S.I.R.F.E.R. Stable Isotope Ratio Facility for Environmental Research	
Methods for NEON vegetation sample receiving, handling, grinding, weighing, encapsulating, and analyzing on IRMS and QC procedures	
Folder Section NEON	Version 1.0
Approved by Steering Committee 10/1/2024	

1. SIRFER Sample Handling

General

Receipt, handling, and analysis of samples at SIRFER should be conducted only by the SIRFER facility manager or a designated analyst who has read the SIRFER SOPs and received training from SIRFER personnel.

Sample Receipt

1. The designated SIRFER point of contact (POC, the SIRFER facility manager) will receive advance email notification of each sample shipment in the form of a NEON shipment manifest. This email and attachments should be filed in the personnel's University of Utah email account and SIRFER official email account (SIRFER@utah.edu).
2. SIRFER personnel shall monitor tracking information for the sample shipment and collect the package from the Biology Department's main office on the day of arrival.
3. Upon receipt, SIRFER personnel shall inspect the sample shipment for damage. Any severe external damage to the packaging materials shall be noted and photographically documented.
4. Upon opening the sample shipment, SIRFER personnel shall inspect the condition of the samples. Any damage to the sample containers, unreadable label information, or other unfavorable conditions that might compromise the samples shall be documented and disclosed to NEON in the form of a 'reply-all' response to the manifest email.
5. The SIRFER facility manager will create a new Job Number for the newly arrived shipment using a FileMaker Pro database. Each Job Number will be a unique record containing the client's name, contact information, the total number of samples received, what type of analysis or analyses are requested by the client, and whether any additional service(s) is required before analysis – i.e., grinding, acidification, etc. The Job Number database is stored on SIRFER's secure fileserver and backed up regularly.
6. The SIRFER facility manager will create and assign unique "SIRFER IDs" for all samples. The unique SIRFER IDs are recorded in a working copy of the NEON manifest so they are linked to the original NEON sample codes. SIRFER IDs are used to track the samples and related data throughout the preparation and analysis workflow, and to link records with the NEON codes during data reporting.
7. Samples will be transferred to a bin labeled with the associated Job Number. The labeled bin will be stored inside the dry cabinet (25 °C, <5% humidity). Bins are filed inside the cabinet in numerical order of the Job Numbers.

Sample Analysis

1. Samples should be analyzed according to their place in the first-in, first-out queue, as indicated by the Job Number sequence. However, analysis of NEON samples should occur no later than 90 days following sample receipt, and the SIRFER facility manager should monitor the job queue and prioritize NEON samples if necessary to meet this goal.
2. The SIRFER facility manager will assign Job Numbers to individual technicians and only assigned technicians can handle these particular samples. The assigned technician is the responsible person to prepare (e.g., grind, acid digest, weigh, etc.) samples before analysis. Technicians will follow the methods described in the SIRFER SOPs to perform any required sample preparation(s).
3. Once the assigned technician has prepared the samples for analysis; s/he will inform the SIRFER facility manager. Depending on their place in the job queue, prepared samples will then be analyzed on SIRFER's isotope analysis instruments.

Data Correction and Reporting

1. Data correction and evaluation of quality control metrics should follow all methods described in chapters 5 and 6 of this SOP.
2. NEON data reports follow a fixed, custom format. The SIRFER facility manager will assign a technician to prepare measured sample data following the required NEON format.
3. The SIRFER facility manager sends the completed data report to the SIRFER director (Bowen), who reviews and verifies the report. The SIRFER director then uploads the report to the NEON Data Portal and notifies the SIRFER facility manager.
4. Once the data report has been uploaded, the SIRFER facility manager creates an invoice for the Job Number and send that invoice to SIRFER Accountant for verification and submission to NEON.

Sample Archival

1. The sample must be archived for 60 days after data reporting.
2. After the archival period samples can be destroyed or discarded following standard lab safety procedures.

2. Selection of samples for grinding and method selection

General

1. SIRFER technicians will check each NEON sample to see whether it has already been ground or not.
   1.1. Most samples will arrive already ground (~40 mesh).
   1.2. Some NEON samples will arrive already marked for further grinding with a red dot on the cap.
   1.3. Other NEON samples may come in coin envelopes or scint vials and need grinding, but this is not indicated on the sample containers.
2. Samples that are already ground (~40 mesh) will be placed in a sample bin labeled with the SIRFER Job Number and "ready for weighing".
   2.1. After all samples in the job have been evaluated, those in the "ready for weighing" bin will be dried.

2.1.1. Remove sample container caps and place containers in the drying oven (65º C) overnight. Caps should be placed beside the vials so that each cap can clearly be associated with the vial it came from.
2.1.2. Remove samples from the oven and recap vials using the original caps.
2.1.3. Return to the sample bin.

3. Any samples that are not already ground or need further grinding will be placed in a separate sample bin labeled with the SIRFER Job Number and "grinding". Once grinding is complete these samples will be transferred to the job's "ready for weighing" bin and combined with the dried, no-grinding-needed samples (samples needing grinding are dried during the grinding protocol (2.1)).
   3.1. If the SIRFER technician finds that the sample size of sample that would otherwise need grinding is less than 5 mg, they will report this to the SIRFER facility manager and place the samples in question in the "ready for weighing" bin; these samples will be prepared without further griding to eliminate potential for loss of sample material.

2.1. Grinding samples using a mortar and pestle

Scope
This method describes the preparation of solid samples as homogeneous powders for isotope ratio analysis via continuous flow isotope ratio mass spectrometry (CF-IRMS). Samples are ground using a mortar and pestle. The method applies to easily pulverized samples. Note that some types of samples do not lend themselves to grinding with a mortar and pestle. For example, stems are not easily pulverized. For such samples, the use of a ball mill for grinding is more appropriate.

Training
Users should be trained to use this step-wise method until they are proficient.

Equipment
- Mortar and pestle
- Liquid nitrogen (LN_2) and dewar
- Spatula
- Forceps
- Glass vials, typically 1-dram or 3-dram size
- Write-on labeling tape and (optional) clear adhesive tape, to cover label.
- Permanent marker
- Laboratory wipes
- 95% ethanol
- Non-latex exam gloves

Method
1. Dry Samples
   1.1. Remove sample container caps and place containers in the drying oven (65º C) overnight. Caps should be placed beside the vials so that each cap can clearly be associated with the vial it came from.

1.2. Remove samples from the oven and recap vials using the original caps.
2. Grind Samples
   2.1. Don gloves and clean workspace.
   2.2. Place the dried sample into the mortar using forceps or gloved hands.
   2.3. Add enough liquid nitrogen to the mortar to just cover the sample.
   2.4. Allow the liquid nitrogen to evaporate until there is just enough remaining to be visible.
   2.5. Quickly grind the frozen sample with the pestle. The sample should grind to a very fine powder.
3. Remove the ground sample from the mortar using the spatula and store it in a labeled vial.
   3.1. The sample can be returned to the original sample container after checking that no residual un-ground sample material is present in the vial.
4. Clean the mortar, pestle, forceps, and spatula using 95% ethanol and laboratory wipes.

2.2. Grinding samples using a ball mill

Scope
This method describes the preparation of solid samples for IRMS analysis that are not easily pulverized. For such samples, the SIRFER lab uses a ball mill.

Training
Users should be trained to use this step-wise method until they are proficient.

Equipment
- Restech MM200 Ball Mill
- Canisters and metal balls
- Spatula
- Forceps
- Glass vials, typically 1-dram or 3-dram size
- Write-on labeling tape and (optional) clear adhesive tape, to cover label.
- Permanent marker
- Laboratory wipes
- 95% ethanol
- Non-latex exam gloves

Method
1. Dry samples.
   1.1. Remove sample container caps and place containers in the drying oven (65º C) overnight. Caps should be placed beside the vials so that each cap can clearly be associated with the vial it came from.
   1.2. Remove samples from the oven and recap vials using the original caps.
2. Put the dried samples in the ball mill canisters.
   2.1. Don gloves and clean workspace.
   2.2. Use large canisters for sample size larger than 100 mg.
   2.3. Use small canisters for sample size less than 100 mg.
   2.4. When using large canisters, two samples can be prepared and ground simultaneously.

2.5. When using small canisters, four samples can be prepared and ground simultaneously.
2.6. Because the two arms of the ball mill must be balanced, a similar sample mass should be used in each canister.
3. Add grinding balls of appropriate size to each canister.
3.1. For large canisters use one large ball with a 1.25 cm diameter.
3.2. For small canisters use 2 small balls of 0.2 cm diameter.
4. Secure the lids of the canisters and make sure they are tight. Use labeling tape to seal the lid. Write down the sample IDs on the labeling tape.
5. Placed the canisters in the ball mill and hand tighten the thumb screws to secure.
6. Set the frequency knob to 30 and the time interval to 1 minute. Press the start button.
7. Remove samples and open the canisters. Make sure to keep the label tape with IDs matched with the appropriate canister.
8. Use a scoop and/or spatula to remove the sample material from each canister and transfer it to a labeled vial.
8.1. The sample can be returned to the original sample container after checking that no residual un-ground sample material is present in the vial.
9. Clean the canisters thoroughly using 95% Ethanol and laboratory wipes.

3. Weighing

3.1. Weighing Samples

Scope
This method describes the weighing and encapsulation of powdered samples for isotope ratio analysis. Samples are carefully weighed into small tin capsules using a microbalance. A microbalance is typically readable to 1 μg and reproducible to ≤ 1 μg. Samples are routinely weighed to 1 μg before analysis. The method applies to dry, powdered samples that will be analyzed using CF-IRMS.

For ^{13}C, and ^{15}N, organic sample analysis, a subsample of a powder is accurately weighed (0.001 mg) into a tin capsule, which is then carefully closed to prevent loss of sample material. If weighed samples are not analyzed immediately, they are placed into a dry storage cabinet (at 25 °C with a humidity level of less than 5%).

Training
Users should be trained to use this step-wise method until they are proficient.

Equipment
- Microbalance
- Glass plate
- Small paintbrush
- Forceps – flat and angled tip
- Spatula or small scoops
- Small (typically 3.5 × 5 mm) pressed tin capsules
- Plastic 96-well plate

- 70% ethanol
- Laboratory wipes
- Non-latex exam gloves

Target Weights

All NEON vegetation samples will initially be weighed and analyzed at a target weight of 5.000 (+/- 10%) mg.

If subsequent reanalysis is required due to low N yield from a sample, the target weight will be increased, based on the yield obtained in the initial analysis, to achieve a yield of at least 0.015 mg N. The new target weight will be determined by the SIRFER facility manager and communicated to the staff member responsible for preparing the sample for re-analysis.

Each sample tray should ***only*** contain one type of sample (normal-weight or low-N) and should be labeled as such by circling the appropriate designation on the weigh sheet (see below).

Method

1. Don gloves and clean workspace.
2. Using the angled forceps, place an empty capsule onto the microbalance pan. Close draft shield and tare the balance.
3. Remove the capsule from the tared microbalance and place it onto the (clean) glass plate.
4. Add dry, powdered sample with a small spatula or scoop to the desired mass.
   4.1. Check mass often to determine if more or less powder should be added to capsule.
   4.2. If powder needs to be removed, hold capsule with angled forceps, tip gently, and tap the "crook" of the forceps lightly on glass plate to dislodge powder. Be sure to turn capsule upright and tap lightly onto glass plate to resettle powder.
5. Put the filled capsule back onto the microbalance pan and close draft shield.
6. Record the final mass of the weighed sample to the nearest 1 µg (0.001 mg) on the SIRFER weighsheet (see attached SIRFER weighsheet) along with the SIRFER ID (found on the sample container).
   6.1. SIRFER ID goes on the row/column corresponding to the letter/number indicating the sample position in the 96-well plate.
   6.2. Sample weight is recorded in the cell below the SIRFER ID.
7. Open the draft shield and remove the weighed, filled capsule; place it onto the (clean) glass plate.
8. With flat forceps, pinch closed the top third of the weighed, filled capsule.
9. Fold the capsule over to close the top. Fold over twice, if possible.
10. Using the angled forceps, push the folded capsule off the flat forceps and continue to collapse and fold the capsule to form a small, dense cube or sphere.
11. Place the encapsulated sample into the 96-well tray. If some powder may have been lost during encapsulation, check mass of sample before placing into tray.
12. Clean the forceps, spatula/scoop, and glass plate using 70% ethanol and laboratory wipes between each sample.
13. If any powder is spilled onto microbalance pan, use a clean paintbrush to gently sweep away material.

SIRFER Weighsheet

SIRFER EA-IRMS Sample Loading Template: NEON Vegetation C&N												
Job #				Tray#			Analyst:					
Primary Reference 1 (PLRM-1):							Primary Reference 2 (PLRM-2):					
Secondary Reference (SLRM):							Instrument:				Type:	Normal
Date loaded:				Date Analyzed:				Export File:				Low-N
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	Blank	COND	COND	COND	COND	PLRM-1					PLRM-2	
B				SLRM	SLRM					PLRM-1		
C			PLRM-2					SLRM	SLRM			
D		PLRM-1					PLRM-2				SLRM	SLRM
Comments:												

3.2. Weighing Reference Materials

Scope
This method describes the weighing and encapsulation of powdered reference materials for isotope ratio analysis. Reference materials are prepared after weighing unknown samples for a run and added to the 96-well plate containing the weighed samples. Materials are carefully weighed into small tin capsules using a microbalance. A microbalance is typically readable to 1 µg and reproducible to ≤ 1 µg. Reference materials are routinely weighed to 1 µg before analysis. The method applies to dry, powdered reference materials that will be analyzed using CF-IRMS.

A subsample of each reference material is accurately weighed (0.001 mg) into a tin capsule, which is then carefully closed to prevent loss of material. If weighed materials are not analyzed immediately, they are placed into a dry storage cabinet (at 25C with a humidity level of less than 5%).

Training
Users should be trained to use this step-wise method until they are proficient.

Equipment
- Microbalance
- Glass plate
- Small paintbrush
- Forceps – flat and angled tip
- Spatula or small scoops
- Small (typically 3.5 × 5 mm) pressed tin capsules
- Plastic 96-well plate
- 70% ethanol
- Laboratory wipes
- Non-latex exam gloves
- Working vials of three internal lab reference materials (stored in the "C-N reference materials" bin):
    - PLRM-1 (Primary Laboratory Reference Material-1) = UU-CN-3 (glutamic acid)
    - PLRM-2 (Primary Laboratory Reference Material-2) = UU-CN-2 (glutamic acid)
    - SLRM (Secondary Laboratory Reference Material) = Spinach (ground spinach leaf)

Method
1. Weigh ~0.500 mg (±20%) of PLRM-1 and PLRM-2 into tin capsules following the same techniques described in section 3.1 (Weighing Samples).
    1.1. One sample capsule is added to each well of the sample tray corresponding to the reference material ID (refer to the weigh sheet).
    1.2. Record the mass of the PLRM sample in the weigh sheet cell below the ID.

2. Weigh SLRM in tin capsules following the same techniques described in section 3.1 (Weighing Samples). Seven SLRM capsules should be weighed per batch to constitute a mass 'ladder', with target weights of 0.2, 0.35, 0.5, 0.75, 1.5, 3, and 7 mg (±20%).
    2.1. One sample capsule is added to each well of the sample tray corresponding to the reference material ID (refer to the weigh sheet).
    2.2. Record the mass of the SLRM sample in the weigh sheet cell below the ID.
3. Additional capsules of any C-N reference materials should be prepared and placed in the cells designated as "COND" on the weigh sheet.
4. An empty tin capsule should be prepared (folded) and placed in the cell designated as "BLANK" on the weight sheet.
5. Once samples and reference materials are all weighed, secure the lid of the 96 well tray with label tape, fold and secure the weigh sheet to the tray with tape, and place the tray and weigh sheet into the dry storage cabinet.

4. CN-EA Solids Analytical Method

Scope
This method describes the measurement of carbon (C) and nitrogen (N) isotope ratios of organic and inorganic solid materials using an isotope ratio mass spectrometer (IRMS) coupled to an elemental analyzer (EA), operated in continuous flow (CF) mode. The method applies to the instruments known as Mad-Eye Moody and Saltbush Bill.

Materials analyzed using this method must be accurately weighed and encapsulated into tin capsules. Note that additional methods are required prior to this method, including sample preparation and sample weighing. Data generated from this analysis method should be corrected using a data reduction method.

Training
Operators should be trained and proficient with daily maintenance procedures (i.e., leak checking, insert changing) and the Isodat software. Operators should be trained to use this step-wise method until they are proficient. A quick-start checklist is provided as an appendix to this method as a guide for trained users; however, the checklist is not a substitute for training.

Instrumentation
Finnigan Mat Delta+ Advantage or Finnigan Mat Delta+ IRMS
Carlo Erba CHN EA1110
ThermoFinnigan Conflo III
Ascarite trap (for low-N sample analysis)

Method
1. Verify Instrument Set Up
    1.1. Check the IRMS front panel for source and pump lights. All should be green, indicating the source and pumps are on.
    1.2. Verify the O_2 pressure using the marked gauge on the front of the EA.

1.3. Open the Acquisition software and then open the appropriate sequence table (e.g., i.e. "NEON N_2_CO_2 +He_1.seq").
1.4. Open the Instrument Control software. Select the EA-Conflo configuration and N_2 as the gas type from the dropdown menus at the bottom of the screen.
1.5. Begin a scan on N_2 backgrounds (masses 28 and 29). Record initial voltages and time in the instrument start checklist.
1.5.1. If signal is not present, confirm source and pumps lights are illuminated, close Isodat software, restart the computer, and begin scan.
1.6. Check the combustion, reduction, and GC temperatures on the EA. Record temperatures in the instrument start checklist.
1.7. Open the front panel of the EA and verify that helium flow is set to the black mark on the gauge.
1.8. Check and record the gas tank pressures for the helium tanks as well as the reference gas tanks in the instrument start checklist.
1.8.1. The following five gas tanks must have >400 psi before beginning an analytical sequence. If tanks are low, inform facility manager:
1.8.2. Helium Primary (1°) and Secondary (2°) (He inert carrier gas tanks)
1.8.3. Oxygen (O_2 combustion aid)
1.8.4. Nitrogen (N_2 reference wall gas tank)
1.8.5. Carbon Dioxide (CO_2 reference wall gas tank)
1.9. Review and fill out the instrument eLog (.xls files) accessible from the computer desktop. Record the Job Number, analyst, and the number of samples to be analyzed.
1.9.1. Use the eLog to determine whether the oxidation column insert needs to be changed.
1.9.2. Oxidation column inserts are changed following a soil run or following analysis of 100 organic samples.
1.9.3. If the insert should be changed, proceed to Step 2. Otherwise, proceed to Step 3.
1.9.4. Save eLog file and then close Excel.
1.10. Close isolation valve between autosampler and oxidation column.

2. Verify trap setup. For normal operation, a magnesium perchlorate trap is used after sample combustion and reduction to trap water. For low-N samples, this trap is replaced with a split trap including both $Mg(ClO_4)_2$ and Ascarite, which traps the majority of the CO_2 produced in the EA. Prior to analysis verify that the trap installed in the EA is correct for the type of samples (normal or low-N) being analyzed.
3. Replace Insert (as needed). Smaller diameter quartz inserts protect the oxidation column against samples that attack and weaken the quartz glass. They can be cheaply and quickly changed to preserve the life of an oxidation column.
3.1. Turn off the source and then close the mass spectrometer (MS) valve finger tight (approximately 3 turns).
3.2. Loosen and remove the autosampler from the oxidation column. A wrench may be necessary because the oxidation column is hot.
3.3. Use the end of a larger Sharpie marker to remove the old insert while wearing oven mitts. Transfer the 1000 °C insert to an asbestos cooling pad. Note whether there was any quartz wool stuck to the bottom of the old insert.
3.4. Pack a new insert with a small piece of quartz wool (*not glass wool*). If quartz wool was stuck to the bottom of the old insert, also add more directly to combustion column.

3.5. Carefully lower the insert into combustion column. Use a Sharpie marker to mark the top of oxidation column then quickly remove the insert.
3.6. Use as glasscutter to score the insert below the Sharpie mark by a few millimeters. Caution – insert is hot! Once cut, the insert should sit BELOW the lip of the oxidation column.
3.7. Carefully lower the cut insert into the combustion column then reattach the autosampler.
3.8. Check for He leaks using the leak detector:
3.8.1. Turn on leak detector and remove wand from sleeve.
3.8.2. Allow leak detector to auto "zero." When lights have stopped moving and one green light appears, leak detector is ready.
3.8.3. Move wand over autosampler connection and monitor for helium leaks. Make any necessary corrections/adjustments and verify the autosampler/oxidation column connection is leak-free before proceeding.
3.9. Open MS isolation valve 3 turns. Wait 10 minutes (load samples while waiting; see Step 3) before turning on source.

4. Load Samples
4.1. With autosampler isolation valve **closed**, open vacuum valve for 10-15 seconds to evacuate autosampler.
4.2. Close vacuum, then loosen and flip open screw clamps securing autosampler lid.
4.3. Fill autosampler with helium to "pop" open autosampler lid. Once lid is open, verify there are no samples from previous analytical sequence in wells.
4.4. Check carousel size (i.e., 50 or 100-position carousel). Change carousel, if necessary, depending on sample tray size.
4.5. Verify the appropriate carousel size has been selected on autosampler control box. Use toggle switch to select correct carousel size.
4.6. Check alignment of carousel opening over oxidation column by manually advancing carousel one position using control box.
4.6.1. Adjust alignment as needed using the "fine movement" toggle located on the back of the autosampler control box.
4.6.2. Carousel alignment should be adjusted so that one advance sweeps past the oxidation column opening and just into the next sample well. This helps avoid sample hang-ups.
4.7. Advance carousel so that position 50 (or 100) is over the oxidation column.
4.8. Load samples from tray into the carousel in the order detailed by weigh sheet. One sample should be loaded into each well.
4.9. Close the autosampler lid. Secure lid with screw clamps.
4.10. Verify the auto-sampler valve is still **closed** and then open the vacuum valve. Tighten lid screw clamps again.
4.11. Close vacuum valve, fill autosampler with helium to marked pressure on gauge, then open vacuum valve again and leave open for a minimum of 5 minutes. (Enter sample information into analytical sequence table while waiting; see Step 4).
4.12. After 5 minutes, close vacuum valve.
4.13. Fill autosampler with helium to marked pressure on gauge.
4.14. Open autosampler isolation valve. If necessary, turn on source.
4.15. Continue monitoring N_2 backgrounds (masses 28, 29, and 30) for at least 200 seconds after autosampler isolation valve has been opened before proceeding.

5. Enter Sample Information
   5.1. Enter sample identifiers (unique SIRFER ID) into Identifier 1 column of analytical sequence table.
   5.2. Note which wells hold reference materials by typing "RM" into Identifier 2 column of the analytical sequence table. Optional one wt% Acetanilide standard can be added at the beginning of the run.
   5.3. Enter sample masses into Amount column of analytical sequence table.
   5.4. Verify the correct analytical method is selected for each line of the analytical sequence table (i.e., "fake" method for wall gas source conditioners, dual gas method for samples).
      5.4.1. Tip – Sometimes the Isodat Acquisition software is buggy. If you notice that the analytical sequence table has several grid boxes shaded (selected) with navy blue fill and you are unable to "deselect" them, try this trick: Click on line 1 of the sequence table. Right click and select "insert row above." Highlight the new row, right click, and delete it. Cells in the sequence table should now be modifiable.
   5.5. Save analytical sequence table.
6. Check Instrument Configuration
   6.1. Once N_2 backgrounds are flat (i.e., not changing) or slowly decreasing, stop scan in Instrument Control software. Note background voltages and time in instrument start checklist.
   6.2. Turn on N_2 gas (Reference 2) and then peak center by selecting the center scan button in the top left corner of the screen.
   6.3. Record peak center value and the mass 28 voltage in the instrument start checklist.
   6.4. Turn off N_2 gas (Reference 2) and turn on CO_2 gas (Reference 1).
   6.5. Check jump from N_2 to CO_2.
      6.5.1. Click on the "Jump" button.
      6.5.2. Highlight line 1 (N_2 to CO_2) and click on the green check mark.
      6.5.3. Click "yes" when asked "Are you sure?" and confirm that CO_2 reference gas (Reference 1) is on.
      6.5.4. Record the previous jump value and the new jump value.
      6.5.5. If new jump valve is the same as previous value, save and continue.
      6.5.6. If new jump value is not the same as previous value, switch to N_2 gas configuration and then repeat jump check until the same jump calibration is returned twice. Save and record final jump value in instrument start checklist.
   6.6. Peak center on CO_2 by selecting the center scan button in the top left corner of the screen.
   6.7. Record peak center value and the mass 44 voltage in the instrument start checklist.
   6.8. Turn off CO_2 reference gas, then switch back to N_2 gas configuration. Verify that backgrounds voltages for masses 28, 29, and 30 are still low.
   6.9. Close Instrument Control software. Leave only Acquisition software open.
7. Start Instrument
   7.1. Verify that autosampler isolation valve is **open**.
   7.2. If necessary, manually advance carousel using autosampler control box so that Well 1 (or the first well with a sample) will be the first well analyzed after instrument start.
   7.3. In the Acquisition software, use the mouse to highlight the rows in the analytical sequence table that will be analyzed.

7.3.1. As you do this, verify that the green peak center "check" mark is present for every line.
7.3.2. Verify again that the "fake" analysis method is only selected for wall gas Source Conditioners.
7.4. Select "Start" to begin acquisition.
7.5. Enter job number for File name. Re-enter job number as Folder name.
7.6. Verify that results will be exported as .xls file.
7.7. Locate waving flag and verify check mark for "Measure Only Selection" (i.e., the lines you highlighted) then select OK.
7.8. The first sample analyzed is an EA conditioner that should be used to check the "timing" of the acquisition.
7.8.1. Verify the sample drops from the autosampler into the oxidation column and that flash combustion occurs, producing sharp peaks that are clearly separated from each other and from the square reference gas peaks.
7.8.2. Verify that the Acquisition software records peaks for this sample.
7.8.3. If either of these criteria is not met, stop the run and alert the SIRFER Facility Manager.

8. Complete Post-Acquisition Checks
8.1. Open Work Space software.
8.1.1. Click on "Results" tab.
8.1.2. Find your Job Number and double click.
8.2. You will see a chromatogram for every acquisition (i.e., for every line in the analytical sequence table).
8.2.1. Double click to open each chromatogram and verify the peaks look as expected.
8.2.2. Make notes for any sample acquisition that was unusual (no peak, very large peak, etc.).
8.3. Check the autosampler carousel and verify that every capsule dropped.
8.4. Oxidation columns must be re-built after approximately 600 samples; note how many samples are on the current column and then schedule a column rebuild as needed.

Appendix 1: EA-IRMS Background for Carbon and Nitrogen Isotope Ratio Analysis

Biological and soil samples are analyzed for carbon and nitrogen isotopes using continuous-flow Isotope Ratio Mass Spectrometry (CF-IRMS). Here, samples are analyzed using an elemental analyzer (EA) coupled to an IRMS through an open split interface. A weighed sample is combusted in the presence of O_2 forming H_2O, CO_2, and N_2 gases that are pushed along in a helium (He) stream. Halogen or sulfur-containing gases are chemically removed and incomplete combustion products (i.e., CO_x, CO, etc.) are reduced, followed by the removal of excess O_2 and all water. The final product gasses, N_2, and CO_2, are separated on a gas chromatography column before entering the IRMS. Samples are analyzed alongside pure gases (Ultra High Purity UHP N_2 and CO_2) for calibration purposes.

Appendix 2: Instrument start checklist

	No.	Action
☐	1	Open sequence table in Acquisition.
☐	2	Start scan and record N2 backgrounds (masses 28 and 29).
☐	3	Record gas tank pressures and column temperatures.
☐	4	**Close** autosampler isolation valve.
☐	5	Fill out e-log. New insert required? Follow steps 6-13. Insert ok? Skip to step 14.
☐	6	**Turn off source.**
☐	7	Close mass spec valve.
☐	8	Remove autosampler from oxidation column.
☐	9	Replace insert.
☐	10	Replace autosampler and check for leaks with gas detector.
☐	11	Open mass spec valve and wait 10 minutes.
☐	12	While waiting, enter sample information into Acquisition.
☐	13	After 10 minutes, turn on source.
☐	14	Open vacuum valve to evacuate autosampler **with isolation valve closed**.
☐	15	Close vacuum valve, then loosen clamps and backfill autosampler with He.
☐	16	Open autosampler lid and load samples.
☐	17	Close autosampler lid and evacuate autosampler; tighten clamps.
☐	18	Close vacuum valve, fill autosampler with He, then open vacuum valve and leave open.
☐	19	Enter sample info into Acquisition or begin correcting data from previous run.
☐	20	After **5 minutes**, close vacuum valve and fill autosampler with He.
☐	21	**Open** autosampler isolation valve.
☐	22	Monitor backgrounds for at least 200 seconds.
☐	23	Once backgrounds are flat, stop scan. Record background values.
☐	24	Turn on N2 reference gas and peak center. Record value.
☐	25	Turn off N2 reference gas and turn on CO2 reference gas.
☐	26	Check jump from N2 to CO2. If value is the same as previous, save. If value is different from previous, do not save. Repeat steps 24-26.
☐	27	Record saved jump value in sequence.
☐	28	Peak center on CO2 reference gas. Record value.
☐	29	Switch to N2 and check backgrounds again.
☐	30	Verify that autosampler **isolation valve is open**.
☐	31	Close all programs and windows except Acquisition.
☐	32	Highlight rows and start sequence.
☐	33	Watch first timing sample to verify all peaks are present.

Date

Job #

Analyst

Instrument: SBB

Helium tanks

Primary:

Secondary:

N2 tank

CO2 tank

Ox. Temp

Red. Temp

GC Temp

Jump check

Previous:

New:

Peak center		Backgrounds		
mV	Peak return	Mass	@	@
N_2		28		
CO_2		29		

START TIME

Comments:

5. Quality Metrics on Analysis

1. The Primary Laboratory Reference Materials (PLRM) used for calibration of vegetation isotope data are UU-CN-2 and UU-CN-3 (both glutamic acid).
2. The Primary Laboratory Reference Material used for calibration of vegetation N% and C% is UU-CN-2.
3. A blank (empty tin capsule) is included in each run. No CO_2 or N_2 gas peak should be detected for the blank capsule; if either peak is detected the run should be stopped or the data discarded and samples re-analyzed.
4. Our primary quality control criteria are based on analyses of an independent, secondary laboratory reference material (SLRM), Spinach. The criteria represent the 2 standard deviation range observed for replicate analyses of these reference materials over multiple years, and include:
   4.1. Measured, calibrated SLRM value within ± 0.4‰ of the known values for $\delta^{15}N$ and $\delta^{13}C$.
   4.2. Measured, calibrated SLRM %C and %N within 0.6% of the known values.
   4.3. The standard deviation of 2 or more analyses of SLRM within the run < 0.4‰ ($\delta^{15}N$ and $\delta^{13}C$) and < 0.6% (N% and C%).
5. Currently accepted known values for these reference materials are shown below.

ID	Type	$\delta^{15}N$	$\delta^{13}C$	N%	C%
UU-CN-2	PLRM	−4.6‰	−28.18‰	9.52%	40.81%
Spinach	SLRM	−0.4‰	−27.41‰	5.95%	40.53%
UU-CN-3	PLRM	9.3‰	-12.35‰		

6. Quality control criteria for individual sample analyses are based on data completeness and peak areas. The criteria include:
   6.1. All sample peaks must be present.
   6.2. Sample peak areas must not exceed the maximum SLRM peak area in the run by more than 25%.
   6.3. Sample peak areas must not be more than 25% smaller than the minimum SLRM peak area for the run.
   6.4. Sample N yield calculated from the measured peak area must be 0.015 mg or greater to report nitrogen content and isotope data from the analysis. If N yield falls below this threshold the sample will be re-weighed and analyzed using the low-N protocols.

6. EA-IRMS Data Processing

Scope

This method describes the processing of "raw" data collected during isotope ratio analysis of samples via EA-IRMS. The method applies to all EA-IRMS instruments running the Isodat software.

Method

1. Collect data from instrument
   1.1. After a run, find the shortcut folder on the computer desktop containing results from the analysis method used.
   1.2. Find your Job Number and double click.
   1.3. Move the "raw" data file in the Excel folder to a flash drive for transfer to your computer.
2. Process data using the SIRFER R scripts
   2.1. If needed, obtain the latest release of the R scripts from the GitHub repository: https://github.com/SPATIAL-Lab/SIRFER/releases
   2.2. Save a copy of the "raw" Excel data file from the flash drive to the SIRFER/data folder on your computer. Save another copy of the "raw" data to Working Files.
   2.3. Open the "SIRFER" R-project on your local computer.
   2.4. Run the NEON vegetation data-processing script using the command process.veg("filename.xls"), where filename is the name of the excel data file for the run.
   2.5. Check the following output:
      2.5.1. Nitrogen and carbon isotope drift corrections will be applied if the trends for both PLRMs are of the same sign and have R^2 values > 0.3. Check the drift correction slope reported in the console, if any, to determine whether a correction was applied. Compare these values with the drift graphs in the plot window to verify that the fit is consistent with the observed data.
         2.5.1.1. If any inconsistencies are found, create a new issue at https://github.com/SPATIAL-Lab/SIRFER/issues and reference the data file name. Gabe will investigate and either verify that the correction is valid or update the scripts to resolve the issue. Wait for the issue to be resolved before continuing.
      2.5.2. Nitrogen and carbon linearity corrections will be applied based on a piecewise regression of the drift-corrected SLRM isotope values against ln(PeakArea) if the R^2 value for this relationship is > 0.3. Check the regression breakpoint and R^2 reported in the console, if any, to determine whether a correction was applied. Compare these values with the linearity graph in the plot window to ensure that the fit is consistent with the observed data.
         2.5.2.1. The form of the correction is: $c = m - (ln([a - t]) * s + i)$, where c is the corrected isotope value, m is the measured value, a is the sample peak area, t is the maximum (threshold) peak area for the correction, and s and i are the regression slope and intercept fitted to the SLRM values. Where $a > t$, no correction is applied.
         2.5.2.2. If inconsistencies are found, create a new issue at https://github.com/SPATIAL-Lab/SIRFER/issues and reference the data file name. Gabe will investigate and either verify that the correction is valid or update the scripts to resolve the issue. Wait for the issue to be resolved before continuing.
      2.5.3. Review the quality control metrics reported in the console.
         2.5.3.1. Any run-level metrics that exceed the values stipulated in Chapter 5 will

be marked with an asterisk (*), indicating the batch will need to be re-analyzed.

2.5.3.2. Any individual samples which do not meet the yield and peak area criteria stipulated in Chapter 5 will be listed in the console and will need to be re-analyzed if additional sample material is available.

2.5.3.3. Samples with N yields that fall below the minimum threshold will be reported separately and should be re-analyzed using the low-N method described in Chapters 3 & 4 if additional sample material is available.

2.5.3.4. Quality control flags for samples and runs will be automatically recorded based on these metrics and are auto-populated during data reporting.

2.6. Raw and reduced sample and reference material data will be automatically written to the vegetation C/N database; if previously reduced values for the same run are present in the database a copy of the superseded values will be saved in a subsidiary directory.

3. When all samples for a job have been analyzed, generate a data report using the SIRFER R scripts.

3.1. Save a copy of the sample manifest file in the SIRFER/data folder on your computer.

3.2. Generate a data report by running the command report.veg("filename.csv"), where filename is the name of the sample manifest file.

3.2.1. By default, data for analyses which did not pass one or more QC checks will be excluded from the report. Nitrogen data (isotope values and N%) are also excluded for any analyses where the N yield fell below the QC threshold value.

3.2.2. If the manifest includes samples that did not pass QC but need to be reported (e.g., because reanalysis was not possible), include the argument "flagged = TRUE" in the function call, which will report flagged values where no unflagged data are available for the same sample. In this case N data will also be reported for samples with N yields below the QC threshold value if no value is available for the same sample using the low-N method.

3.3. The script will match data from the manifest and the vegetation C/N database and produce two formatted .csv reports containing sample data and SLRM data.

3.4. Check the following console output to ensure that valid data are reported for all samples:

3.4.1. List of samples (if any) contained in the manifest but missing in the database (i.e., not analyzed; should be none).

3.4.2. Number of samples in the manifest and number of samples reported. The number reported may be less than the manifest total if some samples failed QC but could not be reanalyzed, in which case the report should be re-generated using the "flagged = TRUE" argument.

3.4.3. Number of samples excluded from the data report because they have one or more quality flags.

3.5. Check the plots showing the sample and SLRM values:

3.5.1. Sample isotope and wt% values are within reasonable ranges for the sample types.

3.5.1.1. For terrestrial vegetation samples, normal $\delta^{13}C$ values range from -32 to -12‰ and $\delta^{15}N$ values from -10 to +10‰.

3.5.1.2. For terrestrial vegetation samples, normal N% values range from 0.1 to 8% and C% values from 30 to 60%.

3.5.1.3. Individual values well outside these ranges may suggest sample contamination, and the sample should be reanalyzed.

3.5.1.4. A prevalence of values outside these ranges within a given run may suggest calibration problems, and the batch should be reanalyzed.

3.5.2. SLRM isotope and wt% values are consistent with expectations (boxes showing 50% and 100% of the acceptable ranges described in Chapter 5 are shown on the plots).

3.6. Submit the data reports for review by another analyst or the Facility Manager.

3.6.1. This individual will add their name/initials to the "reviewedBy" column before data submission.

3.7. Data submission will be conducted by the Facility Manager as described in Chapter 1.

7. IRMS QC Monitoring and Maintenance

General

The SIRFER facility manager is the primary party responsible for quality assurance of IRMS organic/inorganic sample measurements, including the maintenance of reference materials and regular monitoring of instrument performance.

Maintaining SIRFER Reference Materials

1. The SIRFER facility maintains a collection of internal standards for carbon and nitrogen isotopes, and concentration analysis (UU-CN-3, UU-CN-2, and Spinach), which have been calibrated relative to international isotope standards (USGS40, and USGS41). These internal standards are stored and handled using techniques that ensure that they are not subjected to any contamination. In addition, the facility maintains stocks of certified international standards. The international standards are archived in an isolated desiccator filled with desiccant. Internal standards are distributed in small aliquots and stored in a separate desiccator in the lab (or, as is more common, next to the weighing stations when they are in use). To avoid cross-contamination, only one aliquot of internal standards can be used at any point in time. The stock of calibrated international standards is replenished by ordering new standards from the IAEA, NIST, and/or USGS after exhausting the current sets of standards.
2. As long as they stay dry and uncontaminated, solid organic and inorganic isotope reference materials can be archived for long periods of time because they do not disintegrate.
3. The aliquot of internal reference materials is stored in capped 20-ml glass scintillation vials and sealed with Parafilm. After an aliquot of internal reference standard becomes exhausted, the technician(s) report to the SIRFER facility manager in order to have that particular internal standard refilled.
4. The SIRFER facility manager closely monitors the instrument calibration parameters (slope, intercept) and QC reference values to identify any systematic shifts following the re-filling of an internal reference standard. If a significant shift is identified, sample analyses are discontinued while the facility manager conducts tests that check the working bottle values against values of the reference standards taken directly from the archive container. If a discrepancy is found, the working bottle is discarded and a new working bottle is filled directly from the archive bottle.

5. SIRFER internal reference materials cover a range of nitrogen isotope values from −4.6‰ to +9.3‰; values range from −28.18‰ to -12.35‰ for carbon. If necessary SIRFER does have the capacity to increase the isotopic range for nitrogen and carbon up to +49.6‰ and 23.96‰ respectively (using SIRFER internal lab reference material UU-CN-1).

Daily Monitoring

1. As a component of the lab's quality assurance practices, the SIRFER facility manager reviews QC parameters on a daily basis and stores the daily QC reference values in the "RM" database.
2. A zero blank is performed daily for both nitrogen and carbon dioxide reference gases. This is to check the fidelity of the instrument. For both gases, the cutoff is 1 standard deviation less than 0.2‰ after 4 cycles of repetitive injection of the same reference gas.
3. A wall-gas linearity check is performed on a weekly basis on all of the IRMS instruments. The SIRFER facility manager will only allow samples to be run on an instrument if the linearity number is below ± 0.1 ‰/volt for both carbon and nitrogen isotopes.

Annual Calibration Check

1. SIRFER internal reference standard values are checked against international standards at the beginning of each calendar year (ideally in the first half of January).
2. SIRFER internal standards are run as 'unknown' samples replicated at least 15 times on two separate instruments. In these analytical batches, the international standards (USGS40 and USGS 41) are analyzed and used for data calibration in place of the PLRMs. Data are otherwise evaluated using the normal SIRFER data reduction procedures, with USGS40 being used as the PLRM for elemental % values.
3. Average measured values of the internal standards serve as the primary criteria for the evaluation of the check.
   3.1. δ^{13}C and δ^{15}N values should be within 0.2‰ of the previously calibrated values.
   3.2. N% and C% should be within 0.3% of the previously calibrated values.
4. If the check values lie outside of the accepted range, sample analysis is stopped and a recalibration of the SIRFER internal standards is conducted according to the following guidelines.
   4.1. A series of dedicated calibration runs are conducted on at least 2 separate IRMS instruments.
   4.2. At least three sets of USGS40 and USGS41 are weighed, along with 15 replicates of SIRFER internal reference materials.
   4.3. Data are reviewed and processed by hand to remove any linear drift or linearity effect.
   4.4. USGS40 and USGS41 values are used to derive a 2-point linear calibration line. The calibration line is used to calculate values for the SIRFER internal standards. Average calibrated SIRFER internal standard values from three or more recalibration runs can be adopted when the precision of calibrated values from 3 or more runs is better than 0.2‰ for δ^{13}C and δ^{15}N values.
5. Recalibrated internal standard values are updated in the "RM Excel" file in the Dropbox and "RM" database. An archive copy of the previous version of the file is saved, noting the date

of depreciation of the previous values.

6. Each year at the end of January a summary file for SLRM analyzed in all NEON vegetation analysis batches will be extracted from the SIRFER dropbox. This summary file will be uploaded to the NEON data portal.

Change Log

Version	Date	Changes	By
1.0	10/1/2024	Original version	Suvankar Chakraborty, Gabe Bowen