Organic Matter (MAOM)

## Summary:

Mineral-associated organic matter (MAOM) pool represents the organic fraction bound to iron minerals. MAOM is extracted by mineral dissolution with 0.5 M HCl. The MAOM extracts are analyzed for TOC and TDN concentrations, as well as molecular characterization using FT-ICR-MS.

## Materials:

* 0.5 M HCl
* Fresh soil
* Orbital shaker
* 15 mL centrifuge tubes
* 0.45 μm pre-rinsed syringe filters (filter rinsing protocol in WEOM Sample Prep and Filtering Protocol)

## Protocol:

1. Weigh 0.25 g fresh soil in 15 mL centrifuge tubes and mix with 10mL of 0.5M HCl (1:40 w/v ratio).
2. Shake for 1 hour at 200 rpm on an orbital shaker.
3. Centrifuge at room temperature for 20 min at 3300 RCF.
4. Filter supernatant using a pre rinsed 0.45 μm syringe filter.
   1. 6 mL of sample poured into 15 mL centrifuge tube for FT-ICR-MS analysis.
   2. Remaining sample filtered into 15 mL centrifuge tube for TOC/TN analysis.
5. Filtered samples are stored at -20 °C until analysis

# Microbial Biomass Carbon and Nitrogen

(chloroform fumigation direct extraction [CFDE])

Modified from: Hofmockel protocol (Modified from Suding Lab protocol, modified from S. E. Hobbie, 5 May 1998)

## Summary:

Extracting soils following the sequential microbial biomass protocol allows quantification of both active and inactive biomass in a sample. All salt extractable organic Carbon is removed in the beginning background extraction. The soil pellet then has liquid chloroform added to lyse all biomass. A salt extraction is then performed again, and TOC values of the extract are processed into Microbial Biomass Carbon and Nitrogen. Nitrogen extracts are taken from the background sample and outsourced for analysis.

## Materials:

* 0.5M K2SO4 (87.13g K2SO4 per 1L extractant made in ultrapure water) (CAS# 7778-80-5)
  + Always test background concentrations of new lots of K2SO4!
* Buchner Funnel
* Ethanol cleaned forceps
* Whatman No.42 filter paper, pre-leached 3x with 0.5M K2SO4 (0.5M) and 1x with ultrapure water; (Whatman Part#1442-047)
  + See Rinsing Filters section below
* Vacuum Pump
* 50mL centrifuge tubes
* Centrifuge
* BrandTech Dispensette
* Orbital Shaker
* Acid washed polypropylene funnels
* Ethanol-free chloroform (for analysis of carbon) (CAS# 67-66-3)
* Black garbage bag
* Viton/Butyl Gloves

## Protocol:

*Subsample soil:*

1. Subsample 8±0.1g into labelled 50 mL centrifuge tubes (siteid\_core#\_depth\_rep\_MicB)
   1. Gravimetric water content will also be essential for final calculations on a dry weight basis.

*Data to collect:*

1. Weight of soil for each sample on the processing mastersheet
2. Time onto and off the shaker 1st extraction (by batch)
3. Time of chloroform addition (by batch)
4. Time of chloroform evacuation (by batch)
5. Time onto and off the shaker 2nd extraction (by batch)

*Rinsing Filters:*

1. Gather the vacuum pump, Erlenmeyer filter flask, number of filters needed, K2SO4, Nano pure water, Buchner funnel, and tweezers.
2. Set up a tray with bench paper. Wipe the plastic side of the bench paper with ethanol.
3. Set up vacuum, Erlenmeyer flask, and Buchner funnel.
4. Place a stack of 5-8 Filters in the funnel.
5. Pour enough DI water into the funnel to cover the filters. Turn on the vacuum pump. Wait a few seconds after the funnel appears to be empty to ensure all liquid is pulled thought (you will hear the vacuum noise change).
6. Once the water has been pulled through, repeat step 5 with K2SO4, rinsing filters with K2SO4 3 times.
7. Turn off the vacuum and use tweezers to carefully remove the filters.
8. Spread the filters out on the tray with the clean bench paper. Place in the incubated shaker at 30C (0 rpm) until dry. Once dry place rinsed filters into a bag to be used for the extraction.

*Extraction:*

* 1. Work in batches (when possible randomized experimental treatments among batches, but do not mix isotopically labelled and natural abundance samples in order to prevent cross contamination)
  2. Include multiple (minimum 3 per day) empty 50mL conical tubes. Carry these through the entire process as blanks.
  3. Add 24 mL K2SO4 to subsample using the dispensette. Cap well.
  4. Place on shaker for 2 hours at 200 rpm/low at room temp (20°C)
  5. Balance samples and centrifuge @ 3300 RCF for 20 minutes.
  6. Place funnels in support racks and insert a pre-leached filter in each funnel.
  7. Remove sample from centrifuge and place “BACK” centrifuge tubes with matching site, core, and depth below funnel.
  8. Pour supernatant into funnel, take care to check that filtrate is dripping directly into the tube.
  9. Once supernatant is filtered through, use ethanol clean forceps to transfer filter paper back into conical tube with soil pellet.

1. From the “BACK” centrifuge tube, pour approximately 10 mL into the “NEXT” centrifuge tube with matching site, core, and depth.
2. Store both “BACK” and “NEXT” extracts at -20°C, the tubes containing the soil pellet and filter paper move onto the fumigation step.
3. Rinse and acid wash funnels for next extraction.

**FUMIGATIONS MUST BE DONE IN THE FUME HOOD**

**\*CHLOROFORM IS A KNOWN CARCINOGEN\***

**Wear Viton/Butyl gloves with disposable nitrile gloves inside during steps 15-18. Always wear a lab coat and safety glasses with side guards when working with chloroform.**

1. Add 2mL chloroform to soil pellet and filter.
2. Add additional labeling to tube and cap if needed, chloroform will cause ink to run if it comes in contact with ink
3. Let sit in the dark in the hood for 24 hours: cover the tube racks with a black garbage bag (darkness prevents the chloroform from breaking down).
4. After 14 hours, remove garbage bags and caps and allow to vent 46 hours. Be sure to keep track of which cap goes to each tube.
5. Note: Be sure to place notice on the fume hood that chloroform containing samples are inside
6. Extract the sample as above: as per steps #1-11.
7. The filter from the second extraction can be discarded.

a) For all experiments leveraging isotopic labeling the leftover soil pellets should be archived at 20°C. This includes 1abeled samples and the unlabeled controls.

b) For experiments not requiring the pellet: At the end of the extraction open all tubes inside the fume hood and leave to dry completely to remove any trace of chloroform before disposing of tubes containing filter and soil.

## References:

S. E. Hobbie, (1998). Chloroform Fumigation Direct Extraction (CFDE) Protocol for Microbial Biomass Carbon and Nitrogen. Retrieved from <https://web.stanf>

# Shimadzu TOC/TN Protocol

**Sample preparation and machine instructions**

## Summary:

The Shimadzu Total Organic Carbon/Total Nitrogen (TOC/TN) unit allows quantification of Carbon and Nitrogen content of a liquid sample. After combusting a sample at 720 Celsius on a platinum catalyst, the gas is passed through an infrared detector to measure Carbon content, and then through a chemiluminescent Nitrogen detector.

1. **Preparing Samples** 
   * Sample volume should be at least 5 mL (sufficient for three injections)
   * When running salt solutions, make sure the salt concentration is less than 10g/L (i.e., > 10 times dilution for 0.5 M K2SO4).
2. **Running standard curves and check standards**

* Use the NPOC and TN check standard curves for MONet samples. See recipes below for check standard preparation.
* Best practice is to run a standard curve regularly to ensure stable measurements. Also disperse 25/10 standards paired with a water every 6 to 10 samples. If the check standards vary by more than ~5%, or if a series of check standards show consistent machine drift, consider re-running the standard curve.

1. **Running the machine**

* A water reservoir is located behind the autosampler, use only ultra-pure water to fill it.
* A waste container is located underneath the TOC, check before each run and inform Tom Wietsma if it is full.
* Ensure the humidifier inside the door of the TOC is filled to the line with ultra-pure water.
* Check the PSI of the Zero Air canister in the service corridor. If it is lower than 500psi, notify Tom Wietsma.

1. **Starting a run**
   * Open the TOC-L Sample Table Editor. Select “New” and the “TOC\_TNb Standard” method.
   * To ensure consistent naming between runs, it is best to copy previous runs and change necessary factors (site ID & core number, dilution values, etc.).
   * To insert new samples, right click on the row header and select “Insert-Sample” or “Insert-Multiple Samples.” Select the proper method. For MONet runs, use the “NPOC\_TN\_50\_20.met” located in the 1000 Soils folder.
   * Adjust the number of samples and sample name and select finish.
   * To adjust the dilution, right click on the row header and select “Measurement Settings,” enter in the proper value and select “OK” to save the setting.
   * Double check that all samples have the correct dilution value, name, and method.
   * To assign vial numbers, click the birthday cake icon. For MONet samples, all 25/10 check standards should be assigned to vial 0. Double check that the vial numbers correspond with the samples.
   * Connect the sample sheet to the instrument by selecting “Connect.”
   * Once the furnace is heated up, the “Start” option will become available.
2. **Exporting your data**
   * On the measurement sheet, select “File, “Ascii Export,” and “Normal.”
   * Select the MONet Z drive and download the simple data into the appropriate folder.
   * Follow the same process for downloading the detailed data, after Ascii Export, select “Detailed.”
   * Ensure the file name follows the proper format for the analysis.
3. **Notes and Cautions** 
   * Ensure all of the items under the “Running the machine” header are completed before starting the instrument.
   * Double check that the tin foil wrapping around each sample vial is secure.
   * Ensure the straw in vial position 0 is at the bottom of the 25/10 standard 1L bottle.

## Calibration Curve:

Make 20mL vials of each standard listed in the table below. Pipette two 9mL vials for each standard listed in the table below (NPOC and TN calibration).

Stock Standard 500/200: 20mL of 1000ppm Carbon Stock + 8mL of 1000ppm Nitrogen Stock +12mL of ultra-pure water.

|  |  |  |  |
| --- | --- | --- | --- |
| **Final C ppm** | **Final N ppm** | Ultra-pure Water (**mL**) | Stock (**mL**) |
| 50 ppm | 20ppm | 18 | 2 |
| 37.5 ppm | 15 ppm | 18.5 | 1.5 |
| 25 ppm | 10 ppm | 19 | 1 |
| 12.5 ppm | 5 ppm | 19.5 | 0.5 |
| 5 ppm | 2 ppm | 18 | 2 |
| 2.5 ppm | 1 ppm | 19 | 1 |
| 1.25 ppm | 0.5 ppm | 19.5 | 0.5 |
| 0.5 ppm | 0.2 ppm | 18 | 2 |

## Check Standards:

Make 1L of 25/10 check standard to place in vial 0 during MONet runs.

25mL 1000ppm Carbon Stock + 10mL 1000ppm Nitrogen Stock

## Sample preparation:

The sample tray can hold 93 vials.

1. Thaw samples in shaker: 30ºC, for Microbial Biomass samples shake at 200rpm for 2 hours.
   1. Always thaw paired samples (e.g., 1\_background and 1\_fumigated, 2\_background and 2\_fumigated, etc.)
   2. Always include sample blanks
2. Dispense at least 5 mL of sample in each Fisherbrand 9mL sample vial.
   1. If dilution is necessary, be sure to adjust the manual dilution value when entering in the sample table editor.
   2. Microbial Biomass follow a 10x dilution: 0.7mL of sample + 6.3mL of ultra-pure water.
      1. Microbial Biomass also requires a 0.5M HCl rinse before check standards. Pipette at least 5mL into a Fisherbrand 9mL sample vial.
3. Sample Positions
   1. For WEOM and MAOM runs, place a water followed by a 25/10 check standard sample between each new core number.
   2. For Microbial Biomass runs, place 0.5M HCl, water, and 25/10 check standard between every 6-10 samples.

# Respiration

**(Soil)**

## Summary:

Soil respiration is measured using the VELP RESPIROMETRIC Sensor 6 System. The RESPIROMETRIC Sensor System allows for the quantification of gases produced during the aerobic degradation of organic material in a sealed chamber. Carbon Dioxide (CO2) reacts with Potassium Hydroxide (KOH) in the alkali collector to produce Potassium Carbonate (K2CO3) and water (H2O). As the reaction takes place, the total gas in the respirometer flask decreases, which is monitored by a pressure transducer in the sensor head. Soil respiration is measured as Biological Oxygen Demand (BOD) and converted to CO2. Respiration incubations occur at 21 degrees Celsius over the course of 5 days, with measurements taking place every 2 hours.

## Materials:

* KOH flakes
* Fresh Soil
* Velp Respirometer
* 1 M HCl
* Milli-Q or DI H2O
* Temperature controlled chamber

## Protocol:

\*Always use acid washed glassware when conducting respiration measurements

1. Weigh 50 g +/- 1g of fresh soil (4mm sieved) into a weigh boat.
2. Carefully transfer the soil into an acid-washed respirometer flask.
3. Add 6 KOH pellets to the alkali collector.
4. Clear the previous run’s data from the respirometer sensor unit by holding down both set and start for 10 seconds.
5. Attach the sensor unit to the alkali collector/flask cap.
6. Place the assembled sensor unit on the flask, without screwing it down, to complete the ‘respirometer’. Leave it unscrewed to allow gas exchange until temperature acclimation is complete.
7. Place the respirometer in the temperature-controlled chamber at 21C for three hours.
8. *Software Preparation:*
9. Launch the Velp software and enter password ‘velp’.
10. Start new analysis File-> New
11. Connect the databox to the PC by clicking service -> Connect databox
12. Click ‘sensor number’ to select a sensor to pair.
13. Fill in sample name, volume (50), analysis (BOD), scale (4000), and duration (5 days).
14. Wait for the 2 hour acclimation to finish.

*Starting Measurement:*

1. Tighten down the sensor unit assembly to the respirometer flask finger tight after it has acclimated for 2 hours (overtightening can cause the seal to break).
2. Press the ‘set’ button to ensure the sensor is in wireless mode. Brackets will appear if in wireless mode. Consult the manual to switch if needed.
3. To start the measurement, click ‘set’ then ‘start’ one after another rapidly. A small white dot will blink on the sensor unit LED screen.
4. Verify the measurement has started. The LED screen on the sensor unit will show ‘on’ if successful. In the software, the ‘unlocked’ icon on the furthest left side of the spreadsheet will change to a ‘lock’ and then a green ‘play’ symbol.
5. Allow measurement cycle to complete.
6. *Data Export:*
7. If missing some data during the run, click ‘set’ twice to send stored data in the sensor unit to the databox.
8. To export data, click a sample, then ‘Report -> Export Data”
9. Save the raw data file.
10. Run R script to plot the data.
11. *Clean up:*
12. Rinse the soil from the respirometer out in a sink with a sediment trap installed.
13. Rinse the glassware and alkali collector with Milli-Q or DI water. Do **not** wet the sensor unit.
14. Acid-wash the flask with 1 M HCL. Pour 250 mL HCL solution into the flask, cap the flask with a regular bottle lid, and shake vigorously 30 seconds. Replace rinse when solution becomes turbid. (HCL rinse located in the flame cabinet in 1206, labelled ‘respirometer wash’).
15. Rinse the glassware 10 times in Milli-Q or DI water.

## References

1. Goldsmith, J., Coburn, J., Neulicht, R., & Bronstein, K. E. (2010). Greenhouse gas emissions estimation methodologies for biogenic emissions from selected source categories: Solid waste disposal, wastewater treatment, ethanol fermentation. U.S Environmental Protection Agency.

2) Velp Respirometer Documents can be found at:

]<https://www.velp.com/en-us/respirometric-sensor-system-for-soil-analysis.aspx>

# Gilson Automated SPE Protocol

## Summary:

Solid Phase Extraction (SPE) is used as a means to rid soil extracts of excess salts and minerals that can interfere with FTICR-MS analysis and may cause ion suppression. SPE is also performed to help concentrate extracted organic matter in lower carbon samples and increase the quality of FTICR-MS data. SPE is automated, using a Gilson GX-274 liquid handler.

## Materials:

* Gilson GX-274 liquid handler
* Culture Tubes
* 2 mL Vials
* 2mL Vial Caps

## System Preparation:

1. Fill one 700mL solvent bottle with Optima LC/MS-grade MeOH and place in the first slot for a solvent bottle.
2. Fill three 700mL solvent bottles with 10mM HCl and place in remaining three solvent bottle slots.
3. Fill all four 1L solvent bottles attached to the solvent pumps with MilliQ H2O.
4. Open "Trilution Liquid Handling" software and open "Applications" tab.
5. Open "Manual" tab and prime solvent pumps.
6. Switch tabs back and select "PNNL Run" from the left side bar in the "Applications" tab.
7. Select method from pull down menu (Either 15mL Rinse or 50mL Rinse)
8. Select "Batch" in the "Mode" tab
9. Enter number of samples in the "#Wells" tab as follows: 1-X# of samples (up to 40 samples)
10. Enter "1-4" in the "#Reservoirs" tab.
11. Place pre-labeled SPE cartridges in their corresponding slots in the movable trays in the center of the instrument and place glass culture tubes in non-movable tray underneath moveable tray.
12. Place pre-labeled 2mL glass Microsolv vials in corresponding slots in right-hand vial tray.

## Sample Preparation:

1. Using the SPE Dilution Sheet for FTICR, dilute samples into 15mL conical tubes with ultra-pure water to standardize NPOC values of each sample.
2. Acidify each sample to pH of 2 using 37% HCl. Using extreme caution, transfer a small amount of HCl to a secondary container to avoid contaminating the large bottle. Pipette 7ul of HCl from the secondary container into each sample.
3. Replace the caps, shake for 15 seconds, and test the pH of each sample using pH test strips.
4. Depending on sample alkalinity and natural buffers within the sample, more 37% HCl may be needed to acidify the sample to pH 2. Add more HCl 1ul at a time until a pH of 2 is reached.
5. Place acidified sample in corresponding slot of the sample tube tray on the left-hand side of the instrument.
6. Check to make sure everything is sitting flush in all the trays. A failure to do so will cause probes to bend and break.
7. Once ready to run sequence press the "Run" button on the bottom left-hand of the Trilution application.

# Metagenomics Extractions

## **Summary:**

Samples are stored at -80 upon arrival. Once DNA is extracted, DNA is sent to JGI for metagenomic sequencing. The DNA extraction method uses the ZymoResearch Kit D6010 and clean and concentrator kit D4013. Samples are quantified on the Qubit 2.0.

**DNA Extraction Protocol: Quick DNA Fecal/Soil Microbe Miniprep Kit D6010**

1. Print Labels for 1.5mL tubes (“Monet, Sample ID, Core Location, DNA)
2. Measure 200mg of soil in ZR Bashing Bead Lysis Tube, do not exceed 200mg as to not overwhelm the tube
3. Remove all the lids and add 750uL of ZymoBIOMICS lysis solution
4. Recap and put in a bead beater for 15 minutes
5. Centrifuge ZR Bashing Bead Lysis Tube in a microcentrifuge at 16000 x g for 1 minute
6. Pipette 350uL of the solution in Zymo-Spin III-F filter, placed in a collection tube and remaining solution into another Zymo-Spin III-F filter with collection tube. Now you have two sets of DNA from one original sample that can be combined later
7. Centrifuge at 8000 x g for one minute (If you get a pellet, pipette supernatant to a fresh tube)
8. Discard the filter
9. Add 800uL of Genomic Lysis Buffer and 400uL of ethanol to the filtrate. Mix well
10. Transfer the solution from both sets for each samples to a single Zymo-Spin IICR filter and collection tube. (Note: You cannot fit the entire solution in the filter, you will need to do the following steps at least twice:)
11. Centrifuge at 10000 x g for 1 minute. Discard flow through
12. Add the rest of the solution from step 9 and repeat step 11
13. Put the filter in a new collection tube
14. Add 200uL of pre-wash buffer to the Zymo-Spin IICR Column and centrifuge at 10000 x g for 1 minute
15. Add 500uL of g wash buffer to the Zymo-Spin IICR Column
16. Centrifuge at 10000 x g for 1 minute. Discard flow through
17. Repeat step 16
18. Get a new collection tube for the filter and change gloves
19. Add 100uL of elution buffer to the filter and let incubate at room temperature for 1 minute
20. Centrifuge at 10000 x g for 1 minute
21. Add 600uL of prep solution to a new III-HRC filter with a collection tube and let incubate for 3 minutes. Then centrifuge at 8000 x g for 3 minutes. Discard flow through.
22. Put the III-HRC filter into a 1.5mL microcentrifuge tube with cap.
23. Discard the filter from step 19 and pipette the eluted DNA to the prepared III-HRC filter
24. Centrifuge at 16000 x g for 3 minutes
25. If samples do not produce good yields, extract multiple sets of the sample and combine at step 19, spinning down the same 100uL elution buffer in each IC filter for that sample.

**DNA Clean and Concentrater-5 Protocol D4013**

1. Add 200uL of DNA Binding Buffer to the eluted DNA
2. Transfer the mixture to a Zymo-Spin column in a collection tube.
3. Centrifuge for 30 seconds at 16000 x g and discard flow through
4. Add 200uL of DNA Wash Buffer centrifuge for 30 seconds at 16000 x g then repeat this step again.
5. Put the filter in a 1.5mL tube with cap
6. Add 100uL of DNA Elution Buffer to the filter, let incubate for 1 minute at room temp
7. Centrifuge at 16000 x g for 1 minute

**Qubit Measurement Protocol**

1. Set up two standards and prepare samples
   1. 190uL of buffer and 10uL of standard
   2. 198uL of buffer and 2uL of sample
2. Vortex tubes briefly
3. Spin down tubes briefly
4. Incubate in the dark for two minutes
5. Follow Qubit prompts and measure standards and then samples, record ng/uL.

# X-ray Computed Tomography (XCT) Data Acquisition and Analysis

*CT Imaging:*

1. Scans to be performed on cores positioned vertically in the XCT scanner
2. Low-resolution scans performed with a voxel size of 0.0825 mm, top and bottom half section images overlap so that they can be merged into a single core image.
3. Two high-resolution scans are also performed; top and bottom sections with a voxel size of 0.0381 mm (these are standalone sections)

*Data analysis:*

1. Raw x-ray images are reconstructed using CT Pro 3D (Metris XT 2.2, Nikon Metrology)
2. Avizo software from ThermoFisher Scientific to analyze the datasets
3. Stitching of the low-resolution top and bottom sections into a single core image. Only merging and visualization is performed on the low-resolution data. Porosity calculations are performed on the high-resolution data, see below.
4. High-resolution volume (i.e., hi-res top, hi-res bottom) sections: cropped (to rectangular prism) to keep the same physical dimensions among datasets for better comparison. The standard cropping size was selected as: 1300x1200x1500
   * Depth determined from the top (0-10 cm) and bottom (max depth to 10 cm above max depth) with the middle section being the remaining depth.
5. Interactive thresholding module applied to segment the pores within the cropped volumes on the high-res images only. From this, we calculate:

* total porosity (%)
* total pore volume (mm3)
* total pore area (mm2)
* min pore volume (mm3)
* max pore volume (mm3)
* mean pore volume (mm3)
* median pore volume (mm3)
* min pore area (mm2)
* max pore area (mm2)
* mean pore area (mm2)
* median pore area (mm2)
* Min pore diameter (mm)
* Max pore diameter (mm)
* Mean pore diameter (mm)
* Median pore diameter (mm)
* Wet bulk density g/cm3 (not calculated by Avizo, calculated from: from literature (Tanaka et al., Earth Planets Space, 63, 103–110 (2011))
* Pore size-frequency distribution (number of pores vs pore diameter)
  1. Output as a list of pore sizes. This will allow for user choice on how to bin pore sizes.

1. In the next step, by applying the axis connectivity module in Avizo, we can separate the connected pores from all of the pores. From this, we can estimate the connected porosity (%).

* The pore connectivity may change based on the direction (X, Y, and Z) within the analyzed volume (in some cases, we will get connectivity only in one direction, either X or Y or Z). Therefore:
* Axis connectivity analysis is performed in all three directions

1. By applying the pore network modeling module, we calculate the flow properties for X, Y, and Z-directions. These are:

* Permeability (µm2)
* Total flow rate (mm3)
* Tortuosity (unitless)

This requires the following parameter to be specified (provided by Mark Rockhold):

Input pressure = 103098 Pa (value as of April 25, 2022)

Output Pressure = 102833 Pa (value as of April 25, 2022)

Fluid Viscosity (Pa.s) = 0.001 (value as of April 25, 2022)

*Images for presentation and manuscript:*

1. XCT image of stitched whole cores
2. Pores (zoomed sections) in Z-direction
3. Connected pores (zoomed sections) in Z-direction
4. Unconnected pores (zoomed sections) in Z-direction
5. Pore network model showing pores and throats (zoomed sections) in X, Y and Z-directions

*Data Upload and File Structure*

Tomography data are placed in the appropriate sub-folder on the 1000 Soils sharepoint site (main tomography folder: /1000 Soils Pilot/Data/Abiotic Measurments/Tomography/ [Tomography](https://pnnl.sharepoint.com/:f:/r/sites/EMSL/Shared%20Documents/Science%20and%20Technology/Science%20Areas/Environmental%20Transformations%20%26%20Interactions%20Group/Research%20Campaigns/FY21/1000%20Soil%20Pilot/Data/Abiotic%20Measurements/Tomography?csf=1&web=1&e=MfbCi7))

* [Data Sheets](https://pnnl.sharepoint.com/:f:/r/sites/EMSL/Shared%20Documents/Science%20and%20Technology/Science%20Areas/Environmental%20Transformations%20%26%20Interactions%20Group/Research%20Campaigns/FY21/1000%20Soil%20Pilot/Data/Abiotic%20Measurements/Tomography/Data%20Sheets?csf=1&web=1&e=VuIiqF): All data generated from steps 5-7, except pore size-frequency distribution
  + Standard format generated by Avizo or template: [1000s\_Tomography\_TEMPLATE.xlsx](https://pnnl.sharepoint.com/:x:/r/sites/EMSL/Shared%20Documents/Science%20and%20Technology/Science%20Areas/Environmental%20Transformations%20%26%20Interactions%20Group/Research%20Campaigns/FY21/1000%20Soil%20Pilot/Data/Abiotic%20Measurements/Tomography/1000s_Tomography_TEMPLATE.xlsx?d=we18255ae27344b5882b04bd5384ff44d&csf=1&web=1&e=LevwcU)
* [Pore Size List](https://pnnl.sharepoint.com/:f:/r/sites/EMSL/Shared%20Documents/Science%20and%20Technology/Science%20Areas/Environmental%20Transformations%20%26%20Interactions%20Group/Research%20Campaigns/FY21/1000%20Soil%20Pilot/Data/Abiotic%20Measurements/Tomography/Pore%20Size%20List?csf=1&web=1&e=gvuRih): pore size-frequency distribution as a list of pore sizes (not binned)
* [Hi-res Bottom Images](https://pnnl.sharepoint.com/:f:/r/sites/EMSL/Shared%20Documents/Science%20and%20Technology/Science%20Areas/Environmental%20Transformations%20%26%20Interactions%20Group/Research%20Campaigns/FY21/1000%20Soil%20Pilot/Data/Abiotic%20Measurements/Tomography/Hi-res%20Bottom%20Images?csf=1&web=1&e=diW3bq): XCT image in Z-direction (clip)
* [Hi-res Top Images](https://pnnl.sharepoint.com/:f:/r/sites/EMSL/Shared%20Documents/Science%20and%20Technology/Science%20Areas/Environmental%20Transformations%20%26%20Interactions%20Group/Research%20Campaigns/FY21/1000%20Soil%20Pilot/Data/Abiotic%20Measurements/Tomography/Hi-res%20Top%20Images?csf=1&web=1&e=tdp5a4): XCT image in Z-direction (clip)
* [Whole Core Images](https://pnnl.sharepoint.com/:f:/r/sites/EMSL/Shared%20Documents/Science%20and%20Technology/Science%20Areas/Environmental%20Transformations%20%26%20Interactions%20Group/Research%20Campaigns/FY21/1000%20Soil%20Pilot/Data/Abiotic%20Measurements/Tomography/Whole%20Core%20Images?csf=1&web=1&e=R4Su4n)
  + Pores (whole volume) in Z-direction
  + Connected pores (whole volume) in Z-direction
  + Unconnected pores (whole volume) in Z-direction
  + Pore network model showing pores and throats (whole volume) in X, Y and Z-directions
* [Powerpoints](https://pnnl.sharepoint.com/:f:/r/sites/EMSL/Shared%20Documents/Science%20and%20Technology/Science%20Areas/Environmental%20Transformations%20%26%20Interactions%20Group/Research%20Campaigns/FY21/1000%20Soil%20Pilot/Data/Abiotic%20Measurements/Tomography/Powerpoints?csf=1&web=1&e=JPBR0S): any summary powerpoints. This is a legacy format and is NOT needed going forward except in special cases.

# General Materials Information

1. Core Liner Cutter (Geoprobe: 206987)
2. Aluminum Foil (Fisher: 15-078-292)
3. Whirlpak bags (Fisher: 01-812-120)
4. Large aluminum pie tins (Waytiffer: B09G6CTWLG)
5. Small aluminum weigh boats (Fisher: NC0325666)
6. Large weigh boats (Fisher: 17-100-421)
7. Kimwipes (Fisher: 06-666-11D)
8. Gloves (Kimtech: 19-149-863)
9. Bench Scraper (MERRY BIRD: COMINHKPR117292)
10. 4mm sieves and base (Fisher: [04-884-1AA](https://www.fishersci.com/shop/products/fisherbrand-u-s-standard-stainless-steel-test-sieves-12-in-dia-3-1-4-in-d/048841AA?keyword=true))
11. Scoopulas (Fisher: NC0124317)
12. Genese Scientific Centrifuge Tubes 50 mL (Olympus 28-108)
13. Genese Scientific Centrifuge Tubes 15 mL (Olympus 28-103)
14. BrandTech dispensette bottle top dispenser (Fisher: 13-689-005)
15. 0.45 um PES Whatan Syringe Filters (Fisher: 09-928-063)
16. 60 mL Syringe (Fisher: 14-955-461)
17. 20 mL Syringe (Fisher: 14-955-460)
18. Whatman No.42 filter paper (Whatman Part# 1442-047)
19. Culture Tubes (Fisher: 14-961-26)
20. Shimiadzu Sample Tubes (Fisher: 14-961-27)
21. 2 mL Vials (Microsolv: 9502S-WCV)
22. 2mL Vial Caps (Microsolv: 9502S-30C-B-M)

# General Chemical Information

1. Ammonium Fluoride (CAS # 12125-01-8)
2. Calcium Chloride (CAS # 10035-04-8)
3. Chloroform Ethanol-free (for analysis of carbon) (CAS# 67-66-3)
4. Ethanol 70% (CAS # 64-17-5)
5. Hydrochloric Acid 37% (CAS # 7732-18-5)
6. Labchem pH standards (Cat # LC122801) (Cat # LC123801) (Cat # LC125051)
7. Methanol (CAS# 67-56-1)
8. Potassium Sulfate (CAS # 1310-58-3)
9. Sodium Bicarbonate (CAS # 144-55-8)
10. Sodium Hydroxide (CAS # 1310-73-2)

# Calculations and Formulas

1. **GWC** **Percent**= x100
2. **Dry Mass** =
3. **Extractant L** = ((Massfresh-Massdry)+ (solvent added mL))/1000
4. **NPOC ug/g** =
5. **TN ug/g** =
6. **CO2-C** = BOD / 32 (O2 Molecular weight) \* 12 (Carbon Molecular Weight) = CO2-C mg/L \* vol. / dry mass
7. **SPE Dilutions =**
8. **Phosphorous Concentrations:**
   1. **Bray =** Soil PO4-P mg kg-1 = (PO4-P mg L-1 in extract - method blank) × 10
   2. **Olsen =** Soil PO4-P mg kg-1 = (PO4-P mg L-1 in extract – method blank) × 20

# General Instrument Information

1) **pH**: METTLER TOLEDO FiveEasy Plus FP20 and METTLER TOLEDO LE407 probe.

1.1) Three-point calibrations are performed bi-weekly, unless standards differ from known PH, in which case the probe is calibrated immediately.

1.2) LabChem PH solutions of 4 (Cat # LC122801), 7 (Cat# LC123801), and 10 (Cat# LC125051) are used for calibrations.

1.3) Electrode is stored in LabChem Electrode Filling Solution (Cat# LC140007) when not in use.

2) **Respiration**: VELP RESPIROMETRIC 6 Sensor System (Cat#  SA102B0176)

2.1) Bottles are 1000 mL, and acid washed in 1M HCL between each use.

2.2) Measurements are recorded on VELP RESPIROSoft v. 2.0.1.0.

2.3) Respiration takes place at 21 Celsius inside an American Biotech Supply Room Temperature Cabinet (Model # T9FB2321205).

3) **Phosphorus**: Spectrophotometer, wavelength 882 nm or automated Flow Injection Analysis system. (Model: FIAlyzer 1000) (KUO Testing Labs)

4) **Extractable Nitrogen**: FIALab FIAlyzer 1001 by KCI Extraction / Cd-Reduction (KUO Testing Labs)

5) **NPOC and TN**: Shimadzu TOC-L with TNM-L Total nitrogen unit.

5.1. Instrument furnace is set to 720 degrees Celsius

5.2. 9 point calibrations are conducted with C ranging from 0.0 ppm to 50 ppm, and N ranging from 0.0 ppm to 20 ppm. Calibrations are performed whenever check standards vary by more than 5% from known concentrations.

5.3. 50/20 C/N check standards are run every 10 samples.

6) **DNA Concentration**: Invitrogen By life Technologies Qubit 2.0 Fluorometer

6.1) Sequencing completed by Joint Genome Institute

7) **SPE:** Gilson GX-274 liquid handler (SKU: 2614102).

7.1) Preventative maintenance is conducted annually.

7.2) Probe, 221 x 1.5 x 1.1 mm, Constricted, Beveled, 0.45 mm Internal Diameter Tip.

7.3) TRILUTION liquid handler database: Jormungand\_DD database version v4.0, DB-build 3.

8) **FTICR**: Scimax (7T) by ESI direction infusion

9) **X-Ray Tomography:** Model XTH320 at 105 Kvand 325 μA with 0.5 mm Cu filter thickness collecting a total of 3142 projections