University of Wyoming
Stable Isotope Facility

Standard Operating Procedures
(NEON abbreviated version)

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Document Change History

NEON Document	Date	Author	Section	Sub-section(s)	Action
UofWyomingSIF_CNiso_20170601	10/31/17	csc			Edited for NEON/Battelle use
UofWyomingSIF_CNiso_20181018					No change
UofWyomingSIF_CNiso_20191009	8/29/19	csc			Update SOP, instruments
UofWyomingSIF_CNiso_20201210	11/7/19	csc			NEON-recommended document updates
	12/2/19	csc			Updated calculations
	10/30/20	csc	Section 5 Sample Preparation	Section 5.3 Weighing Solid Samples	Amended Clarified weighing tolerances. Clarified calibration procedures.
	11/9/20	csc	Section 5 Sample Preparation	Section 5.1 Drying samples Section 5.4 Grinding samples Section 5.6 Grinding samples Section 5.9 Grinding samples	Added instruction that all samples are dried. Sample drying temperature changed to 65 C as stated in NEON/Battelle SOW.
	12/10/20	csc	Glossary	Table 2 Symbols	Added mg and μg to list
			Section 5 Sample Preparation	Section 5.1 Section 5.4 Section 5.6 Section 5.9	Added instruction that all samples are dried. Sample drying temperature changed to the 65 C as stated in the NEON/Battelle SOW.
			Document	Document	Organized document to include NEON specific sections.

NEON Document	Date	Author	Section	Sub-section(s)	Action
UofWyomingSIF_ CNiso_20211124	9/22/21	csc	Document	Section 4.4 Section 5.6 Section 8.7	Changed headings for consistency
	11/5/21	csc cjm	Section 8	Table 8	Updated reference material values
	11/5/21 11/24/21	csc cjm cjm	Document Change History	Document Change History	Updated table to add NEON document column
			Section 4	Section 4.2.1	Added detail for Airtable sample prep requirements
	11/24/21	cjm	Section 5	Section 5.3.4	Added criteria for ground samples
			Section 5 Section 7	Section 5.4	Added criteria for successful decantation of acidified samples. Updated steps 8, 11
				Section 5.5.1	Added step 9 for more detail
				Section 5.6.1	Added drying and grinding clarifications
				Section 5.6.2	Added criteria for ground samples and storage in original container for all
				Section 7.2	Added paragraph explaining CO_2 trapped samples and reasoning to use this method
			Section 8	Table 8	Added values for % data to table 8
			Section 8	8.5.3	Added elemental composition quality control criteria
UofWyomingSIF_ CNiso_20220909	2/20/22	csc cjm	Section 8	Section 8.4	Added new reference material certification procedures.
				Table 8	Updated reference material values.
			Section 5	Section 5.6.1	Included option for desiccant drying cabinet.

NEON Document	Date	Author	Section	Sub-section(s)	Action
UofWyomingSIF_CNiso_20250402	4/2/25	Cjm	Section 1	Section 1.2	Removed Facility Director role and added lead technician role.
			Section 5	Throughout	Updated required PPE
				Section 5.1	Updated thermometer model and oven log book information recorded.
				Section 5.1.1	Updated USDA soil permit number
				Section 5.3.1	Updated preferred grinding method Table 6 Removed references to vegetation samples Removed coffee grinder method
				Section 5.4	Added explanation for acidification and organic carbon precautions.
				Section 5.6	Removed reference to vegetation
			Section 7	Section 7.2	Theory for CO_2 trapping method further explained. QC checks added to recognize isobaric interference.
				Section 7.3	Added analysis types and when/why they are used
			Section 8	Table 8	Added new reference materials
			Section 10	Section 10.5	Updated threshold for reliable data to 300mV

NEON Document	Date	Author	Section	Sub-section(s)	Action
UofWyomingSIF_CNiso_20251027	10/27/25	cjm	Section 2	Table 4	Added centrifuges; removed Thermo MAS Plus autosampler
			Section 5	Section 5.4.2	Added 50mL tube procedure section; added flow chart figures
			Section 8	Section 8	Rearranged subsections
				Section 8.6	Introduced Wyo-Iso script and added into procedures
				Section 8.7	Moved subsection down; Added Wyo-Iso script and database explanations
			Section 9	Section 9.3, Table 11	Added Drift to common corrections; updated weight percent correction description
			Section 10		Removed excel data analysis procedure. This is replaced by Wyo-Iso script

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Glossary of Abbreviations, Symbols, and Terms

These tables contain descriptions and definitions of some of the abbreviations, symbols, and terms found in this document.

Table 1 *Abbreviations*

Abbreviation	Description
AMU	Atomic mass unit
CF	Continuous flow
CF-IRMS	Continuous flow-isotope ratio mass spectrometer
CNS	Carbon Nitrogen Sulfur
EA	Elemental analyzer
EA-IRMS	Elemental analyzer-isotope ratio mass spectrometer
IC	Inorganic carbon
IRMS	Isotope ratio mass spectrometer
OC	Organic carbon
QA	Quality assurance
QC	Quality control
SOP	Standard operating procedure
SQL	Structured Query Language
UW	University of Wyoming
UWSIF	University of Wyoming Stable Isotope Facility
LIMS	Laboratory Information Management System

Table 2 *Symbols*

Symbol	Name	Description
δ	delta	The symbol for stable isotope ratios relative to a standard.
‰	per mil	The unit of stable isotope data. One per mil represents one 'part per thousand' enrichment or depletion from a standard.
%	percent	One part in a hundred.
ºC	degree Celsius	SI unit of measure of temperature.
µl	microliter	SI unit of volume. A microliter is 1/1000000 of a liter.
m	meter	SI unit of length.
mL	milliliter	SI unit of volume. A milliliter is 1/1000 of a liter.
psi	pounds per square inch	A common non-SI unit used to express pressure.
s	second	SI unit of time.
min	minute	An acceptable non-SI unit of time equal to 60 seconds.
^{13}C	carbon-13	A stable isotope of carbon.
^{15}N	nitrogen-15	A stable isotope of nitrogen.
mg	milligram	SI unit of weight. A milligram is 1/1000 of a gram.

µg	microgram	SI unit of weight. A microgram is 1/1000000 of a gram.
^{2}H	hydrogen-2	A stable isotope of hydrogen.
^{18}O	oxygen-18	A stable isotope of oxygen
^{17}O	oxygen-17	A stable isotope of oxygen

Table 3 *Important terms*

Abundance	Abundance is the relative number of atoms of the different isotopes of one chemical element. Abundance is usually expressed as a percentage of the total of all isotopes of a given element.
Atomic Mass Unit	Atomic mass unit or amu, is defined as exactly 1⁄12 the mass of an atom of carbon-12. One amu is equal to approximately 1.66×10^{-24} grams.
Continuous Flow (CF)	Continuous flow is a term for isotope ratio mass spectrometers that are coupled on-line to preparation devices. These instruments represent a “marriage” of chromatography and mass spectrometry, and are similar to conventional organic mass spectrometers in that gas samples are introduced to the IRMS within a stream of an inert gas (helium).
Continuous Flow-Isotope Ratio Mass Spectrometer (CF-IRMS)	In CF-IRMS a sample is introduced into the IRMS using an inert gas that carries the sample gas into the IRMS. Various automated sample preparation devices generate the sample gas, including but not limited to elemental analyzers (EA) and gas chromatographs (GC).
Delta	Delta (δ) is a special unit used to describe the relative deviation of the isotopic ratio of a sample from the known isotopic ratio of a standard. Delta is expressed in the unit of per mil (‰). One per mil represents a one-part-per-thousand difference from a standard.
Elemental Analyzer (EA)	An EA is an instrument that consists of an autosampler, high temperature furnace, chemical trap and a gas chromatograph column. An EA is used primarily to combust material in an oxygen-enriched helium atmosphere producing some or all of the following products: CO_2, H_2O, and N_2.
Isotope Ratio Mass Spectrometer (IRMS)	Isotope ratio mass spectrometers are specialized magnetic sector mass spectrometers that produce precise and accurate measurements of variations in the natural isotope abundance of the light elements H, C, N, O, and S.
Open split interface	The open split interface allows the IRMS to sample the inert gas carrier stream as the stream exits a sample preparation device.
Standard Operating Procedure (SOP)	Standard operating procedures are detailed written procedures designed to standardize an analytical method or procedure.

Section 1 Overview of The Stable Isotope Facility at the University of Wyoming

1.1 General Description

The University of Wyoming Stable Isotope Facility (UWSIF) is a core research facility located in the Berry Biodiversity and Conservation Center at the University of Wyoming. The UWSIF provides quality isotopic analyses for the research community at the University of Wyoming (UW) and other universities and labs nation-wide. The facility is engaged in development of novel analytical techniques, **advises** researchers on sampling and analysis and serves as a hands-on teaching facility for UW students, post-doctoral associates and faculty. The UWSIF is dedicated to ecological and environmental applications of light stable isotopes (H, C, N, O, and S) and is fully equipped and staffed for a broad suite of applications. The UWSIF plays a prominent role in helping on-campus researchers address environmental management and natural resource issues in Wyoming.

The facility is equipped with isotope ratio mass spectrometers, and optical isotope instruments. These instruments are connected to different peripheral devices for automated sample processing, helping to meet the growing demands of researchers and students. The UWSIF has vacuum extraction lines for off-line preparation and purification of sample gases, sample storage freezers, ovens, fume hoods, centrifuges, grinding mills, and other miscellaneous sample preparation equipment.

1.2 Oversight and Management

The UWSIF is administered by a Faculty Director, a Laboratory Manager, and a lead technician. The Faculty Director sets the vision and broad objectives for the lab and provides guidance, leadership and oversight for the operation and long-term growth of the facility. The Laboratory Manger helps to shape the long-term objectives for the facility, provides isotope consultation to researchers across campus, prepares annual reports for University Administration, provides technical guidance to all technicians working in the facility and conducts tours of the facility for visitors. The Laboratory Manager is also responsible for maintaining the instruments, scheduling sample analyses, and overseeing the routine day-to-day operations of the facility. The lead technician assists the laboratory manager in scheduling, sample analysis, administrative tasks and overseeing the routine day-to-day operations. The lead technician is the supervisor responsible when the Laboratory Manager is not present.

Broader oversight of the UWSIF is provided by a steering committee of 6 faculty members from different departments on campus (e.g., Botany, Zoology and Physiology, Geology and Geophysics, Archaeology and Anthropology and Ecosystem Science and Management). The role of the steering committee is to help provide guidance on overall strategic direction of the UWSIF and to provide feedback and oversight on technical challenges, policy issues and financial management of the lab. The steering committee meets with the UWSIF Faculty Director and Facility Director during Spring and Fall semesters. These meetings provide the opportunity to discuss future strategies to be adopted by UWSIF to keep pace with the upcoming technologies. The committee composition reflects balance among participating academic units across campus and allows other new faculty to participate in the evolution of the facility. A major goal is to have participation from faculty with diverse technical requirements and disciplinary backgrounds. Membership is on a two-year rotation, but each member has the opportunity to serve on a continuing basis as necessary to meet the goals for the facility.

1.3 Financial Management

The UWSIF is a non-profit analytical research facility serving the academic community and researchers at the University of Wyoming. Analysis jobs, client billing, monthly, quarterly and annual accounting reports are performed using software by Zoho. Analysis jobs are also managed using a Zoho database system. The UWSIF accounting system is maintained in parallel with the University of Wyoming Office of Research and Economic Development accounting system that is the primary financial accounting system for the UWSIF. The Office of Research and Economic Development keeps track of expenditure amounts and income received to maintain a cash balance. The Office of Research and Economic Development also makes the actual deposits and all payments and monitors paid invoices. The monthly report generated by the Office of Research and Economic Development enables the UWSIF to further analyze the expenditures. By generating the invoices directly, the UWSIF monitors details of sample analyses and jobs to produce a comprehensive picture of the financial and analytical activities in the lab. The UWSIF reconciles the Zoho cash balance with that determined monthly by the Office of Research and Economic Development to ensure all expenses and income have been included in all UWSIF reports.

The income is also reviewed in relationship to expenses incurred by the UWSIF to provide a complete picture of all aspects of facility efficiency. Our system can analyze income by month, quarter or year and provide details for these periods based on specific analyses performed. We can review not only income, but the number of samples and standards demanded by each instrument to evaluate maintenance costs and productivity. We are constantly striving to improve the details needed to build our database in order to establish more useful feedback from the accounting system.

Our financial policy is based on the following criterion:

- The sample costs for on-campus academic clients are intended to cover costs of expendable materials and supplies associated with analysis, routine instrument calibration and maintenance, instrument repair and replacement over the long run, new methods development and wages for undergraduate technicians who conduct routine tasks in the facility.
- Sample costs are evaluated on an annual basis to ensure the lowest feasible per sample charge.

1.4 Training

1.4.1 Laboratory orientation and safety requirements

Prior to beginning technical work at UWSIF, each researcher, analyst or technician attends a laboratory orientation training session, which includes the following:

- UWSIF safety procedures
- University of Wyoming Environmental Safety laboratory rules
- Facility safety equipment

1.4.2 Technical training

Full-time and part-time technicians are fully trained for their specific tasks by the Laboratory Manager or senior technicians. New personnel must show competence in the use of the instruments (as determined by the Laboratory Manager) before they are allowed independent operation of the instruments. All technicians are trained in appropriate data reduction methods, which includes raw data evaluation, instrument stability tests, and the use of reference materials for data corrections.

1.4.3 Training refresher

The UWSIF provides training to all technicians on an annual basis. This training includes:

- A review of the UWSIF Quality Management Plan and the UWSIF Standard Operating Procedures Manual, including new additions or deletions.
- New analytical methods (where appropriate).
- New facility instrumentation (where appropriate).
- A review of all appropriate SOPs.

On occasion, new methodology or instruments are introduced to the facility. When this occurs, UWSIF technicians receive additional training to ensure that the instruments are used in a proper fashion and that laboratory technicians are thoroughly familiar with all new methods.

The Laboratory Manager maintains a log which records all laboratory training.

Section 2 UWSIF Instrument Inventory

2.1 Laboratory Equipment

The UWSIF uses a variety of instruments and equipment to generate stable isotope data. Tables 4 and 5 list the important instruments and equipment.

Table 4 *UWSIF instruments and equipment*

Instruments/Equipment	Quantity
Costech 4010 Elemental Analyzer	1
Costech Zero Blank autosampler	4
Finnigan ConFlo III Interface	1
Finnigan DELTA^{Plus} XP Isotope Ratio Mass Spectrometer	1
LabConCo Freeze Zone Freeze Dry System	1
Binder drying oven	2
Retsch MM-400 Mixer Mill	1
Sartorius SE2 microbalance	3
Mini beader	1
Sartorius Cubis microbalance	1
Thermo ConFlo IV Interface	3
Thermo DELTA V Isotope Ratio Mass Spectrometer	3
Thermo IsoLink Elemental Analyzer	1
VWR Galaxy 16 micro-centrifuge	1
Beckman Coulter Allegra X-22 Centrifuge	1

Table 5 *UWSIF mass spectrometers and peripherals used for soil C and N analyses.*

IRMS	Peripherals	Isotopes	Gas form
Finnigan DELTA^{Plus}XP	Costech 4010 Elemental Analyzer Finnigan ConFlo III Interface Costech Zero Blank autosampler	^{13}C ^{15}N	CO_2 N_2
Thermo DELTA V	Thermo IsoLink Elemental Analyzer Thermo ConFlo IV Interface Costech Zero Blank autosampler	^{13}C ^{15}N	CO_2 N_2

Section 3 Isotope Ratio Mass Spectrometry - General Information

3.1 Isotope Ratio Mass Spectrometer (IRMS) - Principal of Operation

An isotope ratio mass spectrometer consists of three main components: an ion source, an electromagnetic, and a detector.

3.1.1 Electron impact ionization source

Attached to the ionization chamber are the filament (cathode), an electron trap (anode), and ion extraction and focusing plates. The filament emits electrons that travel across the ionization chamber where some of the sample gas molecules collide with the electron beam making a positive ion. In the $Delta^{Plus}$ XP, ≈1 in 1500 gas molecules are ionized, ≈1 in 1100 gas molecules for the Delta V. The electron trap collects all the electrons not involved collisions with the sample gas. The extraction plates accelerate the positive ions out of the ionization chamber and direct the ions toward the exit slits and focusing plates. The accelerating voltage is 3kV. The different focusing plates are designed to focus the ion beam as it leaves the source. The ion exit plate defines the maximum beam width prior to passage down the flight tube.

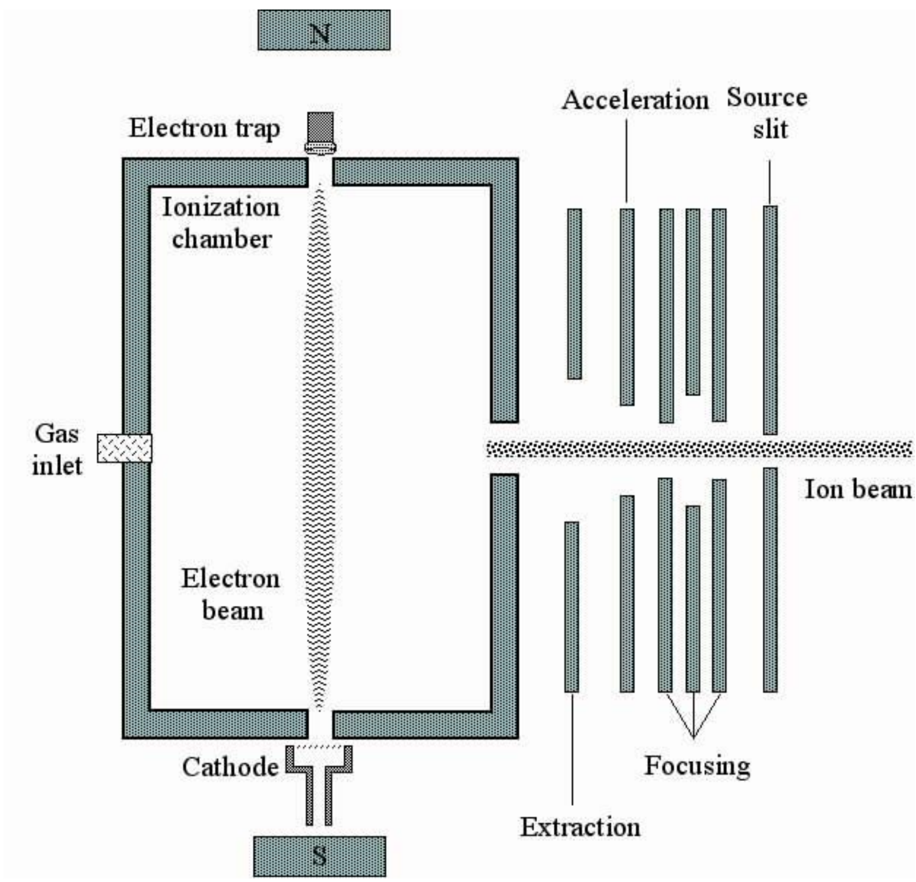

Figure 1. Schematic of the electron impact ionization source.

3.1.2 Electromagnet

The flight tube has a 90-degree fixed deflection path fitted with a permanent magnet having a maximum magnetic field strength of 0.75 Tesla, allowing the measurement of mass ranges up to 70 amu ($Delta^{Plus}XP$) or 96 amu (Delta V). For any given gas, the electromagnet is held constant. At a constant accelerating voltage (*V*) and magnet strength (*B*), the lighter ions will be deflected more than the heavier ions. In the case of CO_2, mass 44 is deflected more than mass 45 and mass 46. In order to analyze another gas such as N_2, the accelerating voltage remains fixed and the magnetic field strength is varied.

These parameters are related by the equation:

$$\frac{m}{z} = r^2 \times \left(\frac{B^2}{2V}\right)$$

Where: *m*/*z* is the mass-to-charge ratio
B is the magnet field strength
r is the radius of the ion circular path
V is the acceleration voltage

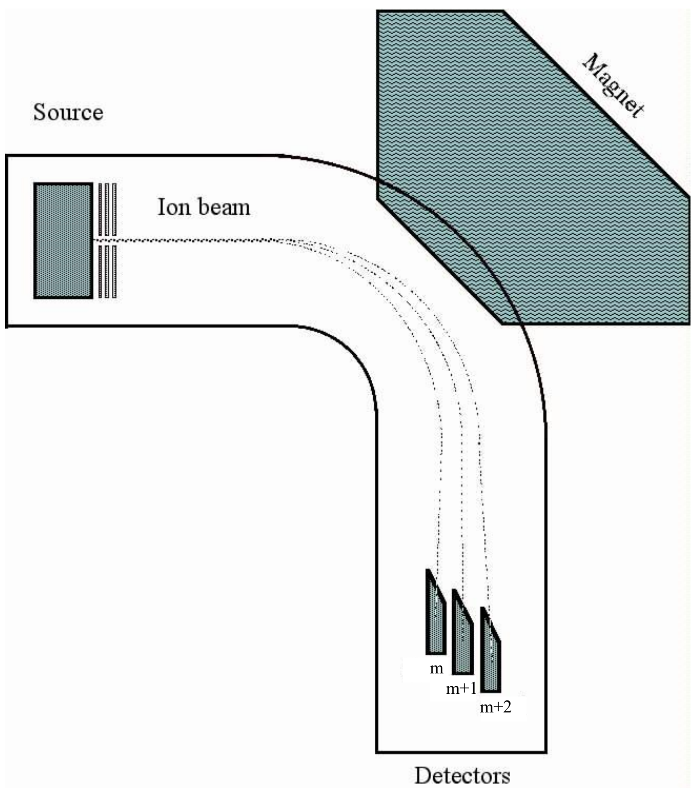

Figure 2. Schematic of the isotope ratio mass spectrometer.

3.1.3 Faraday cup detectors

After leaving the magnet, the separated ion beams are directed to three collectors called Faraday cups. The three collectors consist of one narrow collector (cup 2) and two wide collectors (cups 1 and 3). These cups are grounded through high ohm resistors. When configured for CO_2 and N_2, the resistor on cup 1 = 3 x 10^8 Ω, the resistor on cup 2 = 3 x 10^{10} Ω, and the resistor on cup 3 = 1 x 10^{11} Ω. When the positive ions are detected on a collector, an electron returns to the ion, causing a voltage drop in the resistor that acts as a measure of the ion current. The signals received by the collectors are amplified and transferred to voltage-to-frequency converters. This digitizes the analog signal for processing by the computer. In the case of CO_2, all three masses (44, 45, and 46) are detected at one time, allowing the software to calculate both $\delta^{13}C$ and $\delta^{18}O$ ratios for a given sample.

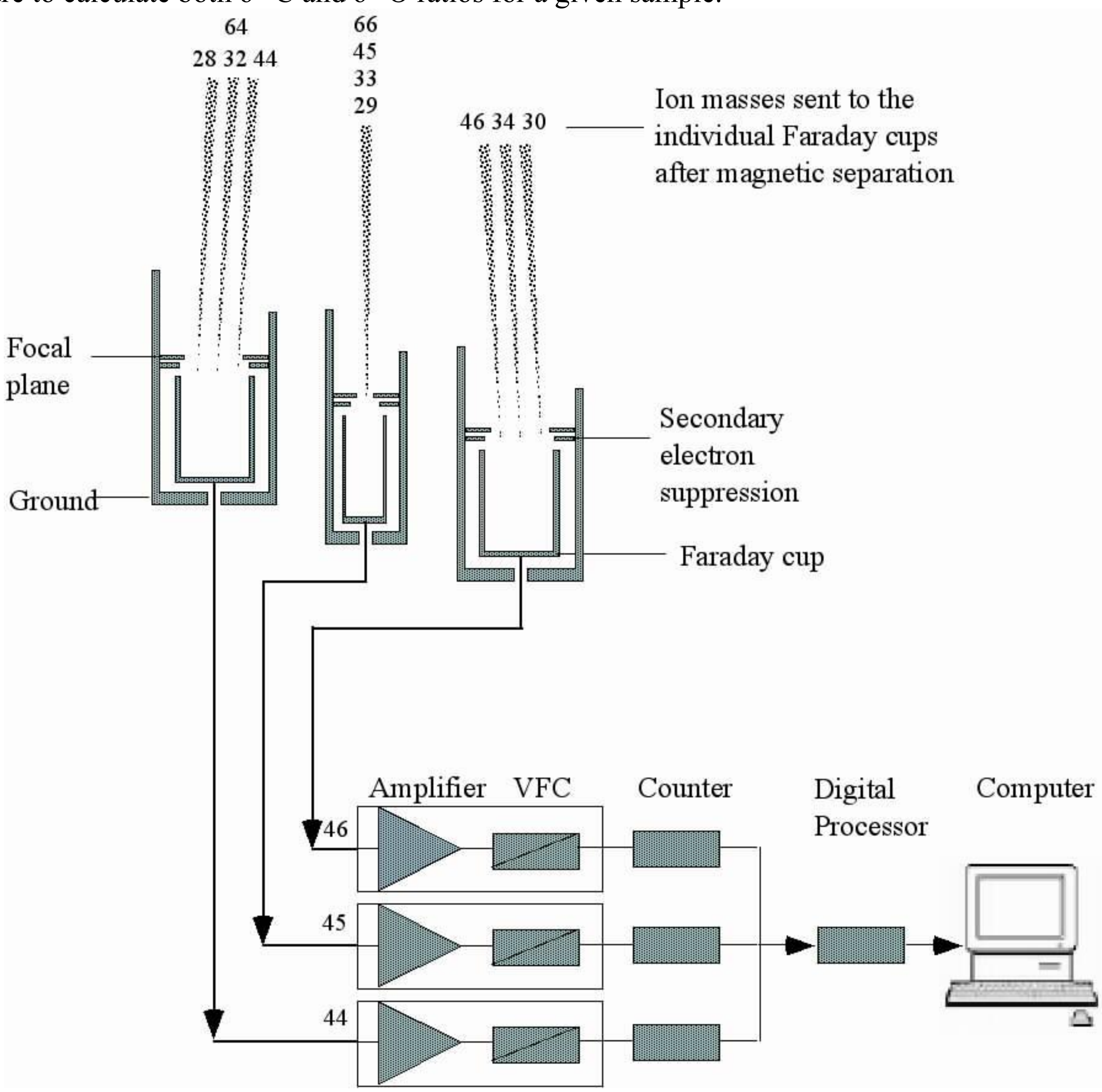

Figure 3. Schematic of the triple Faraday cup detector system.

3.2 The Open Split Interface - Principal of Operation

Prior to isotopic analysis, complex samples (solids, liquids, gases) are converted into simple gases (N_2, and CO_2) through combustion or carbon reduction. When using continuous flow mode, these simple gases are carried to the isotope ratio mass spectrometer by an inert carrier gas (helium). The gas pressure in the ionization source effects the precision and accuracy of the isotope measurement, so it is very important to keep the pressure in the ion source constant at all times. Continuous flow isotope ratio mass spectrometers achieve this using an open split. An open split uses a small capillary to “sniff” the carrier gas/sample gas from an open cell, which is at atmospheric pressure. Because the ionization source is at a very low vacuum, the “sniffed” gas is pulled into the ion source at a constant rate.

The ion source of the mass spectrometer works best with helium flows of about 0.2 - 0.3 $mL \cdot min^{-1}$. Since sample gas flows entering the open split cell range from 2-140 $mL \cdot min^{-1}$, this gas flow needs to be reduced to avoid too much gas entering the ion source. This sample gas “splitting” is one of the main purposes of the open split interface.

Mass spectrometers requires molecular flow. This is accomplished in the open split cell. Gases produced in the EA are transported by helium carrier gas flowing at 80-100 $mL \cdot min^{-1}$ and enter the open split cell. Since the mass spectrometer capillary is so small (0.1mm ID) only a small portion (<10 %) of sample and carrier gas can enter the mass spectrometer. This essentially reduces the flow to ~0.3 $mL \cdot min^{-1}$ and allows the ion source to operate at 10^{-6} mbar pressure.

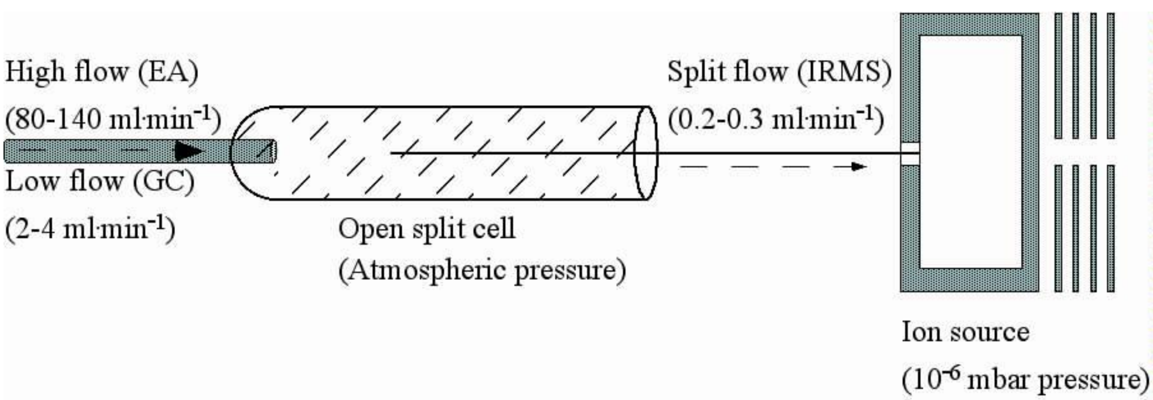

Figure 4. Schematic of the open split interface.

3.2.1 Finnigan ConFlo III interface

The ConFlo III interface consists of two open split cells, one dedicated to reference gases and the other to sample gases. Inside the reference open split cell are four fused silica capillaries, one for the N_2 reference gas, one for the CO_2 reference gas, one for helium make-up gas, and one connected directly to the mass spectrometer source. The reference gas capillaries are attached to pneumatic pistons that allow moving of the capillaries in and out of the helium. The helium make-up and mass spectrometer capillaries are fixed. The sample gas open split cell consists of three fused silica capillaries, one coming from the elemental analyzer, one for helium dilution, and one connected directly to the mass spectrometer source. The helium dilution capillary is attached to a pneumatic piston that allows computer selected sample dilution. The capillary from the elemental analyzer and the capillary connected directly to the mass spectrometer source are fixed. If bulk dilution of the sample is required, the split dilution valve opens and flow to the open split cell is reduced from 80-100 $mL{\cdot}min^{-1}$ to 8-10 $mL{\cdot}min^{-1}$.

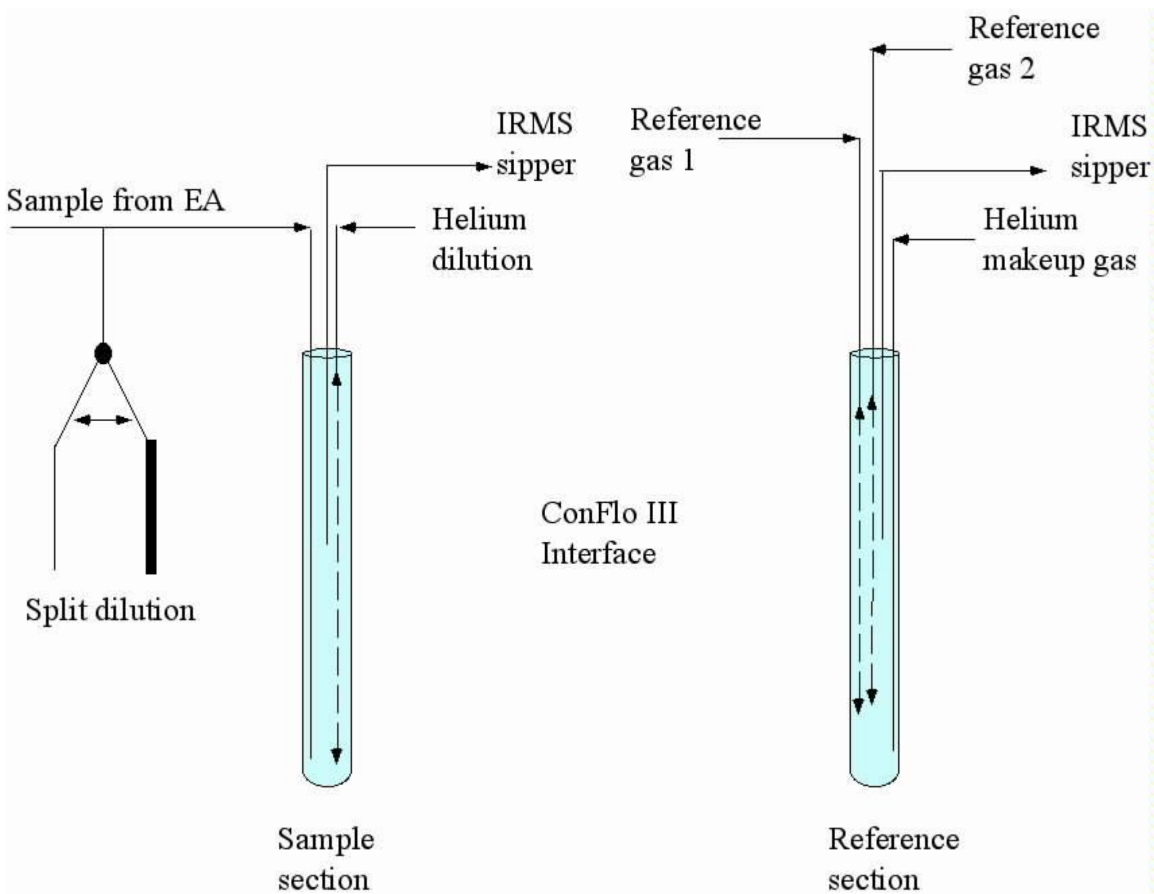

Figure 5. Schematic of the Finnigan ConFlo III open split interface.

3.2.2 Thermo ConFlo IV interface

The ConFlo IV open split interface has five permanently-connected reference gases, low and high carrier flow inputs, and incremental helium dilution. Incremental dilution allows the selection of a wide range of helium dilution options (sample or reference).

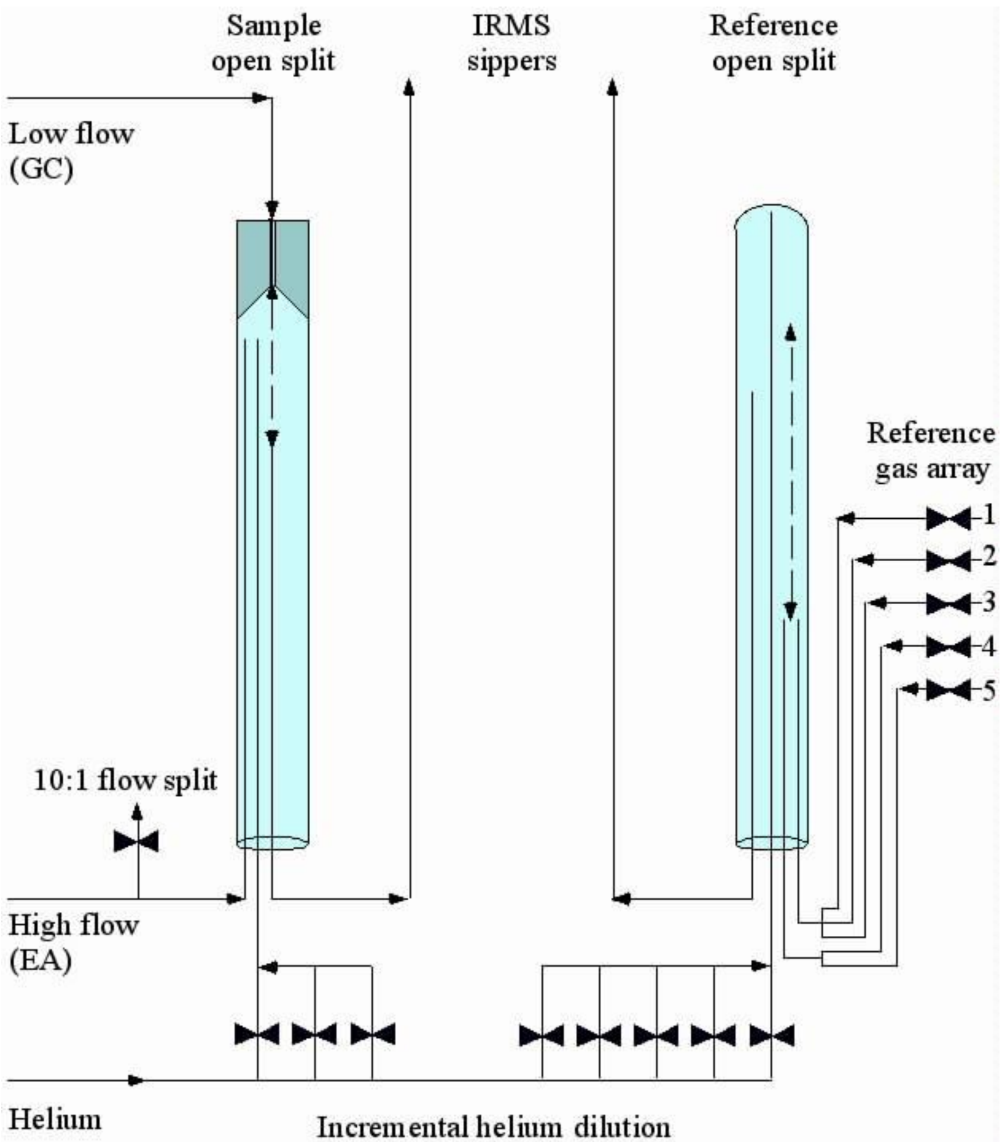

Figure 6. Schematic of the Thermo ConFlo IV open split interfacee

3.3 The Elemental Analyzer - Principal of Operation

An elemental analyzer is a scientific instrument which determines the elemental composition of a sample. The basic principle of an elemental analyzer is as follows:

The sample is weighed into a tin capsule, which is dropped into an oxygen-enriched high temperature combustion reactor. The resulting combustion gases pass through reagents, producing CO_2, H_2O, N_2, and oxides of nitrogen. These reagents also remove halogens, sulfur, and phosphorus. The combustion gases then pass through a reducing reactor to remove excess oxygen and reduce oxides of nitrogen to elemental nitrogen. Water is then chemically removed. The resulting gases enter a gas chromatograph where the N_2 and CO_2 are separated. In stand-alone instruments, the separated gases are then sent to a thermal conductivity detector (TCD) for elemental analysis. If the elemental analyzer is attached to an IRMS, the gases go from the TCD to the IRMS via an open split interface for isotopic analysis.

Some versions of elemental analyzers have single reactors. These single reactors combine the chemistry of a double reactor system in a single reactor. The advantage of this system is the reduction of system volume, which allows for quicker analytical times. The disadvantage is that the single reactor has a much shorter lifetime.

3.3.1 Costech 4010 elemental analyzer

The Costech 4010 elemental analyzer operates based on the principal of flash combustion in which a sample contained within a tin capsule is dropped into a combustion reactor held at 1020 °C. For nitrogen and carbon analysis, the combustion reactor contains chromium oxide as an inorganic oxygen source and silvered cobaltous/cobaltic oxide for removal of halogens and sulfur. When the tin capsule is dropped into helium temporarily enriched with oxygen, the tin ignites and flash combustion occurs. Flash combustion raises the temperature of the sample to >1700 °C. The encapsulated sample, depending on its composition, combusts, generating one or more of these gases: N_2, N_xO_x, CO_2, and H_2O. The reduction reactor contains reduced copper wires for the reduction of nitrogen oxides to N_2 and the removal of excess O_2. An adsorption trap containing magnesium perchlorate removes the H_2O. The remaining N_2 and CO_2 gases travel through a chromatographic column (Porapak Q) and then move to the ConFlo III open split interface. See Figure 7 for a detailed schematic of an elemental analyzer. See Section 3.2.1 for a detailed description of the ConFlo III interface.

Instrument temperatures:

Combustion reactor	1020 °C
Reduction reactor	650 °C
Chromatographic column	60 °C

Flow rates:

Helium	80-100 $mL \cdot min^{-1}$
Oxygen	25-30 $mL \cdot min^{-1}$

3.3.2 Thermo Scientific IsoLink elemental analyzer

The Thermo Scientific IsoLink elemental analyzer operates based on the principal of flash combustion in which a sample contained within a tin capsule is dropped into a combustion reactor held at 1020 °C. For nitrogen and carbon analysis, the combustion reactor contains chromium oxide as an inorganic oxygen source and silvered cobaltous/cobaltic oxide for removal of halogens and sulfur. When the tin capsule is dropped into helium temporarily enriched with oxygen, the tin ignites and flash combustion occurs. Flash combustion raises the temperature of the sample to >1700°C. The encapsulated sample, depending on its composition, combusts, generating one or more of these gases: N_2, N_xO_x, CO_2, and H_2O. The reduction reactor contains reduced copper wires for the reduction of nitrogen oxides to N_2 and the removal of excess O_2. An adsorption trap containing magnesium perchlorate removes the H_2O. The remaining N_2 and CO_2 gases travel through a chromatographic column and then move to the ConFlo IV open split interface. See Figure 7 for a detailed schematic of an elemental analyzer. See Section 3.2.2 for a detailed description of the ConFlo IV interface.

Instrument temperatures:

Combustion reactor	1020 °C
Reduction reactor	650 °C
Chromatographic column	variable

Flow rates:

Helium	100-120 mL·min^{-1}
Oxygen	25-30 mL·min^{-1}

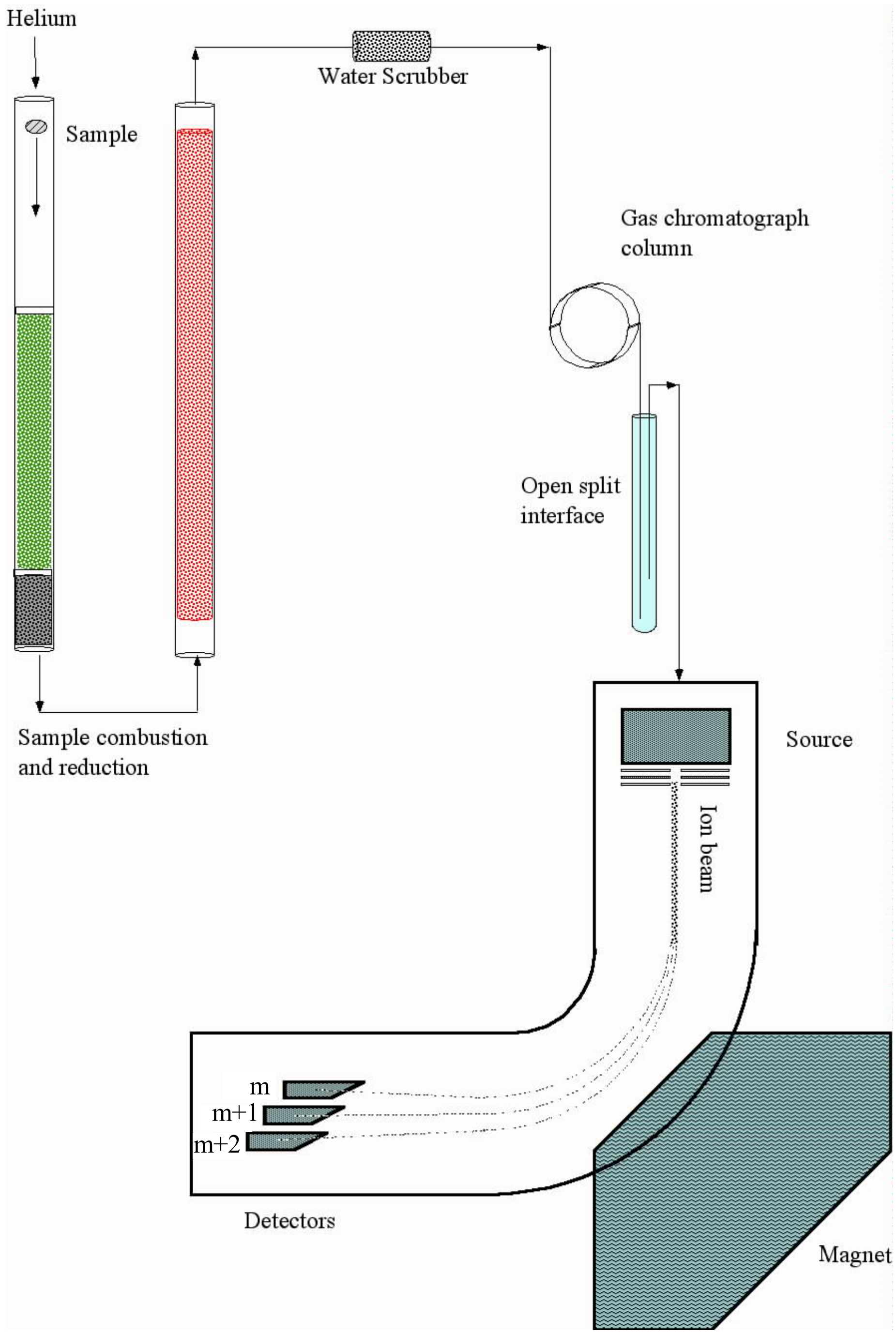

Figure 7. Schematic of the elemental analyzer coupled to an IRMS.

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3.4 NEON Specific Instrumentation

There are no special instruments used for NEON sample analysis.

Section 4 Sample Handling - General Information

4.1 Receiving Samples and Sample Check-in

When samples arrive at UWSIF, all sample shipping containers are opened and carefully checked for any sample spillage. In the event there is sample spillage, the following procedures will be followed:

1. Broken sample containers are carefully removed.
2. If any sample can be saved, it is placed into a new container.
3. After checking the sample manifest, missing samples are noted in the sample log

4.1.1 Quarantine sample handling

Any soil samples being received from a quarantined area need special treatment. See internal UWSIF Soil Import Standard Operating Procedures for proper handling of quarantine samples.

4.2 Sample Information Management Systems

UWSIF uses Zoho and Airtable software platforms to manage samples once the samples are received at UWSIF. Airtable allows UWSIF to track the status of the samples through the entire analytical process. Zoho assigns unique run and sample identification numbers to the samples, stores analytical data, and generates accounting information.

4.2.1 Airtable LIMS

Samples are tracked from the moment they arrive at the laboratory using Airtable LIMS. Airtable LIMS lists the number and type of samples, sample preparation steps (drying, grinding, weighing, acidification), and analytical analyses to be performed. Progress for each job is tracked continuously. All jobs are managed on a daily and weekly basis by the lab manager and lab technicians. The following information is available in Airtable LIMS:

- Job number
- Client
- Job status
    - Checked in
    - Sample preparation required
    - Sample preparation started
    - Analysis started
    - Waiting on reruns
    - QAQC in progress
- Days in queue
- Sample material
- Instrument used
- # of samples
- # of runs
- Runs completed
- # of reruns
- Due date

4.2.2 Zoho database

UWSIF uses the Zoho platform to manage the financial aspects of analysis. Through Zoho, samples are tracked from the moment they arrive at the laboratory to the time results are reported. For sample tracking, the Zoho system works as follows:

1. New samples are submitted to the lab via a Routing Sheet. Each Routing Sheet is assigned a job number (e.g., 2017-0031).
2. Every sample is assigned a unique UWSIF sample number associated with the assigned job number (e.g., 2017-0031_7).
3. The samples also retain client supplied sample identification information.
4. For sample traceability, all results are filed under the assigned job number. Multiple runs within a job are named sequentially: 2017-0031 Tray 1, followed by 2017-0031 Tray 2, and so on.
5. Analytical results are sorted and stored in different folders according to analysis type and client's last name.

Zoho keeps track of all financial and analytical information for analytical runs. The Zoho database provides the following information:

- The specific analyses to be performed
- Any sample prep that needs to be charged (grinding & acidification)
- The number and type of samples
- The cost for the analysis
- Contact and billing information for the client
- Generates invoices for the services provided
- Stores analytical results

4.2.3 UWSIF final reports

Analytical final reports are typically emailed to clients as an excel file. Clients can also find their data on the UWSIF website at the UWSIF PORTAL. More meta-data is stored on OneDrive and is shared with the client when a report is sent.

4.3 Storage of Samples

Samples are stored in dry cabinets before and after sample preparation processes are completed. After the analysis is complete, all remaining sample material is discarded. UWSIF will store unused sample material for a maximum of six months.

4.4 NEON Specific Sample Handling and Tracking Procedures

4.4.1 Receiving Samples and Sample Check-in

In the event there is sample spillage or missing samples from a NEON shipment, the following procedures are followed:

1. Broken sample containers are carefully removed.
2. If any sample can be saved, it is placed into a new container.
3. All missing samples are noted in the receipt form.
4. NEON is informed of any shipping issues or missing samples.

4.4.2 NEON Quarantined domains

Soils from the following sites need to be treated as quarantine samples, see internal UWSIF Soil Import Standard Operating Procedures:

Sites Name
DSNY
JERC
OSBS
GUAN
LAJA
GRSM
ORNL
DELA
LENO
TALL
CLBJ
JORN
PUUM

4.4.3 UWSIF final reports

UWSIF generates specially formatted reports for NEON. These reports are automatically uploaded to NEON when the analysis is complete.

4.4.4 Storage of Samples

Unless special arrangements are made, UWSIF discards all NEON samples six months after the analysis is complete, and the data have been sent to NEON.

Section 5 Sample Preparation - General Information

5.1 Drying of Samples

After the samples are checked-in, the samples are placed into a 65 ºC drying oven overnight. All sample containers are placed into the drying oven such that the container closures are open so moisture can escape the container. After overnight drying, the containers are fully closed and stored in a dry cabinet until further sample processing.

The drying oven temperature accuracy is checked monthly using a certified temperature meter (Omega HH506A Digital Thermometer). The monthly checks are recorded in the drying oven logbook. The Omega HH506A Digital Thermometer is externally calibrated on an annual basis. The temperature tolerance is ± 2 ºC. In the event that the drying oven does not meet the stated temperature tolerance, the drying oven is labeled as "OUT OF SERVICE" until repairs are made. A secondary drying oven is then checked for accuracy and used until repairs are made on the primary oven.

A drying oven logbook is maintained which contains the following information:

- Job # PI
- Oven #
- Reason
- Date Ground
- Date/time in Oven
- Date/time out Oven
- Monthly temperature checks

5.1.1 Special drying/sterilizing requirements of quarantined samples

Plant and soil samples which are collected in USDA-APHIS restricted areas require additional heating steps in order to sterilize the samples. These USDA-APHIS sterilizing steps occur at a temperature above the temperature requirements as stated in Section 5.1 (see USDA-APHIS Compliance Agreement #WY 1-001 and USDA-APHIS Permit P525-23-107-60002). Samples which go through the USDA-APHIS sterilizing protocol do not need to be further dried. See internal UWSIF Soil Import Standard Operating Procedures.

5.2 Sample Labeling

When a sample is removed from bulk containers for analysis and placed in another container, it is not necessary to mark the individual sample, but it is necessary to label the container used to transport the sample for final isotope analysis. When sub-sampling from a bulk container and transferring the sub-sample to a tin capsule for isotope analysis, the tin capsule need not be labeled, but the 96-well tray holding the tin capsule must be labeled with the appropriate UWSIF identification information.

5.3 Grinding Samples

5.3.1 Grinding methods and sample material

The type of material that a sample is composed of determines the grinding method used. The grinding methods listed in Table 6 can be used for a variety of sample types. The grinding method selected is determined by the nature of the sample. The most commonly used method is noted as "preferred".

Table 6 *Sample type grinding methods*

Grinding method	Soil type	
	Hard or Clayey Soil	General Soil
Mortar/pestle		preferred
Mixing mill	preferred	
Mini Beader	preferred	

5.3.2 Required sample consistency

A properly ground soil sample should have the following characteristics:

- Fine powder
- Homogeneous

5.3.3 Grinding samples: mortar/pestle method

The mortar is a bowl, typically made of hard wood, metal, ceramic, or hard stone. The pestle is a heavy and blunt club-shaped object. The substance to be ground is placed in the mortar, where the pestle is pressed and rotated onto it until the desired texture is achieved.

Materials needed:

70 % ethanol
forceps
labeling tape
laboratory wipes
liquid nitrogen
markers
Ceramic mortar and pestle
spatula
storage containers

Liquid nitrogen is very cold.
PPE required:

Safety glasses
Insulated gloves are also required for additional protection when working with liquid nitrogen
Chemical resistant gloves should be worn when working with ethanol.

Procedure: Grinding with a mortar and pestle

1. Dry the samples overnight in a drying oven (65 ºC).
2. Place a dried sample into the mortar.
3. Add enough liquid nitrogen to cover the sample.
4. Allow the liquid nitrogen to evaporate until there is just enough left to be visible.
5. Quickly grind the sample with the pestle. If done correctly, the sample will be a very fine powder.
6. Remove the ground sample and store in an appropriate container.
7. Clean the mortar and pestle with 70 % ethanol.
8. Return the samples to the dry cabinet after grinding.

Note: some samples do not lend themselves to using this method. For example, some soils are very clayey and become very hard when dried making it difficult to use a mortar and pestle. For such samples, the use of a ball mill or mini beader is more appropriate and much easier.

5.3.4 Grinding samples: mixing mill method

Mixing mills operate on the principle of exposing a sample to a moving ball, which crushes the sample to a fine powder. Samples are placed into stainless steel grinding jars along with a steel ball. The ball mill rapidly shakes the grinding jar. The inertia of the grinding balls causes them to impact the sample within the grinding jars with high energy and pulverizes it. The movement of the grinding jars combined with the movement of the balls result in the intensive mixing of the sample.

Materials needed:

- 70 % ethanol
- labeling tape
- laboratory wipes
- ball mill
- stainless steel grinding jars and stainless-steel balls
- storage containers

PPE required:
Chemical resistant gloves should be worn when using ethanol.
Safety glasses should be worn when using ethanol and using the ball mill.

Procedure: Grinding a sample with a mixing mill

1. Dry the samples overnight in a drying oven (65 ºC).
2. Place the sample (or an appropriately sized subsample) into the bottom of a grinding jar.
3. Drop a stainless-steel ball in with the sample and fit the top on tightly.
4. Use labeling tape to label each jar.
5. Grind the sample using the ball mill for 60 seconds at a frequency of 30 oscillations per second.
6. Check to make sure the sample has been sufficiently ground to a fine powder. If not, grind again.
7. Carefully transfer the ground sample to a suitable container.
8. Label the container.
9. Clean the grinding jar with 70 % ethanol after each use.
10. Return the samples to the dry cabinet after grinding.

5.4 Carbonate Removal from Soils - Acidification Method

If present, inorganic carbon (IC) must be removed from soils to determine the organic carbon (OC) content and isotopic value. A rinse method using 3N phosphoric acid is used to remove the IC from the sample. This method is used for 2 reasons. One is to ensure complete removal of IC from sample and the other is to reduce the risk of causing damage to the analytical instrument if HCl was used.

Precautions:
Loss of organic carbon. Depending on the sample matrix, more soluble forms of organic carbon can be lost during the acidification process.

Loss of nitrogen. When soil is acidified using this method, inorganic nitrogen compounds such as nitrate and ammonium will likely be broken down and lost. If nitrogen isotope analysis of the soil is desired, there must be an additional analysis of non-acidified soil for nitrogen measurements.

Materials needed:

2 mL Micro-centrifuge tubes
50mL centrifuge tubes
3N phosphoric acid
Glass funnel
deionized water
drying oven
Erlenmeyer flask
hardened glass filter paper
ring stand
pH paper
vortex mixer
Centrifuge for 2mL micro-centrifuge tubes
Centrifuge for 50mL centrifuge tubes

5.4.1 Standard Acidification Procedure (2mL micro-centrifuge tube method)

Phosphoric acid is a hazardous corrosive chemical that can cause burns.

PPE required:
Chemically resistant lab coat
Safety glasses
Chemically resistant gloves

Procedure: Acid washing of soil samples for isotopic analysis

Acidification steps (see Figure 8 Soil acidification step flow chart**)**

1. Weigh out ≈100 mg of finely ground soil into clearly labeled 2 mL micro-centrifuge tubes.

2. Cautiously add 3N phosphoric acid to each micro-centrifuge tube up to the 1.5mL mark. Do not close the tube cap. Note: the sample will bubble as carbonate is converted to CO_2.
3. Close the lid when bubbling stops.
4. Using a vortex mixer, mix each tube for 10 seconds. Open the lid after mixing.
5. Cover with foil and place into the fume hood **overnight**.
6. Measure the pH of the supernatant with pH paper.
7. If the pH is <4, skip to step 9.
8. If the pH is >4, decant liquid and repeat steps 2-7 until the pH is <4 or process has been repeated 3 times.
    a. If the pH does not drop below 4 after 3 acid rinses, go to Section 5.4.2.

Rinsing steps (see Figure 9 Soil acidification rinsing step flow chart**)**

Caution: The next steps involve removing the supernatant. Pay close attention to avoid sample loss.

9. Centrifuge at 14000 rpm for 1 min.
10. Carefully decant or filter the supernatant into a waste beaker.
    a. If the supernatant can be decanted cleanly, proceed to step 11
    b. If decanting causes loss of floating material, proceed to filtration steps below.
11. Rinse the sample with distilled water. Vortex until fully mixed (use spatula if material does not mix properly)
12. Centrifuge tube at 14000 rpm for 1 minute.
13. Carefully decant/filter the supernatant into a waste beaker.
14. Repeat steps 11-13, ≈ 6 times.
15. After the final rinse, test the pH to confirm that all the acid has been removed (≈pH 6-7).
16. Carefully decant the remaining supernatant into a waste beaker.
17. Go to step 24.

Filtering steps (see Figure 10 Soil acidification filtering step flow chart**)**

Caution: Only glass filters can be used for filtering. Never use cellulose filters as they can interfere with $\delta^{13}C$ values.

18. Fold glass filter paper in half twice.
19. Place glass funnel, supported by ring stand, in the neck of an Erlenmeyer flask.
20. Place folded glass filter paper in glass funnel.
21. Carefully pour contents of tube onto filter paper.
22. Allow the liquid to pour into Erlenmeyer flask.
23. Rinse the soil on the filter with DI water
24. Place the sample or filter into a drying oven at 65 °C for 24 hours.
    a. If the sample was filtered, carefully scrape the soil from the filter into a labelled glass scintillation vial after it is oven-dried, without removing any pieces of the filter.
25. Grind soil again, following section 5.3 (or 5.6.2 if samples contain *Toxicodendron* spp.)

Reference:
Connin, S.L., R.A. Virginia, and C.P. Chamberlain. 1997. Carbon isotopes reveal soil organic matter dynamics following arid land shrub expansion. Oecologia 110:374–386.

5.4.2 High-Carbonate or Tightly bound Carbonate Soils-Acidification Procedure (50mL Centrifuge Tubes)

The standard acidification procedure can occasionally be insufficient when removing IC, particularly in soils with high IC content or tightly bound IC.

The 50mL Centrifuge Tube procedure should be used if one of the following is true.

- The pH of the supernatant in the standard procedure remains > 4 after 3 acid rinses (step 8).
- Measured **δ^{13}C values are unusually high** (e.g., > −13‰ or higher than expected for the specific soil region).

The following modifications should be made to the standard procedure (section 5.4.1) to ensure complete removal of IC.

- Use 50mL centrifuge tubes instead of 2mL micro-centrifuge tubes
- Weigh same amount of soil ~100mg
- Increase the acid and rinse volumes to 25mL.
- Set centrifuge to 4200 rpm for 4 minutes

Note: A different/larger centrifuge must be used with the 50mL tubes

Acidification steps

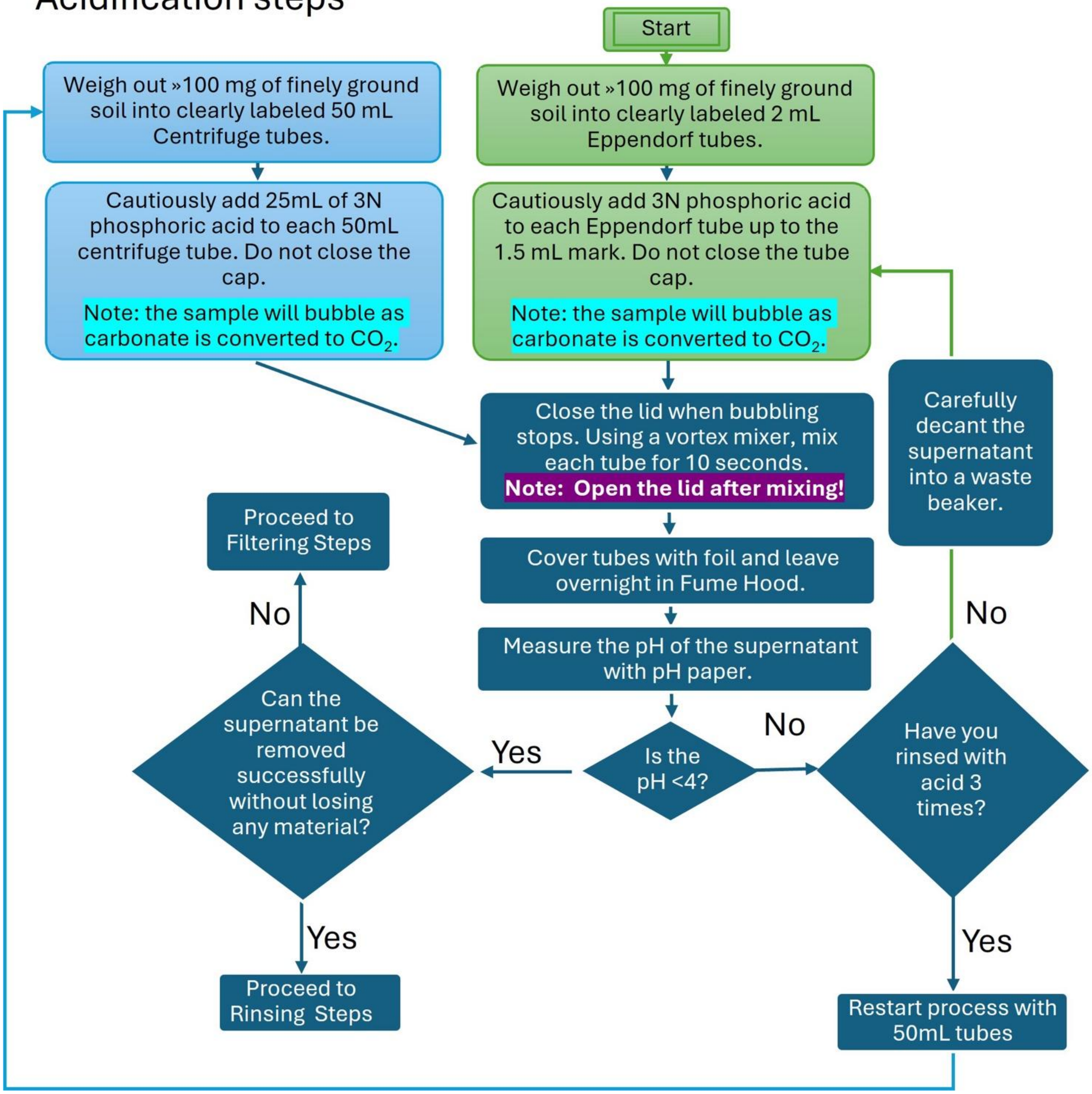


Figure 8 Soil acidification step flow chart

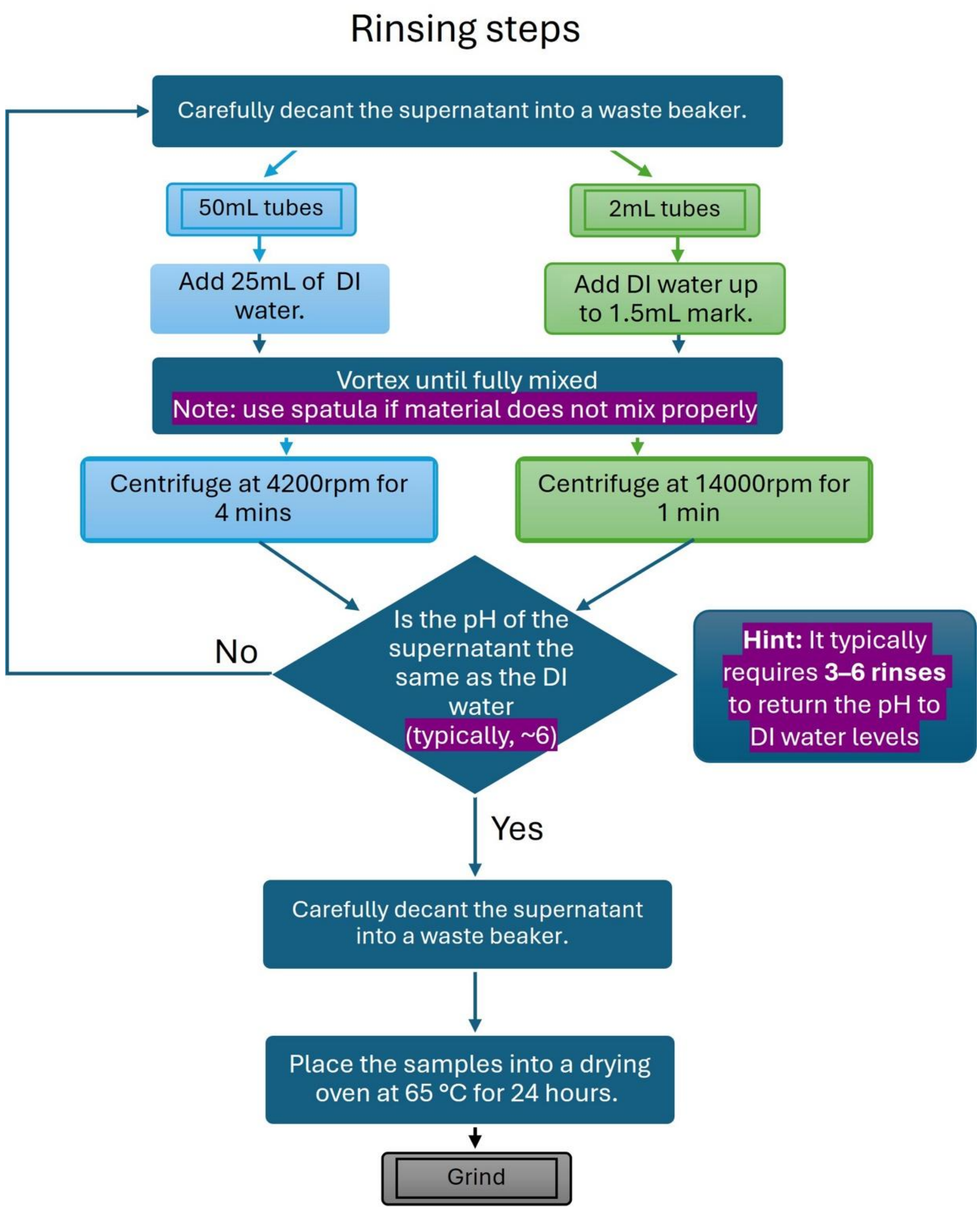


Figure 9 Soil acidification rinsing step flow chart

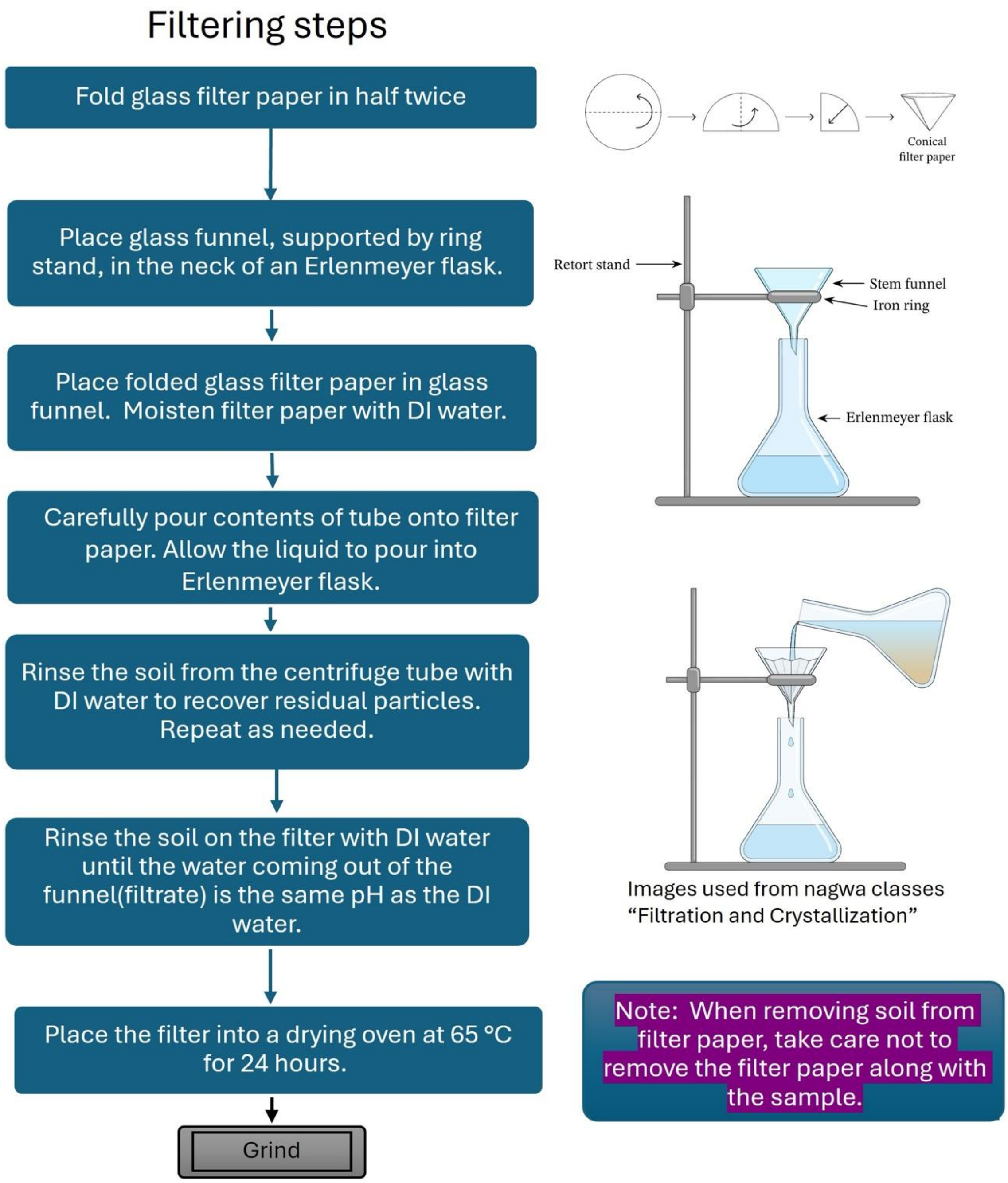


Figure 10 Soil acidification filtering step flow chart

5.5 Weighing Solid Samples

UWSIF uses microbalances to accurately weigh all samples. The Sartorius SE2 microbalance is readable to 0.1 µg and reproducible to ± 0.25 µg. Monthly calibration checks are performed using NIST traceable calibration weights from Henry Troemner, LLC. The Henry Troemner calibration weights have a stated tolerance of ± 5 µg. On an annual basis, all microbalances are calibrated by the Colorado Scale Center (www.coloradoscalecenter.com).

For $\delta^{13}C$ and $\delta^{15}N$ analysis of solid samples, a 2-50 mg subsample of the unknown is weighed into a tin sample container. The weight depends on material type and organic carbon content. If organic carbon content is not known, samples are tested at differing weights to determine the optimal sample weight for reliable detector signal. Typically, samples are recorded to 0.001 mg. If the samples are not analyzed immediately, the weighed samples are placed into a dry storage cabinet.

5.5.1 Weighing procedure

Materials needed:

70 % ethanol
Forceps
Laboratory wipes
Spatula
Tin capsules

Required PPE:
Safety glasses

1. Using only clean forceps, place an empty tin capsule onto the balance pan and tare the balance.
2. After taring, remove the capsule from the balance and place it onto a clean glass plate. Add dried ground sample with a small spatula to the desired weight.
3. Put the filled capsule back onto the balance pan and weigh the sample.
4. Depending on the microbalance used, either type the sample weight into the client sample sheet or press "PRINT" to electronically transfer the weight from the balance to the client sample sheet.
5. Remove the capsule from the balance pan.
6. With flat-jawed forceps, pinch the top third of the capsule.
7. Fold the capsule over to close the top. Do this step twice if possible.
8. Push the folded capsule off the forceps and continue to collapse and fold the capsule until you form a small, dense cube.
9. Inspect tin to make sure there are no protrusions or tears/holes. Wrap in extra tin if needed.
10. Place the cube into a 96-well sample tray, following the client sample sheet.
11. Clean the tools and glass plate.

When the weighing session is complete, store the tray in a dry storage cabinet until EA-IRMS analysis.

5.6 NEON Specific Sample Preparation Procedures

5.6.1 Drying of received samples

After NEON samples are checked-in, the samples are placed into a 65 ºC drying oven overnight. Samples should be dried before any sample prep methods are performed including grinding. All sample containers are placed into the drying oven such that the container closures are open so moisture can escape the container. After overnight drying, the containers are fully closed and stored in a dry desiccant cabinet until further sample processing procedures. If samples need to be transferred to a smaller box, they are clearly labeled with Job# and PI. Containers are stored in desiccant cabinet until all analyses are performed for those samples. Samples are logged in and out using the Desiccant Cabinet Log. The data recorded in the log are, Job #, PI, Quantity, Date/Time In, Desiccant Good, and date/time out.

If samples cannot be stored in a dry desiccant cabinet, they are stored fully closed and placed in a storage cabinet. If any required sample processing steps cannot be performed within **3 days** of the overnight drying and cannot be stored in the desiccant cabinet, the samples are returned to the drying oven for another overnight drying just prior to any additional sample processing. If possible, UWSIF schedules sample processing of NEON samples in order to minimize multiple oven drying steps.

5.6.2 Grinding samples

For all NEON soil samples that require additional grinding at UWSIF, the method used is chosen based on Table 6. Some NEON samples require special grinding procedures. Those methods follow:

Grinding soils marked as containing material from a *Toxicodendron spp*.

Toxicodendron is a genus of flowering plants in the sumac family, *Anacardiaceae*. **Poison ivy**, **poison oak**, and the **lacquer tree** belong to this genus. The genus produces the skin-irritating oil urushiol, which can cause an allergic reaction. Special precautions must be taken during grinding of soil samples that are marked as containing material from a *Toxicodendron* plant. The following grinding methods apply.

NOTE: *Toxicodendron spp*. cause rashes if touched and can be an irritant to the airway and eyes if particulates become airborne.

Mortar/pestle method

Required PPE:
Gloves
Safety glasses
Grinding must be done in fume hood or a dust mask must be worn.
NOTE: Liquid nitrogen is very cold.
Insulated gloves are also required for additional protection when working with liquid nitrogen
Chemical resistant gloves should be worn when working with ethanol.

Materials needed:

70 % ethanol

labeling tape
laboratory wipes
liquid nitrogen
markers
mortar and pestle
nitrile gloves
spatula
storage containers

Procedure: Grinding samples with *Toxicodendron* with a mortar and pestle

1. Dry the samples overnight in a drying oven (65 ºC).
2. Place a dried sample into the mortar.
3. Add enough liquid nitrogen to cover the sample.
4. Allow the liquid nitrogen to evaporate until there is just enough left to be visible.
5. Quickly grind the sample with the pestle. If done correctly, the sample will be a very fine powder.
6. Remove the ground sample and store in original container.
7. Clean the mortar and pestle with 70 % ethanol.
8. Return the samples to the dry cabinet after grinding.

Note: some samples do not lend themselves to using this method. For example some soils are very clayey and become very hard when dried making it difficult to use a mortar and pestle. For such samples, the use of a mixing mill is more appropriate and much easier.

To clean the fume hood:

1. Wipe down the fume hood work surface the 70 % ethanol. Take care that all loose material is collected and disposed of properly.

Mixing mill method

Required PPE:
Gloves
Safety glasses
Grinding must be done in fume hood, or a dust mask must be worn.

Materials needed:

70 % ethanol
face mask
lab coat
labeling tape
laboratory wipes
nitrile gloves

ball mill
stainless steel grinding jars and stainless steel balls
storage containers

Procedure: Grinding samples with *Toxicodendron* with a mixing mill

1. Dry the samples overnight in a drying oven (65 ºC).
2. Place the sample (or an appropriately sized subsample) into the bottom of a grinding jar.
3. Drop a stainless steel ball in with the sample and fit the top on tightly.
4. Use labeling tape to label each jar.
5. Grind the sample using the ball mill for 60 seconds at a frequency of 30 oscillations per second.
6. Check to make sure the sample has been sufficiently ground into fine powder. If not, grind again. **Note: When opening the jar the sample can be pressurized causing the sample to poof into the air. Take extra care when opening the jars with samples that are labeled as containing *Toxicodendron*.**
7. Carefully transfer the ground sample to original container.
8. Clean the grinding jar with 70 % ethanol and laboratory wipes.
9. Return the samples to the dry cabinet after grinding.
10. Wipe down the countertop work surface the 70 % ethanol. Take care that all loose material is collected and disposed of properly.

5.6.3 Carbonate removal

Not all NEON soil samples require carbonate removal. During the first few years of NEON operations, UWSIF tested soil samples from several NEON domains with intermediate pH values to determine if they required acidification to remove carbonates. By comparing carbon isotope values of soils before and after acidification, UWSIF and NEON jointly determined whether or not carbonate removal was necessary for soil samples from that particular NEON site. Below is the list of all sites that are routinely acidified in order to report soil organic carbon.

NEON Domain	NEON site
D02	BLAN
D04	GUAN
D04	LAJA
D06	KONZ
D09	DCFS
D09	NOGP
D09	WOOD
D10	CPER
D10	STER
D11	CLBJ
D11	OAES
D12	YELL
D13	MOAB
D14	SRER
D15	JORN

D15	ONAQ

5.6.4 Determining weights for analysis of soils

The lab uses an Airtable NEON DOMAINS tab that has a list of target weights for soils depending on domain and whether the soil is organic or mineral. This table is a best guess using previous analyses and is not always correct for each site within the domain. If there is any question, a test run should be done at varying weights to determine the ideal amount of sample to achieve optimal detector response.

Section 6 Instrument Operation - General Information

6.1 Delta V

6.1.1 Startup and status

Make sure the following lights are illuminated on the Delta V control panel:
Connection (5)
Vacuum (4)
Secondary (3)
Main (2)
Voltage (10)
Emission (9)

If any of the above lights are not illuminated or are ORANGE, contact the UWSIF staff.

Figure 11. Delta V control panel (from the Thermo user manual).

6.1.2 Initial instrument checks

Go to the following screen on the computer:

Screen: Instrument Control.

1. Select the proper gas (CO_2, N_2) (located in a small box on the lower left on the screen). Wait for the instrument to adjust the source and magnet.
2. Perform a Peak Center (click on the icon located at the upper left of the screen).

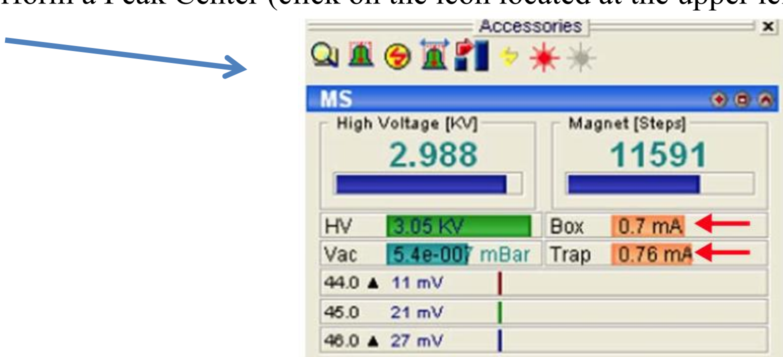


Figure 12. Computer screenshot peak center icon (from the Thermo user manual).

3. Wait for the instrument to finish the peak center routine.

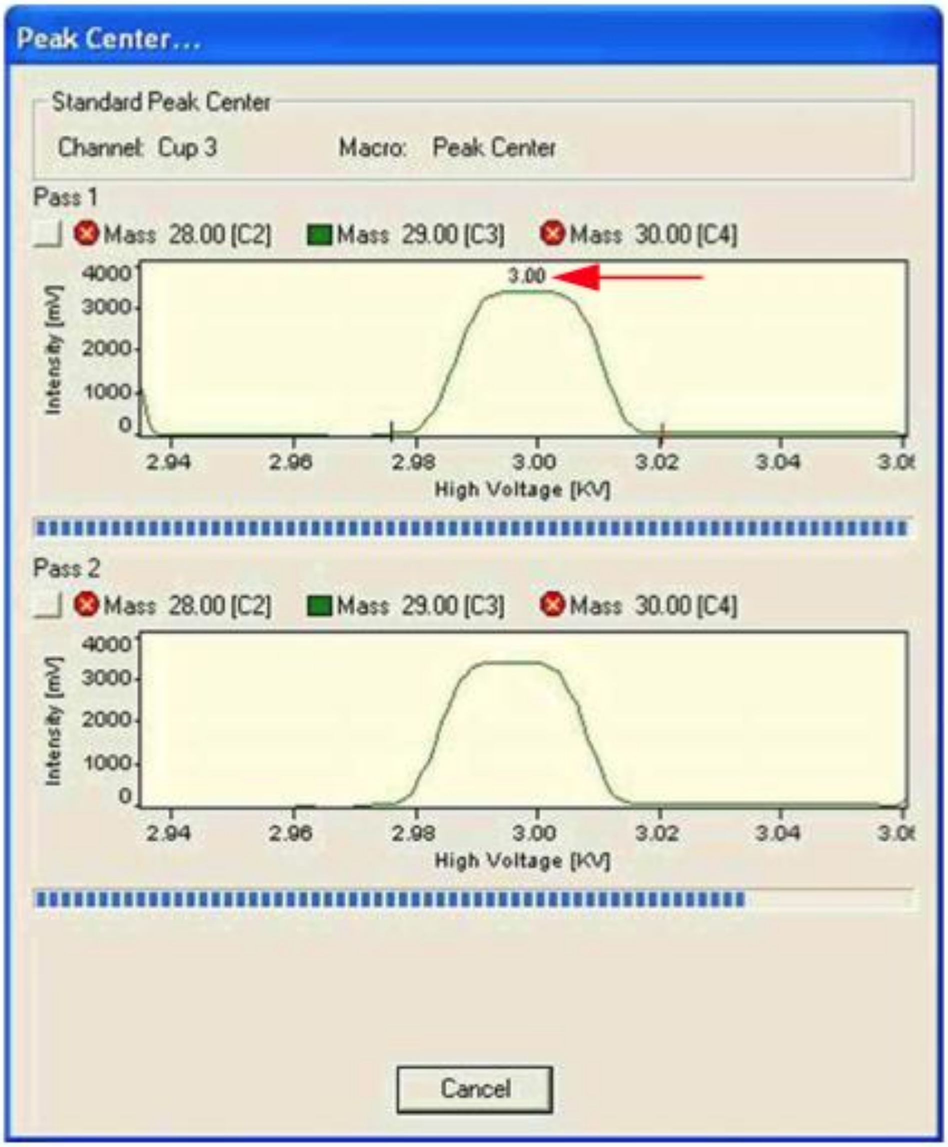


Figure 13. Computer screenshot peak centering (from the Thermo user manual).

6.2 Delta^{Plus}XP

6.2.1 Startup and status

Make sure the following GREEN lights are illuminated on the Delta Plus control panel:

POWER
HOST CONNECTION
TURBO PUMPS > 80%
HIGH VACUUM OK
ACCEL VOLTAGE
EMISSION

If any of the above lights are not illuminated or are RED, contact the UWSIF staff.

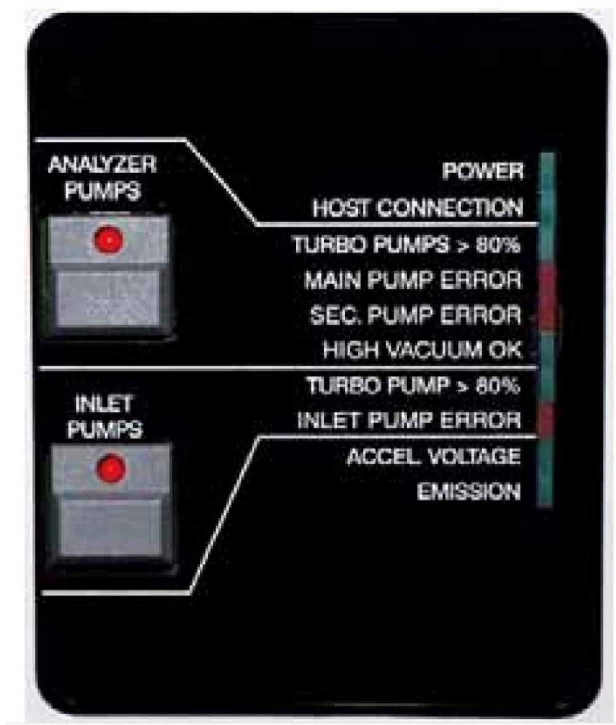


Figure 14. Delta Plus control panel (from the Thermo user manual).

6.2.2 Initial instrument checks

Go to the following screen on the computer:

Screen: Instrument Control.

1. Select the proper gas (CO_2, N_2) (located in a small box on the lower left on the screen). Wait for the instrument to adjust the source and magnet.
2. Perform a Peak Center (click on the icon located at the upper left of the screen).

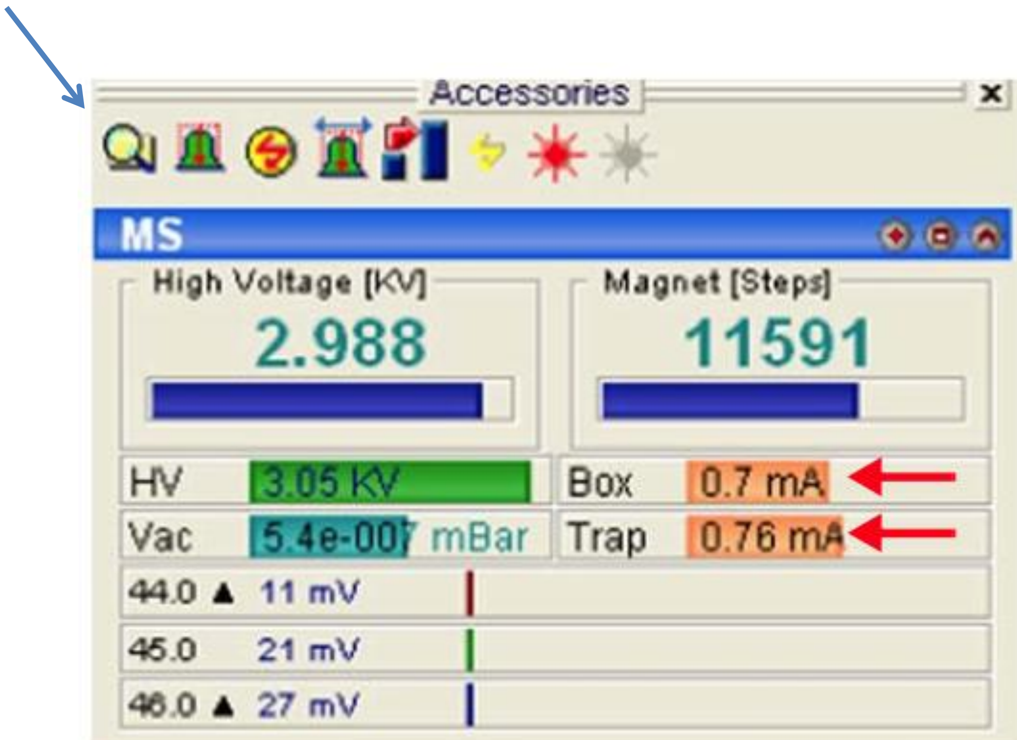


Figure 15. Computer screenshot peak center icon (from the Thermo user manual).

1. Wait for the instrument to finish the peak center routine.

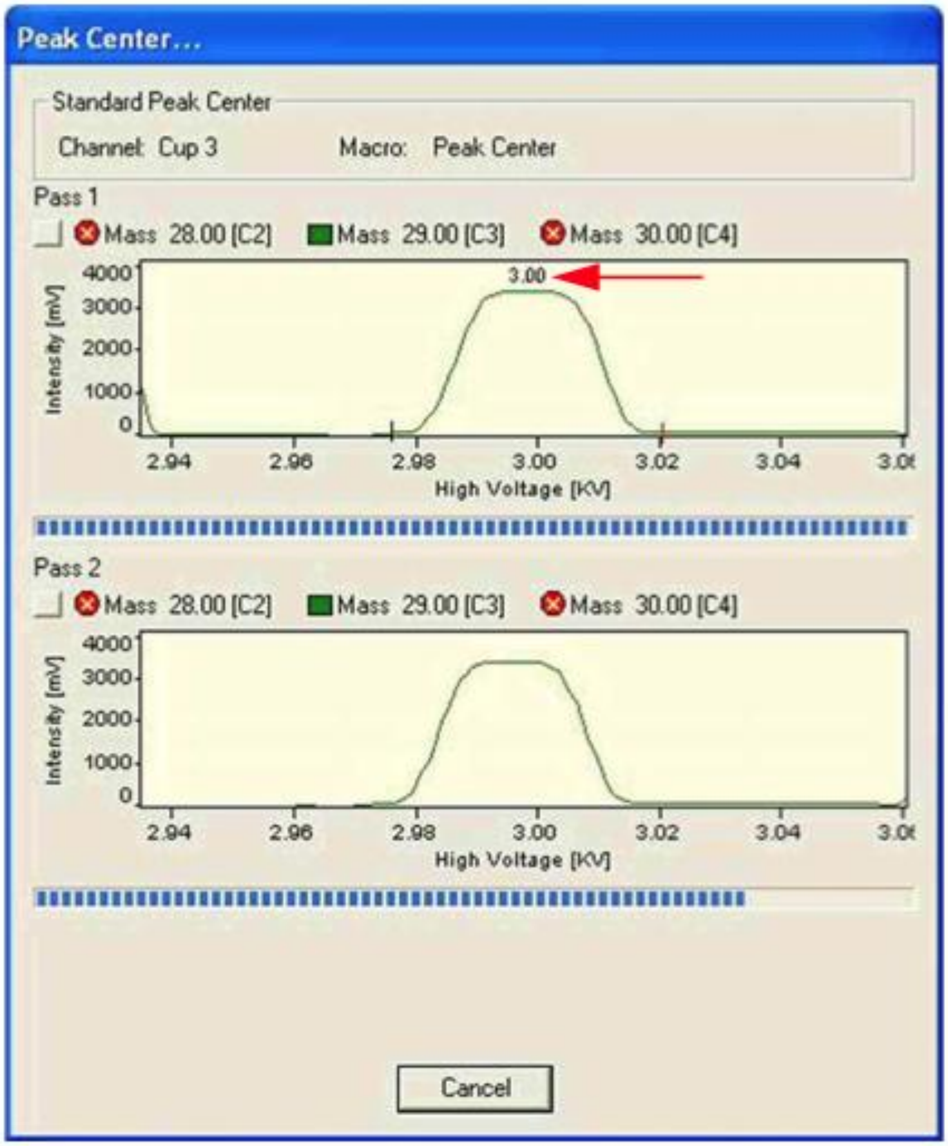


Figure 16. Computer screenshot peak centering (from the Thermo user manual).

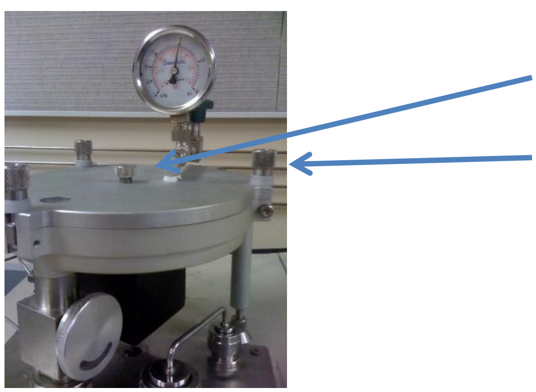
6.3 Zero Blank Autosampler

The samples must not be too large for the carousel and they must not be flat.
The proper weight of the sample should yield peak amplitudes between approximately 2000 and 3000 mV.

1. Release the pressure from the sample carousel. There is a purge valve located on the top of the autosampler lid in the center.
2. After the pressure has been released open the lid by loosening the three thumbscrews. Due to the sealed nature of the lid, always tighten and/or loosen the thumbscrews in a random manner (as you would with lug nuts on a car tire).
3. You may need to change the carousel to 50 or 100 holes depending on the size and or number of the samples. Be sure to change the carousel size on the EA Panel (Costech EA only).
4. Line up the carousel if necessary.
5. Load the samples. The first sample should be located to the right of the combustion chamber.

1st sample position

6. Close the autosampler lid.
7. Leave the purge valve open for 10 minutes to allow for the evacuation of any remaining atmosphere within the sample carousel.



Purge valve

Thumbscrew

8. After the 10-minute purge, close the purge valve and perform a helium leak check.

6.4 ConFlo III

Go to the following screens on the computer and make sure that the listed lights are active (click on the button with the curser to turn on or off):

Screen: ConFlo III

You should select only one reference (Ref I or Ref II).

- Select the reference gas (CO_2).
- Select the reference gas (N_2).

6.5 ConFlo IV

Go to the following screens on the computer and make sure that the listed lights are active (click on the button with the curser to turn on or off):

Screen: ConFlo IV Diagnosis
- MS Cap. light is green.

Screen: ConFlo IV Interface
- Ref I Status On/Off is green.
- Ref II Status On/Off is green.

Screen: ConFlo IV Interface
- Select sample port HF II

Figure 17. Computer screenshot ConFlo IV settings (from the Thermo user manual).

6.6 Instrument On/Offs

- Check the background of mass 28 by right clicking on the center bar within the MS panel located on the left side of the computers display screen and clicking on JUMP TO MASS and entering 28.
- After the instrument changes have taken place, right click the center bar again and perform a PEAK CENTER check. If the peak center is erratic wait for stabilization then retest.
- Run an On/Off test. An On/Off test consists of multiple injections (10) of a reference gas through the ConFlo III or ConFlo IV interface. After the On/Off test has been completed, check the standard deviation of the On/Off peaks. Skip the first two peaks and highlight the rest of the appropriate column (for example ^{13}C or ^{15}N) and then right click the highlighted area and choose the CALCULATE option. If the standard deviation of the last On/Off test preformed is below 0.05 then you are able to move on to the next step. If the value is higher than 0.05, but the standard deviations are decreasing it is possible to start the run. It is important to check the first few reference materials to make sure the values are reasonable. Normally three On/Off sets are more than sufficient.

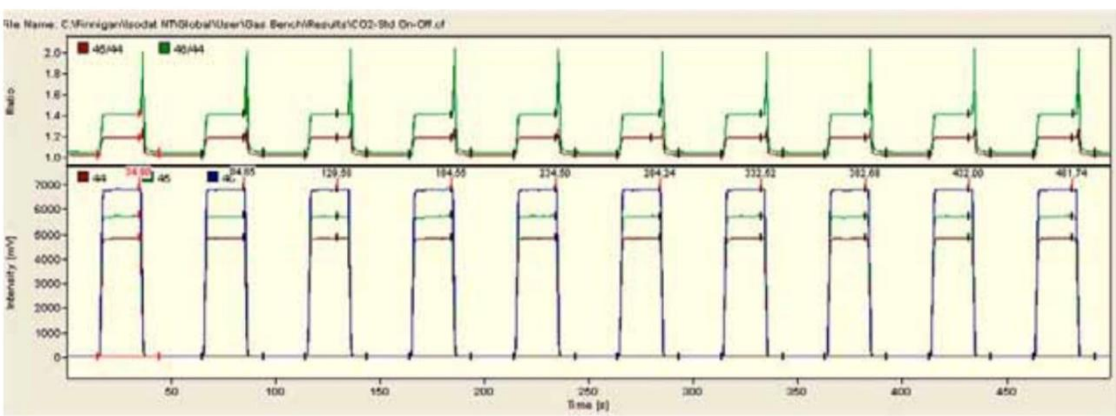


Figure 18. Chromatogram of the On/Off test (from the Thermo user manual).

6.7 NEON Specific Instrument Operation Procedures

There are no special NEON instrument operation procedures.

Section 7 Mass Spectrometer Analysis - General Information

7.1 $\delta^{13}C$ and $\delta^{15}N$ Analysis of Solids

Carbon and nitrogen isotopic analysis of solids is performed using an Elemental analyzer coupled to an Isotope Ratio Mass Spectrometer (EA-IRMS). The elemental analyzer operates based on the principal of flash combustion in which a sample contained within a tin capsule is dropped into a combustion reactor held at 1020 °C. For nitrogen and carbon analysis, the combustion reactor contains chromium oxide for oxidation and silvered cobaltous/cobaltic oxide for removal of halogens and sulfur. When the tin capsule is dropped into helium temporarily enriched with oxygen, the tin ignites and flash combustion occurs. Flash combustion raises the temperature of the sample to >1700 °C. The encapsulated sample, depending on its composition, combusts generating one or more of these gases: N_2, N_xO_x, CO_2, and H_2O. The reduction reactor contains reduced copper wires for the reduction of nitrogen oxides to N_2 and the removal of excess O_2. An adsorption trap containing magnesium perchlorate removes H_2O. The remaining N_2 and CO_2 gases travel through a chromatographic column (Porapak Q) for separation and then move to the ConFlo III or ConFlo IV open split interface.

Instrument temperatures:

Combustion reactor:	1020 °C
Reduction reactor:	650 °C
Chromatographic column:	60 °C

Flow rates:

Helium:	80-100 mL·min^{-1}
Oxygen:	25-30 mL·min^{-1}

Combustion reaction

$$CHNO \underset{O_2}{\overset{1020\ °C}{=}} CO_2 + N_2 + H_2O + O_2 + NO_x + N_xO_x$$

Reduction reaction

$$NO_x + N_xO_x + O_2 \underset{Cu^+}{\overset{650\ °C}{=}} N_2 + CuO$$

7.2 $\delta^{15}N$ Analysis of Solid Samples with High Carbon Content

Nitrogen isotopic analysis of solids with low nitrogen content is performed using an Elemental analyzer coupled to an Isotope Ratio Mass Spectrometer (EA-IRMS).

The elemental analyzer operates based on the principal of flash combustion in which a sample contained within a tin capsule is dropped into a chromium oxide combustion reactor held at 1020 °C. When the tin capsule containing the sample is dropped into helium temporarily enriched with oxygen, the tin ignites and flash combustion occurs. Flash combustion raises the temperature of the sample to >1700 °C. The encapsulated sample combusts, generating one or more gases: N_2, N_xO_x, CO_2, and H_2O. Silvered cobaltous/cobaltic oxide removes halogens and sulfur. The reduction reactor contains reduced copper wires which reduce nitrogen oxides to N_2 and removes excess O_2. **An adsorption trap containing soda lime and magnesium perchlorate removes CO_2 and H_2O**. The remaining N_2 travels through a chromatographic column (Porapak Q) and then moves to the ConFlo III open split interface.

Instrument temperatures:

Combustion reactor:	1020 °C
Reduction reactor:	650 °C
Chromatographic column:	60 °C

Flow rates:

Helium:	80-100 mL·min^{-1}
Oxygen:	25-30 mL·min^{-1}

The analysis of samples which have high carbon and low nitrogen content (high C:N ratios) requires very large samples in order to get sufficient N2 gas for analysis. It is thought that the ionization efficiency of CO_2 is affected by the large amount of CO_2 gas entering the source.

Ionization occurs when the CO_2 molecules are bombarded with electrons:
CO_2 + e- → CO_2+• + 2e-
Later reactions lead to further fragmentation and creation of metastable ions such as CO+ (mass 28).

It is important to have a fast gas exchange within the source. If there is a large amount of CO_2, the gas exchange can be slow due to enhanced surface activity for more polar molecules within the source. Thus, during analysis of the subsequent sample, the CO+ ions are still being ionized and decaying from CO_2 and giving rise to isobaric interferences when measuring nitrogen. (see Handbook of Stable Isotope Analytical Techniques volume-I, P.A. de Groot (Editor), 2004, Chapter 38, Willi A. Brand)

This interference can be seen in the mass 28 backgrounds. Therefore, mass 28 backgrounds are checked for each nitrogen peak. If the mass 28 backgrounds for the sample exceeds 20mV above the mass 28 baseline, both the sample and previous sample should be rerun using a CO_2 absorption trap.

A chemical absorption trap is used to remove CO_2 produced in the combustion reactor. This makes possible the measurement of samples with high C:N ratios without isobaric interference. If both carbon and nitrogen isotope values are desired, two analyses are required, with the carbon data generated using Section 7.1 and the nitrogen data generated using Section 7.2.

7.3 Types of Analysis

<u>Analysis 1:</u> 010 EA-IRMS organic d15N, d13C, C% & N% - This method reports the carbon and nitrogen isotope values as well as their carbon and nitrogen content.

<u>Uses:</u>

Routine analyses

Used when no special precautions are needed for high carbon content.

<u>Analysis 2</u>: 009 EA-IRMS organic d15N & N% - This method reports the nitrogen isotope value and nitrogen content.

<u>Uses:</u>

Samples with High C:N ratios

It is necessary to use a CO_2 trap for samples with high C:N ratios to minimize the interference seen by samples with high carbon content, thus only nitrogen is measured.

Soils containing carbonate, untreated sample

Since we do not use the carbon values from bulk untreated samples containing carbonate, and such samples often have a higher carbon concentration, it is beneficial—but not always necessary—to use a CO_2 trap to minimize the interference seen by samples with high total carbon content.

<u>Analysis 3:</u> 008 EA-IRMS organic d13C & C% - This method reports the carbon isotope values and carbon content.

<u>Uses:</u>

<u>Nitrogen values already measured or not needed;</u>

<u>Soils containing carbonate, treated samples</u>

For this method the samples can be weighed at a lower weight and the carbon peak is not diluted with helium during IRMS analysis.

7.4 NEON Specific Mass Spectrometer Analysis

There are no special NEON mass spectrometer protocols.

Section 8 Quality Assurance - General Information

8.1 Laboratory Reference Materials

A set of UWSIF laboratory reference materials has been developed and calibrated against IAEA, NIST, and USGS primary reference materials. Calibration on these internal reference materials is conducted annually.

Every analytical run at UWSIF contains multiple quality assurance (QA) and quality control (QC) reference materials. Table 7 defines the analytical roles of these materials.

Table 7 *UWSIF QAQC reference materials defined*

Quality assurance	The quality assurance reference materials are calibrated with international standards using a two-point calibration. These laboratory reference materials are used in routine analyses distributed at the beginning, middle and end of each run. Besides providing a two-point normalization, they help to keep a check on issues like machine drift, non-linearity related to sample size, and column degradation.
Quality control	These reference materials provide a check on the two-point normalization produced by the normalization reference materials. The reference materials have similar chemical composition to the unknown samples being analyzed. They provide a means to check for consistency between runs.

For bulk nitrogen and/or carbon analyses, UWSIF routinely uses a select group of reference materials. About 20 % of all samples analyzed in the lab are quality assurance and quality control reference materials.

Table 8 lists the UWSIF laboratory reference materials commonly used for quality assurance and quality control of nitrogen and/or carbon isotope analysis of plants and soil. The delta values are expressed in ‰.

Table 8 *UWSIF QAQC reference material uses*

UWSIF quality assurance and quality control reference materials currently in use, with known $\delta^{13}C$ and $\delta^{15}N$ values				
Reference material	Analytical use	Material	$\delta^{13}C_{VPDB}$	$\delta^{15}N_{AIR\text{-}N2}$
UWSIF39-Glut 2	quality assurance	glutamic acid	+24.36	+27.88
UWSIF41-Glut 3	quality assurance	glutamic acid	-28.60	-2.38
UWSIF48-Glut 4	quality assurance	glutamic acid	-23.27	-2.86
UWSIF46-Soil 3	quality control	soil	-23.55	+6.12
UWSIF47-Alfalfa2	quality control	plant	-28.43	-0.33

8.2 Handling of Reference Materials

8.2.1 Control and distribution of reference materials

The daily use of all UWSIF reference materials is strictly controlled. The following rules apply:

- International reference materials are distributed only by the Laboratory Manager and are used only for the standardization of UWSIF quality assurance and quality control reference materials.
- Only one reference material (international, quality assurance or quality control) may be in use at any time. At no time should multiple reference material containers be open.

8.2.2 Storage of reference materials

All reference materials (international, UWSIF) are stored in a desiccant cabinet. Indicating Drierite is the hydroscopic compound used to maintain a dry atmosphere inside the desiccant cabinet. Indicating Drierite is blue when dry and changes to pink upon absorption of moisture. The color change is pronounced and clearly visible. The desiccant is checked on a monthly basis to assure that it is still actively drying the cabinet. When the bulk of the Drierite in the desiccant cabinet has turned pink, the drying agent is replaced. The replacement date of the Drierite is recorded on the door of the desiccator.

8.3 UWSIF QAQC Reference Material Certification

On an annual basis, all UWSIF quality assurance and quality control reference materials are compared to appropriate international reference materials to confirm that their accepted isotope values have not changed. Long-term records of these comparisons are maintained for quality assurance purposes. The acceptable criteria of the annual reference materials calibration are based on the average standard deviations of all the annual calibrations of the appropriate international reference materials used for the calibration.

The annual calibration delta value for UWSIF QA and QC reference materials must be within 0.15 ‰ for ^{13}C and 0.20 ‰ for ^{15}N of the accepted known value of the reference material. If the annual calibration of any reference material does not meet the stated criteria, a second calibration is performed. If the material still does not meet acceptable criteria, a new aliquot of the reference material is taken from the bulk supply and a new calibration is performed. If the new aliquot does not meet the stated calibration criteria, the reference material is retired and a new reference material is acquired and calibrated versus internationally accepted reference materials.

Table 9 lists the international reference materials used for calibration of UWSIF laboratory reference materials. Delta values are in ‰.

Table 9 *UWSIF recertification reference materials*

International reference materials with known δ13C values (solid)			
NIST code	Name	Material	$\delta^{13}C_{VPDB}$*
RM 8573	USGS40	glutamic acid	-26.39
	USGS41a	glutamic acid	+36.55
International reference materials with known $\delta^{15}N$ values (solid)			
NIST code	Name	Material	$\delta^{15}N_{AIR\text{-}N2}$*
RM 8549	IAEA-NO3	potassium nitrate	+4.7
RM 8573	USGS40	glutamic acid	-4.52
	USGS41a	glutamic acid	+47.55

*Brand, WA, Coplen T, Vogl J, Rosner M, Prohaska T; Assessment of international reference materials for isotope-ratio analysis; Pure Appl. Chem., 2014; (86(3): 425-457.

8.4 UWSIF New Internal Reference Material Certification

New internal UWSIF reference materials are prepared for certification using the following steps (as appropriate):

1. The new reference material is dried at 65 °C for 24 hours.
2. The dried material is ground using a mixing mill to < 40 mesh.
3. The ground material is further dried at 65 °C for 24 hours.
4. All ground and dried material are combined into one container.
5. The combined material is shaken to further mix the material.
6. The combined material is stored in a dark, cool location.

Certification steps:

Step 1 - Initial isotopic composition and homogeneity

The new reference material is analyzed for isotopic composition and homogeneity using the appropriate instrumentation (EA-IRMS, TC/EA-IRMS, GC-IRMS). The following analytical criteria are required:

1. A minimum of three analytical runs.
2. International reference materials are included in each analytical run.
3. Each international reference material is analyzed a minimum of 3 times within each run.
4. A minimum of 5 replicates of the new reference material are analyzed in each run.

Step 2 – Determining **accepted** known values

1. Accepted values for internal reference materials are determined by the average values from the initial composition and homogeneity tests.

Step 3 – Verification of values by Independent laboratory analysis

The new reference material is sent to at least 2 selected independent analytical laboratories for analysis. The following requests are made of each laboratory:

1. Analyze a minimum of 5 replicates of the new UWSIF reference material.
2. Provide UWSIF with the source/identification and known values of the laboratory reference materials used during the analysis.
3. Provide UWSIF with the corrected isotopic data for all samples, including the laboratory reference materials used.
4. A thorough investigation of analysis and calculations is done to determine if independent lab analyses are acceptable.
5. If values from independent labs differ by more than 0.15 ‰ for ^{13}C and 0.20 ‰ for ^{15}N, then a more thorough investigation is done to determine why values differ and if they should be used.
    - If a legitimate reason cannot be found as to why values differ from lab to lab a new independent analysis must be done.

8.5 Carbon and Nitrogen Quality Control Criteria

8.5.1 Carbon isotope analysis quality control

The quality assurance of carbon isotope analysis is based on the standard uncertainty of the known value of the quality control reference materials analyzed during the analytical run. The standard uncertainty (1-sigma) is calculated from multiple analyses of the quality control reference materials. If the standard uncertainty is greater than 0.15 ‰, the unknowns are re-analyzed until the 2-sigma expanded standard uncertainty of the result is less than 0.3 ‰. The isotopic composition is reported in per mil relative to VPDB on a scale such that USGS40 and USGS41 are -26.39 ‰ and +37.63 ‰, respectively.

8.5.2 Nitrogen isotope analysis quality control

The quality assurance of nitrogen isotope analysis is based on the standard uncertainty of the known value of the quality control reference materials analyzed during the analytical run. The standard uncertainty (1-sigma) is calculated on multiple analyses of the quality control reference materials. If the standard uncertainty is greater than 0.2 ‰, the unknowns are re-analyzed until the 2-sigma expanded standard uncertainty of the result is less than 0.4 ‰. The isotopic composition is reported in per mil relative to AIR-N_2 on a scale such that USGS40 and USGS41 are -4.52 ‰ and +47.57 ‰, respectively.

8.5.3 Carbon elemental composition quality control

The quality assurance of carbon elemental composition analysis is based on the standard uncertainty of the known value of the quality control reference materials analyzed during the analytical run. The standard uncertainty is calculated from multiple analyses of the quality control reference materials. For UWSIF46 (soil 3), if the standard uncertainty is greater than 0.05 %, the unknowns are re-analyzed until the 2-sigma expanded standard uncertainty of the reference material carbon elemental composition is less than 0.1 %. For UWSIF047 (alfalfa2), if the standard uncertainty is greater than 0.9 %, the unknowns are re-analyzed until the 2-sigma expanded standard uncertainty of the reference material elemental composition is less than 1.8 %. The elemental composition is reported in percent relative to USGS40 such that the carbon elemental composition of USGS40 is 40.78 %.

8.5.4 Nitrogen elemental composition quality control

The quality assurance of nitrogen elemental composition analysis is based on the standard uncertainty of the known value of the quality control reference materials analyzed during the analytical run. The standard uncertainty is calculated on multiple analyses of the quality control reference materials. For UWSIF46 (soil 3), if the standard uncertainty is greater than 0.006 %, the unknowns are re-analyzed until the 2-sigma expanded standard uncertainty of the reference material nitrogen elemental composition is less than 0.012 %. For UWSIF47 (alfalfa2), if the standard uncertainty is greater than 0.08 %, the unknowns are re-analyzed until the 2-sigma expanded standard uncertainty of the reference material elemental composition is less than 0.16 %. The elemental composition is reported in percent relative to USGS40 such that the nitrogen elemental composition of USGS40 is 9.51 %.

8.6 Quality Assessment Procedures

University of Wyoming Stable Isotope Facility has developed a QA/QC script (Wyo-Iso) to perform elemental composition calculations, common corrections, isotope normalization and perform routine QA/QC checks. The script automatically flags any data that does not pass the QA/QC criteria (see section 8.5). Implementation of the script ensures consistent, reproducible calculations, while minimizing human bias and errors. A final manual review is performed to ensure the script has not erroneously removed data or applied incorrect corrections.

The scripts were developed using the git version control system. They are hosted on gitlab at https://gitlab.arcc.uwyo.edu/stable-isotope-facility/ea-irms_cns

More information on the Wyo-Iso script can be found in the internal UWSIF How to Process EA-IRMS Data from IsoDat SOP.

In order to ensure that all QC criteria are met for every analytical run, the following information is generated and stored via the Wyo-Iso script:

1. Sample metadata (e.g., type of sample, user information, etc.).
2. The raw results of the analytical run, including all mass spectrometric data.
3. Reduced analytical results including normalized delta values using the quality assurance and quality control reference materials.
4. An assessment of the quality of the analytical run through an analysis of the quality assurance and quality control reference materials.
5. An assessment of the quality of the analytical run through an analysis of the long-term standards database.

The final analytical report contains a complete record of how the unknown samples and the QA and QC laboratory reference materials were analyzed and reduced. The report also provides a record of what correction factors were used for data reduction, and a quality assurance evaluation.

8.6.1 QA/QC Data reduction

Samples are analyzed in batches with each batch containing quality assurance and quality control reference materials. These reference materials are located at the beginning, middle, and end of the analytical run.

8.6.2 Acceptance criteria for reference materials

The Wyo-Iso script uses the statistical procedures listed below to ensure that the reference materials meet all stated criteria:

1. If the standard deviation of the reference materials is less than or equal to the accepted tolerance for $\delta^{13}C$ or $\delta^{15}N$ (see sections 8.5.1 and 8.5.2), the mean delta value of the reference materials is used.
2. Measurements will be flagged as outliers if the values of the reference materials meet the two following conditions:
    a. Deviate from a meaningful range of raw values. The meaningful range prevents finding outliers on a very small scale that are not problematic. We define the meaningful range as 4 times the standard deviation (sd) of a long-term series of measurements of the same material on the same instrument. For each standard, the meaningful range will be defined as the tray median +/- 2 long-term sd.
    b. Deviate from the other measurements of the same material in this tray. This outlier is determined with a Dixon's Q test. Only one outlier can be detected by this method.
3. If any point is determined to be an outlier, the point is excluded for the remainder of the script analysis and the mean delta value of the reference material is recalculated. If the standard deviation of this recalculation is less than or equal to the accepted tolerance for $\delta^{13}C$ or $\delta^{15}N$, the mean delta value from this recalculation is used.
4. Any flagged reference material sample is examined for abnormalities (e.g., poor chromatography, high background, etc.). QC samples found to have abnormalities are looked at closely to determine whether or not the sample can be removed. If a sample is removed, the mean delta value is recalculated.
5. If none of the above apply, the results are rejected, and corrective action is required.

8.6.3 Manual Verification of Script Output.

The analyst examines the following from the output files:

1. Checks data quality via script by observing the following.
    a. Amplitudes, areas, backgrounds
    b. Confirms flagged samples based on the script criteria
    c. Confirms outliers detected
    d. Confirms drift and linearity corrections that were applied were needed.
2. Reviews precision and accuracy tables to ensure run passes QAQC criteria

3. Reviews the flags and determines which samples need to be re-analyzed to achieve acceptable results.

8.6.4 QA/QC Corrective action

If the analyst is unable to solve a problem with the reference material data, the analyst contacts the Laboratory Manager. The Laboratory Manager re-evaluates the data to determine whether or not the re-analysis of some of the samples or the re-analysis of the entire sample set is appropriate.

If a re-analysis of the samples does not give satisfactory results, the script flags the samples and averages all the reference material data and reports the mean value. The script flags the problem materials on the final report, thus advising the client of the problem

8.7 Long-term Reference Material Data Collection Procedures

Once the script data has been verified, the isotope and elemental composition data (if applicable) for all reference material in an individual analytical run is uploaded into an online Structured Query Language (SQL) database via the Wyo-Iso script. The Wyo-Iso database maintains individual run data for each reference material. Long-term averages and standard deviations of isotopic and elemental composition, and sample counts are easily accessed using the reports in Wyo-Iso script.

Using the script to upload finalized reference material data reduces the risk of human error and enables review of each analysis to ensure that the values align with long-term data. Because the script updates that database automatically, the long-term dataset is always current.

More information about the online database can be found in the internal UWSIF How to Manage the SIF Online Database SOP.

8.8 NEON Specific Quality Assurance Procedures

8.8.1 Carbon isotope analysis quality control

For NEON samples, if the stated precision of the quality control reference materials cannot be achieved after 2 re-runs due to systematic issues with the samples, data are still reported to NEON but a quality flag is added to the NEON report for all records in that analytical batch. The quality flag(s) for isotopic and elemental composition (isotopeAccuracyQF, percentAccuracyQF) in the NEON report are changed from 0 (OK, no issue) to 2 (carbon run QA materials do not meet acceptance criteria) or 3 (carbon and nitrogen run QA materials do not meet acceptance criteria), as appropriate.

8.8.2 Nitrogen isotope analysis quality control

For NEON samples, if the stated precision of the quality control reference materials cannot be achieved after 2 re-runs due to systematic issues with the samples, data are still reported to NEON but a quality flag is added to the NEON report for all records in that analytical batch. The quality flag(s) for isotopic and elemental composition (isotopeAccuracyQF, percentAccuracyQF) in the NEON report are changed from 0 (OK, no issue) to 1 (nitrogen run QA materials do not meet acceptance criteria) or 3 (carbon and nitrogen run QA materials do not meet acceptance criteria), as appropriate.

8.8.3 Final analytical reports

The Wyo-Iso final report is generated for all NEON samples, but is not sent to NEON. NEON requires a specific format for uploading data to the NEON data portal. A separate NEON formatted report is generated and sent to NEON.

8.8.4 Corrective action, NEON rerun protocol

For NEON samples, if the analyst is unable to solve a problem with the reference material data, the analyst contacts the Laboratory Manager. The Laboratory Manager re-evaluates the data to determine whether or not the re-analysis of some of the samples or the re-analysis of the entire sample set is appropriate. If a re-analysis of the samples does not give satisfactory results, the analyst averages all the QC data and reports the mean value. The data are sent to NEON with the flags described above to indicate that a required QA parameter (isotopic or elemental composition) has not been met.

On occasion, UWSIF will flag the data from an unknown sample which appears to be wrong (e.g., spurious isotope or weight % values). These samples are generally rerun for clarification. For NEON unknowns, UWSIF will rerun the samples a maximum of 2 additional times. If, after 2 reruns, the data are still not acceptable, they are sent to NEON with the NEON report using record-specific flags (cnIsotopeQF, cnPercentQF) to indicate that a required parameter (isotopic or elemental composition) is outside of laboratory qaqc tolerance and a remark is added to state that the sample was analyzed 3 times.

Section 9 Calculations and Corrections - General Information

9.1 Standard Calculations and Corrections

Actual isotope ratios are very hard to measure accurately. Researchers have found that the measurement of an *actual* isotope ratio can vary between instruments or laboratories or even on different days on the same instrument. To avoid this problem, isotope ratios are generally expressed as *relative* ratios rather than *actual* ratios. A *relative* isotope ratio is found by measuring the isotopic abundance of a sample **and** the isotopic abundance of a known reference material on the same instrument at the same time. Because the final data are expressed as a ratio, any change in the instrument such as changing sensitivity will appear in the analysis of both the sample and the reference material and will cancel in the final data. Analyzing samples in this way makes it possible to compare the isotopic data between instruments and between other laboratories.

Typically, IRMS factory software automatically calculates raw delta values. This process begins with the integration of the sample and working gas peaks. From those areas, isotopic ratios are calculated. Table 10 lists the common calculations and corrections.

Table 10 *Standard Isodat software calculations and corrections*

Delta calculation	Instrument software automatically calculates delta values, based on the measured ratios of the reference and unknown gases.
^{17}O correction: $\delta^{13}C$ determination in CO_2 gas	The mass resolution of typical IRMS instruments does not allow for the separation of isobaric species within the mass spectrometer ($^{13}C^{16}O_2$ and $^{12}C^{17}O^{16}O$ both have $m/z = 45$). When isobaric species interfere with the masses used to determine isotope ratios, a ^{17}O correction for carbon isotope ratios of CO_2 is automatically applied.

9.2 The Delta Calculation

Stable isotopes are usually reported in delta notation (δ), a value that has the units of per mil (‰). Delta values are not absolute isotope abundances but the difference between a sample and an international standard.

Isotopic concentrations are expressed as the difference between the measured ratios of the sample and reference divided by the measured ratio of the reference using the formula:

$$\delta_{\mathrm{sample(raw)}} = \left[\frac{R_{\mathrm{sample}}}{R_{\mathrm{standard}}} - 1\right] \times 1000$$

where: R_{sample} = the measured isotope ratio (abundance) of the unknown sample
$R_{standard}$ = the defined isotope ratio of the international standard
$\delta_{sample(raw)}$ = the raw delta of the sample

9.2.1 Reference gases

Reference gases are required by an IRMS for analyses. All raw delta values obtained for the sample are place onto an internationally accepted scale by the reference gas (Section 9.2). Although it is recommended that a reference gas be assigned it's known delta value(s), it is not necessary if known reference materials which have been placed on an accepted international scale are analyzed during the analysis of the unknowns. The reference gas must have a defined delta value(s) if the analysis protocol does not use known reference materials for normalization. The unknown sample delta values are placed onto an internationally defined standard scale as follows:

$$\delta_{\mathrm{sample(true)}} = \delta_{\mathrm{sample(RM(raw))}} + \delta_{\mathrm{RM(true(i))}} + \left(\frac{\delta_{\mathrm{sample(RM(raw))}} \times \delta_{\mathrm{RM(true(i))}}}{1000}\right)$$

where: $\delta_{sample(true)}$ = the delta value of the sample corrected to an international standard.
$\delta_{sample(RM(raw))}$ = the measured delta value of the sample versus the gas reference material.
$\delta_{RM(true(i))}$ = the known delta value of the gas reference material versus the international standard.

9.3 Commonly Used Corrections

Data correction are procedures for processing and/or converting data obtained directly from the IRMS into data suitable for researchers and for comparison with other laboratories. Some of the important conversions in the data reduction process are linearization (area corrections), IRMS and sample preparation corrections, and the normalization of the raw data to known reference materials. Note: before applying any correction to the data, it must be confirmed that the required correction is systemic and effects all reference materials. All of these factors are integrated into the Wyo-Iso script (see the internal UWSIF How to Process EA-IRMS Data from IsoDat SOP). Table 11 lists common corrections that should be considered when reducing the raw data.

Table 11 *Common user corrections*

Blank	If there is a blank associated with the isotopic measurement, the magnitude and isotopic composition of the blank should be determined. A blank correction can then be applied to the measured data. The average peak area and δ value of the blank measurement can be used to correct the data for any blank contribution.
Linearization	If there exists a relationship between the measured peak area of the laboratory reference material and the known isotopic value, then the results should be linearized to remove this trend. This must be done before the results are normalized.
Drift	If there exists a relationship between time and the known isotopic or elemental composition of the reference material, then the results should be corrected for drift over time. This must be done before the results are normalized.
Memory	Memory effects come about when there is signal carryover between the previous sample and the current sample. Memory effects occur mostly when labeled materials are analyzed.
Scale normalization	Scale normalization is a process that places the raw delta data onto the zero-point of a δ scale.
Weight percent	Measured areas are used to calculate weight percents using a known standard calibration or a kfactor calculation.

9.3.1 Scale normalization

Scale normalization adjusts an isotope-delta scale so that the measured delta values of two reference materials are set to their defined or accepted δ values. Scale normalization accomplishes two things: (1) it anchors the data to an accepted isotopic scale, and (2) it compensates for daily changes in the responses of the instrument. Most analytical laboratories use a two-point normalization.

Using two reference materials, a linear equation of the form

$$\delta_{\mathrm{sample(true)}} = m \times \delta_{\mathrm{sample(raw)}} + b$$

is generated, relating the measured and known isotope values of the two reference materials.

The process is as follows:

Step 1) Derive the slope of the normalizing equation:

$$m = \left[\frac{\delta_{\mathrm{RM1(true)}} - \delta_{\mathrm{RM2(true)}}}{\delta_{\mathrm{RM1(raw)}} - \delta_{\mathrm{RM2(raw)}}}\right]$$

where: $\delta_{\mathrm{RM1(true)}}$ = the accepted delta value of reference material 1
$\delta_{\mathrm{RM2(true)}}$ = the accepted delta value of reference material 2
$\delta_{\mathrm{rRM1(raw)}}$ = the measured delta value of reference material 1
$\delta_{\mathrm{RM2(raw)}}$ = the measured delta value of reference material 2
m = the slope of the normalizing equation

Step 2) Calculate the y-intercept of the normalizing equation:

$$b = \delta_{\mathrm{RM1(true)}} - \left[m \times \delta_{\mathrm{RM1(raw)}}\right]$$

where: $\delta_{\mathrm{RM1(true)}}$ = the accepted delta value of reference material 1
$\delta_{\mathrm{RM1(raw)}}$= the measured delta value of reference material 1
b = the y-intercept of the normalizing equation

Step 3) Normalize the data using the normalizing equation:

$$\delta_{\mathrm{sample(scale)}} = \mathrm{m} \times \delta_{\mathrm{sample(raw)}} + b$$

where: $\delta_{\mathrm{sample(raw)}}$= the measured delta value of sample
$\delta_{\mathrm{sample(scale)}}$ = the scale normalized delta value of sample

9.3.2 Single-point offset

In the event that only a single reference material is used for isotope corrections, apply a simple offset correction. This offset correction is calculated as follows:

$$\delta_{\mathrm{sample(offset)}} = \delta_{\mathrm{RM(true)}} - \left[\left(\sum \delta_{\mathrm{RM(raw)1}}, \ldots, \delta_{\mathrm{RM(raw)n}}\right) \div n\right]$$

where:

$\delta_{\mathrm{RM(true)}}$ = the accepted delta value of reference material
$\delta_{\mathrm{RM(raw)n}}$ = the measured delta values of reference material
n = the number of measured values of the reference material
$\delta_{\mathrm{sample(offset)}}$ = the offset corrected delta value of the sample

9.3.3 Linearity (area)

Corrections for instrument non-linearity are based on analyzing a reference material of known isotopic composition using various weights that span the expected weight percent range of the samples. The linearity correction equation has the form:

$$\delta_{sample(linear)} = \delta_{sample(raw)} - (m \times Area + b)$$

where: $\delta_{\text{sample(raw)}}$ = the measured isotope value of the sample
$Area$ = the measured area of the sample peak
$\delta_{\text{sample(linear)}}$ = the linear corrected delta value of the sample
m = the slope of the linear correction regression trendline
b = the y-intercept of the linear correction regression trendline.

To correct the data:

Step 1) Calculate residuals of the reference material using the equation:

$$\delta_{\text{RM(residual)}} = \delta_{\text{RM(raw)}} - \delta_{\text{RM(true)}}$$

where: $\delta_{\text{RM(true)}}$ = the accepted isotope value of the reference material
$\delta_{\text{RM(raw)}}$ = the measured isotope value of the reference material
$\delta_{\text{RM(residual)}}$ = the linear residual of the reference material

Step 2) Derive a regression trendline of the reference material peak area vs. the reference material linear residuals:

where: m = the slope of the regression trendline
b = the y-intercept of the regression trendline
R^2 = the coefficient of determination of the regression

At UWSIF, if the value of R^2 is <0.7, no correction is applied. If the R^2 is >0.7, the following calculation is performed:

Step 3) Calculate the residual corrected isotope values of the reference material and unknowns using the equation:

$$\delta_{\text{sample(linear)}} = \delta_{\text{sample(raw)}} + (Area \times m + b)$$

where: $Area$ = the measured peak area of the sample
$\delta_{\text{sample(raw)}}$ = the measured isotope value of the sample
$\delta_{\text{sample(linear)}}$ = the linear residual corrected isotope value of the sample

9.3.4 Drift

Corrections for instrument drift are based on the delta values of a reference material over time. The drift correction equation has the form:

$$\delta_{sample(drift)} = \delta_{sample(raw)} - (m \times Time + b)$$

where: $\delta_{\text{sample(raw)}}$ = the measured isotope value of the sample
$Time$ = the measured time of the sample peak (sequence line number)
$\delta_{\text{sample(drift)}}$ = the drift corrected delta value of the sample
m = the slope of the drift correction regression trendline
b = the y-intercept of the drift correction regression trendline.

To correct the data:

Step 1) Calculate residuals of the reference material using the equation:

$$\delta_{\text{RM(residual)}} = \delta_{\text{RM(raw)}} - \delta_{\text{RM(true)}}$$

where: $\delta_{\text{RM(true)}}$ = the accepted isotope value of the reference material
$\delta_{\text{RM(raw)}}$ = the measured isotope value of the reference material
$\delta_{\text{RM(residual)}}$ = the drift residual of the reference material

Step 2) Derive a regression trendline of the time of analysis vs. the reference material residuals:

where: m = the slope of the regression trendline
b = the y-intercept of the regression trendline
R^2 = the coefficient of determination of the regression

At UWSIF, if R^2 is <0.7, no correction is applied. If the R^2 is >0.7, the following calculation is performed:

Step 3) Calculate the drift residual corrected isotope values of the reference material and unknowns using the equation:

$$\delta_{\text{sample(drift)}} = \delta_{\text{sample(raw)}} + (Time \times m + b)$$

where: $Time$ = the measured time of the sample (sequence line number)
$\delta_{\text{sample(raw)}}$ = the measured isotope value of the sample
$\delta_{\text{sample(drift)}}$ = the drift residual corrected isotope value of the sample

9.3.5 Blank

If there is a blank associated with the isotopic measurement, the magnitude and isotopic composition of the blank should be determined. A blank correction can then be applied to the measured data. The average peak area and δ value of the blank measurement can be used to correct the data for any blank contribution.

Note: Blanks typically are very small. Blank corrections should only be applied if the isotopic compositions of the blank determinations are consistent. Because integration of very small peaks can be problematic, UWYOSIF defines a minimum acceptable sample peak size of 50mV so that a small blank peak should have minimal effect on a sample peak one hundred times larger.

To correct for the presence of a blank:

$$\delta_{\text{sample(blk)}} = \frac{\left[\left(\delta_{\text{sample(raw)}} \times Area_{\text{sample(raw)}}\right) - \left(\delta_{\text{blk(raw)}} \times Area_{\text{blk(raw)}}\right)\right]}{\left(Area_{\text{sample(raw)}} - Area_{\text{blk(raw)}}\right)}$$

where: $\delta_{\text{sample(raw)}}$= the measured isotope value of the sample
$\delta_{\text{blk(raw)}}$ = the measured isotope value of the blank
$Area_{\text{sample(raw)}}$ = the measured peak area of the sample
$Area_{\text{blk(raw)}}$= the measured peak area of the blank
$\delta_{\text{sample(blk)}}$= the blank corrected isotope value of the sample

9.3.6 Weight percent calculation: kfactors

If weight percent values of the samples need to be calculated, kfactors can be generated and used to calculate the unknown weight % values. The *kfactors* are calculated using the known weight percent of a standard, the measured peak area of the standard, and the known weight of the standard.

Step 1) Calculate a *kfactor* for each measurement of the weight percent standard:

$$kfactor_{\text{standard}} = \frac{wt\%_{\text{standard(true)}} \times wt_{\text{standard}}}{Area_{\text{standard(raw)}}}$$

where:
$wt\%_{\text{standard(true)}}$ = the known weight percent value of the standard.
$Area_{\text{standard(raw)}}$ = the measured peak area of standard
wt_{standard} = the known weight of the standard
$kfactor_{\text{standard}}$ = the weight percent *kfactor* for the standard

Step 2) Average the calculated *kfactors* for the standard.

Step 3) Correct each measured weight percent value:

$$wt\%_{\text{true}} = \frac{kfactor_{\text{standard}} \times Area_{\text{sample(raw)}}}{wt_{\text{sample}}}$$

where:
$Area_{\text{sample(raw)}}$ = the measured peak area of the sample
wt_{sample} = the known weight of the sample
$wt\%_{\text{true}}$= the *kfactor* corrected weight percent of the sample

9.3.7 Weight percent calculation: known standards

Weight percent values of unknown samples can be calculated based on the quantity of carbon and nitrogen contained in weight percent standards. The calculation is done using the known weight percent of a standard, the measured peak area of the standard, and the known weight of the standard. The following example is for the determination of carbon content.

Step 1) Calculate the amount of carbon for each weight percent standard:

$$carbon_{\text{standard}} = wt\%_{standard} \times wt_{standard}$$

where: $wt\%_{\text{standard}}$ = the known weight percent value of the standard
wt_{standard} = the weight of the standard (mg)
$carbon_{\text{standard}}$ = the amount of carbon in the standard (mg)

Step 2) Generate a linear equation expressing the relationship between the carbon peak area and mg carbon for the standard:

where: m = the slope of the $\text{carbon}_{\text{standard}}$ vs $\text{peakarea}_{\text{standard}}$ linear trendline
b = the y-intercept of the $\text{carbon}_{\text{standard}}$ vs $\text{peakarea}_{\text{standard}}$ linear trendline

Step 3) Calculate the amount of carbon for each sample:

$$carbon_{sample} = peakarea_{\text{sample}} \times m + b$$

where: $carbon_{sample}$ = the amount of carbon in the sample
$peakarea_{\text{sample}}$ = the measured peak area of the sample carbon peak
m = the slope of the $\text{carbon}_{\text{standard}}$ vs $\text{peakarea}_{\text{standard}}$ linear trendline
b = the y-intercept of the $\text{carbon}_{\text{standard}}$ vs $\text{peakarea}_{\text{standard}}$ linear trendline

Step 4) Calculate the weight percent of carbon in each sample:

$$wt\%_{\text{sample}} = \frac{carbon_{\text{sample}} \times 100}{wt_{\text{sample}}}$$

where: $wt\%_{\text{sample}}$ = the calculated weight percent of the sample
wt_{sample}= the measured weight of the sample

9.3.8 Weight percent statistics

Uncertainty in weight percent measurements is expressed as the percent error of a known weight percent standard. Calculation of the percent error requires the determination of the absolute error. The absolute error is defined as the average of the absolute difference between the known value of a weight percent standard and the individual measurements:

$$Err_{\text{abs}} = \frac{\left[\sum \left| wt\%_{\text{standard(raw)1}} - wt\%_{\text{standard(true)}} \right| \ldots \left| wt\%_{\text{standard(raw)n}} - wt\%_{\text{standard(true)}} \right| \right]}{n}$$

where: $wt\%_{\text{standard(raw)1}}, \ldots\ wt\%_{\text{standard(raw)n}}$ = the individual measured weight percent values of the standard.
$wt\%_{\text{standard(true)}}$ = the known weight percent value of the standard.
n = the number of measured weight percent values of the standard.
Err_{abs} = the absolute error of the standard.

The percent error associated with the weight percent standard is defined as the absolute error divided by the known weight percent value for the measured weight percent standard expressed as a percent:

$$Err_{\text{rel}} = \left(\frac{Err_{\text{abs}}}{wt\%_{\text{standard(true)}}} \right) \times 100$$

where: $wt\%_{\text{standard(true)}}$ = the known weight percent value of the standard.
Err_{rel} = the percent error of the weight percent standard

9.3.9 Outlier test

The UWSIF uses the Dixon Q test to determine outliers. This test is primarily used for small data sets (between 3 and 10 data points). It can be used to test whether the minimum value is an outlier, the maximum value is an outlier, or either the minimum or maximum value is an outlier. To apply the Dixon Q test, the data are arranged in order of increasing values. Q_{cal} is calculated as follows:

$Q_{\mathrm{cal}} = gap/range$

where: *gap* = the absolute difference between the data point in question and the closest data point to it.
range = the absolute difference between the largest and smallest data points.
Q_{crit}=Dixon Q test critical values

Step 1) Arrange the data points in either ascending or descending order.

Step 2) Calculate Q_{cal} for the suspected outlier.

Step 3) Compare Q_{cal} to Q_{crit} for a chosen confidence (α) interval (see Table 12).

If Q_{cal} is larger than Q_{crit}, the data point is considered to be an outlier.

Note: only one point may be rejected from a data set using the Dixon Q test.

Table 12 *Dixon Q test critical values*

Sample size	3	4	5	6	7	8	9	10
α = .10	0.941	0.765	0.642	0.560	0.507	0.468	0.437	0.412
α = .05	0.970	0.829	0.710	0.625	0.568	0.526	0.493	0.466
α = .01	0.994	0.926	0.821	0.740	0.680	0.634	0.598	0.568

9.4 NEON Specific Calculations and Corrections

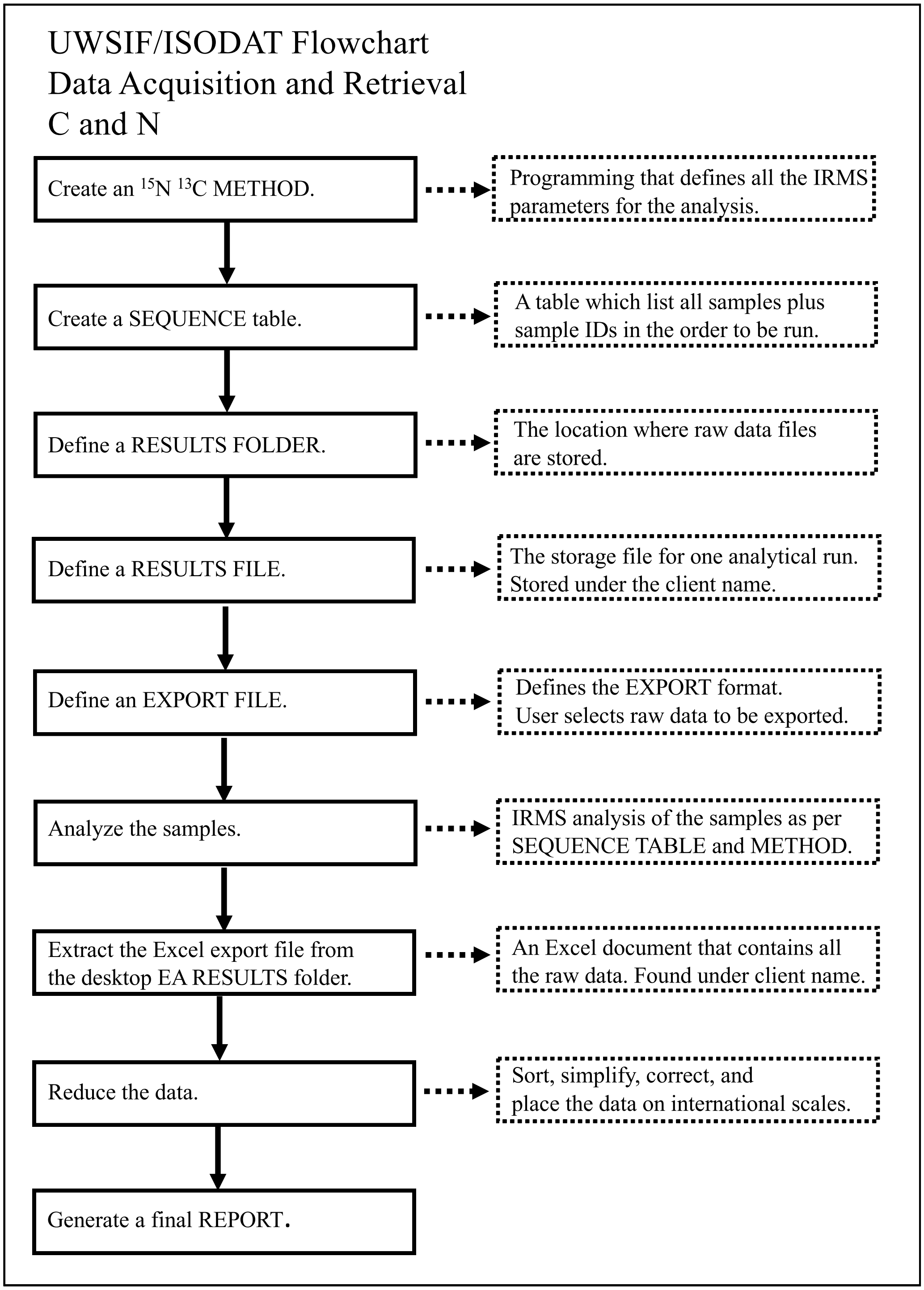
There are no special NEON calculations and corrections.

The following corrections are used for NEON samples (when appropriate):

Scale normalization (Section 9.3.1)
Linearity (Section 9.3.3)
Drift (Section 9.3.4)
Weight percent calculation: known standards (Section 9.3.7).
Outlier test (Section 9.3.9)

Note: Elemental composition calculations: the use of known standard versus kfactor methods

Section 9.3.7 (known standards) is the method UWSIF generally uses for calculating elemental composition of NEON samples. If systematic errors occur that makes it impossible to use this method for elemental composition calculations, UWSIF will use the kfactor method (Section 9.3.6). An example of a systematic error would be losing a known standard sample during analysis (e.g., autosampler error).


UWSIF/ISODAT Flowchart
Data Acquisition and Retrieval
C and N
Create an ^{15}N ^{13}C METHOD.
Programming that defines all the IRMS parameters for the analysis.
Create a SEQUENCE table.
A table which list all samples plus sample IDs in the order to be run.
Define a RESULTS FOLDER.
The location where raw data files are stored.
Define a RESULTS FILE.
The storage file for one analytical run. Stored under the client name.
Define an EXPORT FILE.
Defines the EXPORT format. User selects raw data to be exported.
Analyze the samples.
IRMS analysis of the samples as per SEQUENCE TABLE and METHOD.
Extract the Excel export file from the desktop EA RESULTS folder.
An Excel document that contains all the raw data. Found under client name.
Reduce the data.
Sort, simplify, correct, and place the data on international scales.
Generate a final REPORT.

Final data checks:

1. To check the run, copy the average corrected value for the check reference materials into the appropriate cell on the "EAMS Run" template (see Figure 18). The **accuracy** of the Lab QC Check material (the difference between the corrected value and the actual value) must be below 0.4 ‰ for $\delta^{15}N$ and below 0.3 ‰ for $\delta^{13}C$. If this is not the case, the run is not accepted and all samples in the run are re-analyzed.

Section 10 Elemental Analyzer Maintenance – General Information

10.1 Building a Combustion Reactor

The combustion reactor should be replaced every 1000-1200 samples, or as needed.

Materials needed:

- 1.8 cm x 45 cm quartz tube
- 70 % ethanol
- chromium oxide
- funnel
- laboratory wipes
- push rod
- quartz wool
- ruler
- silvered cobaltous/cobaltic oxide
- tray

Procedure:

1. Mark the outside of the combustion tube at the following distances (from the bottom): 4 cm, 10 cm, 11 cm, 21 cm, 22 cm.
2. Insert the column plug.
3. Insert 1 cm of quartz wool into the bottom of the quartz tube. Using the push rod, push the quartz wool into the quartz tube until the quartz wool hits the 11 cm mark (the reactor plug).
4. Using a funnel add silvered cobaltous/cobaltic oxide through the bottom of the quartz tube until the reagent reaches the 4 cm mark. Gently tap the tube to compact the reagent. Add more reagent if necessary.
5. Add additional quartz wool to fill the bottom of the quartz tube.
6. Through the top of the quartz tube, add 10 cm of chromium oxide. As you add the reagent to the tube gently tamp the tube to compact the reagent to avoid separation when the reactor is heated to 1020 °C.
7. Add approximately 1.0 to 1.5 cm of quartz wool on top of the chromium oxide.
8. Prepare a quartz insert by placing 1 cm of quartz wool into the bottom of the tube, followed by 2-3 mm of chromium oxide.
9. Slide the insert into the top of the combustion reactor. The insert should extend past the top of the combustion reactor by 4 to 5 cm.
10. With a marker, draw a line around the quartz insert where it extends above the combustion reactor.
11. Remove the quartz insert and cut it to the proper length.
12. Insert the cut quartz insert into the combustion reactor.
13. Install a black sealing ring at the top with the flat portion down.
14. Clean the outside of the combustion tube with laboratory wipes and 70 % ethanol.

10.2 Building a Reduction Reactor

The reduction reactor should be replaced every 1000-1200 samples, or as needed.

Materials needed:

- 1.8 cm x 45 cm quartz tube
- 70 % ethanol
- analytical grade pure copper (0.3mm mesh wire)
- funnel
- laboratory wipes
- quartz wool
- tray

Procedure:

1. Insert 1.5 to 2.0 cm of quartz wool into the bottom of the quartz tube. Place the bottom of tube against the tray and use the push rod to tamp down the quartz wool.
2. Using a funnel add 35 to 40 cm of pure Cu wires to the quartz tube. As you add the copper wire to the tube, gently tamp the tube to compact the copper to avoid separation when the reactor is heated to 650 °C. Note: a vortex mixer can also be used to compact the copper wire. As wire is added to the quartz tube, a 2-3 second vortex mixing will easily compact the copper wires in the quartz tube.
3. Add quartz wool to fill the rest of the open tube.
4. Install a black sealing ring at the top with the flat portion down.
5. Clean the outside of the reduction tube with a laboratory wipe and 70 % ethanol and set the tube aside.

10.3 Building/Preparing Quartz Inserts

Be sure to wear safety glasses and quartz glassblower glasses while making new inserts.

Materials needed:

- glass saw
- natural gas/oxygen torch
- standard wall 15 mm OD quartz tubing
- tube scorer

Procedure:

1. Cut the quartz tubing to 12-inch lengths.
2. Using the glass saw, cut notches at 1.5 inch and 2.5 inch from the bottom on one side of the tube. Cut a notch at 2 inches from the bottom on the opposite side of the tube.
3. Fire polish the notches with the torch.
4. Using the torch, heat the bottom of the insert and collapse the opening to roughly ½ the original diameter. Closing the opening helps keep the contents of the quartz insert within the insert. Heat the completed insert to 850 °C overnight.

10.4 Leak Checking an Elemental Analyzer

Whenever consumables are replaced in an elemental analyzer, a leak test is required to ensure that the instrument is not leaking.

Procedure:

1. Place a plug over the Vent-M, release pressure on the helium regulator on the EA (turn black/silver knob counterclockwise).
2. If there is a leak the pressure on the helium gauge will decrease.
3. The most common source of leaks will be at the carousel connection to the combustion reactor, seals in water trap, or the carousel lid seal. Make sure to restore helium pressure before checking for leaks.
4. If there is no leak, remove plug on Vent-M and restore helium pressure.

If it is determined that a leak exists, a helium leak detector is used to isolate the leak. Once the leak is fixed, repeat the leak test procedure.

10.5 Replacing the Elemental Analyzer Reactors

The combustion reactor should be replaced every 1000-1200 samples. The reduction reactor is replaced as needed, depending on the size of the samples analyzed. Increases in the m30 signal during ^{15}N analysis can be used as an indicator that it is time for the replacement of the reduction reactor.

Materials needed:

- prepared combustion reactor (with quartz insert)
- prepared reduction reactor

Be sure to wear leather gloves and safety glasses while changing reactors.

Procedure:

1. Turn off the source by clicking on the gray symbol at the top left side of the Isodat screen.
2. Close the mass spectrometer by turning the source SGE valve carefully until you see the vacuum pressure in Isodat drop to 10^{-9} mbars. Do not over-tighten.
3. Put the EA into "Stand-by" mode.
4. Set both reactor ovens to 500 °C. Wait for both ovens have reached 500 °C or below.
5. Take out the old reactors by unscrewing the bottom and then the top.
6. Insert new reactors. Make sure all O-rings are still in good shape before reusing. (O-rings should be flexible and cracks should not be visible.)
7. Make sure the reactor braces are secure.
8. Put the EA into "Work" mode.
9. With a helium leak detector, check all seals and openings including the carousel. You may have to retighten or replace O rings in order for there not to be leaks. (Be sure to turn off the helium before retightening anything. You may need to wait about ten minutes for the pressure to release before opening anything again.)
10. Set the two oven temperatures to their original temperatures (1020 °C, 650 °C). Wait for the ovens to reach their working temperatures.
11. Open the mass spectrometer by turning the source SGE valve carefully until you see the vacuum pressure rise to approximately 10^{-6} mbars.
12. Turn on the source by clicking on the red sun symbol at the top left of the Isodat screen.
13. Ensure that the nitrogen background is below 400 mV by performing a jump to mass 28 and a peak center.

10.6 Replacing the Quartz Insert

The quartz insert is a disposable tube placed into the top of the combustion reactor to catch residue from sample combustion. The insert must be replaced periodically.

Materials needed:

- forceps
- prepared quartz insert
- Sharpie felt pen
- tube scorer

Procedure:

1. Turn off the Delta^{Plus} XP or Delta V source.
2. Loosen the purge valve on top of carousel.
3. Loosen the fittings connecting the carousel to the combustion reactor. Move the carousel to the side so the old insert can be removed without obstruction.
4. Carefully remove the used insert from the reactor. Care should be taken when handling the insert because it is at 1020 ºC.
5. Insert 1 cm of quartz wool into the bottom of the new insert.
6. Add 2-3 mm of chromium oxide to the top of the quartz wool.
7. Slide the quartz insert into the top of the combustion reactor. The insert should extend above the top of the combustion reactor by 4 to 5 cm. With a felt pen marker, draw a line around the quartz insert where it extends above the combustion reactor. Remove the quartz insert and cut it to the proper length. Insert the shortened quartz insert into the combustion reactor.
8. Reposition the carousel onto the combustion reactor and secure the connection. Let the carousel purge for approximately 5 minutes to remove the atmosphere from the carousel headspace. Then close the purge valve.
9. Allow about 7 minutes for the air to purge the EA plumbing.
10. Perform a leak check.
11. Turn on the Delta^{Plus} XP or Delta V source.

10.7 Replacement of the Water Trap

The water trap requires replacement when the appearance of magnesium perchlorate changes from dry granular to off-white non-granular. The timing of the maintenance is dependent on the moisture generated from the combustion of the samples. The entire procedure should be performed over a secondary container, such as a tray, to capture any spilled material. Safety glasses, rubber gloves, and a lab coat/apron are required.

Materials needed:

- analytical grade magnesium perchlorate
- forceps
- funnel
- quartz wool
- spatula
- tray

Procedure:

1. Turn off the Delta^{Plus} XP or Delta V source.
2. Remove the magnesium perchlorate water trap. Hold the glass tube and loosen the red caps on both ends of trap and remove. Check the silicon seals for cracks. Replace the seals if necessary.
3. Clean the water trap by removing the quartz wool from each end of the tube. Push all the used magnesium perchlorate out of the tube into a waste container. Rinse the tube with deionized water, followed by a rinse with 70 % ethanol, and finally blow the tube dry with compressed air.
4. Add 5 to 7 mm of quartz wool to one end of the clean glass tube.
5. Using a funnel, fill the glass tube with magnesium perchlorate granules and gently tap the tube on the bench top to compact the granules. Be sure to leave approximately 5 to 7 mm of space in order to insert quartz wool into the open end.
6. Add 5 to 7 mm of quartz wool to fill the glass tube.
7. Install the newly prepared water trap in reverse. Remember the Teflon face of the seal goes against the steel seat inside the red cap and the silicon face goes against the glass water trap tube.
8. Perform a leak check.
9. Turn on the Delta^{Plus} XP or Delta V source.

10.8 NEON Specific Elemental Analyzer Maintenance

There is no NEON specific elemental analyzer maintenance protocols.

Section 11 Troubleshooting - General Steps

11.1 Troubleshooting the Elemental Analyzer

Elemental analyzers required periodic maintenance and occasional troubleshooting. Table 13 lists some of the common elemental analyzer problems and possible solutions.

Table 13 *Troubleshooting guide*

Observation	Potential problem	Solution
High nitrogen blank	Air leak Oxygen contaminated	Leak test the instrument Replace the cylinder
Nitrogen peak tailing	Bad combustion	Smaller sample size Increase the oxygen Remove residues
Carbon peak tailing	Bad combustion	Remove residues Smaller sample size Increase the oxygen
Bad peak separation	Copper exhausted GC column temperature	Replace reduction reactor Decrease temperature
Peak between nitrogen and carbon peaks	Oxygen contaminated	Check the oxygen blank
Peaks elute late	Leak in system Water trap exhausted	Perform a leak test Replace the magnesium perchlorate

11.2 Troubleshooting: Helium and Oxygen Gases

The nominal pressure of gas supplies should be: 100 kPa for helium and 100 or 125 kPa for oxygen.

Table 14 *Solving EA chromatography problems*

Symptoms and solutions to EA chromatography problems	
Symptom	Solution(s)
A negative peak between the nitrogen and carbon peaks.	Set both pressures to the same value to eliminate the negative peak and to have good separation between the nitrogen and carbon peaks.
Tailing on the nitrogen peak. Bad separation between the nitrogen and carbon peaks. Split or tailing on the carbon peak.	Decrease the weight of the sample. Increase the oxygen quantity. Remove the residue. Change the combustion reactor.

12.3 Troubleshooting: Flash Combustion

There are two parameters that can be modified to optimize flash combustion: the oxygen flow and the oxygen injection time.

The normal values are 100 mL·min^{-1} and 4 seconds, respectively.

Table 15 *Optimizing flash combustion*

Steps to optimize flash combustion	
Oxygen flow rate	Run 1: oxygen flow rate 80 mL·min^{-1}, injection time 4 seconds. Run 2: oxygen flow rate 120 mL·min^{-1}, injection time 4 seconds. Set the oxygen flow rate to the lowest level that still allows flash combustion.
Oxygen injection time	Run 1: oxygen flow 100 mL·min^{-1}, injection time 4 seconds. Run 2: oxygen flow 100 mL·min^{-1}, injection time 13 seconds. Set the oxygen injection time to the lowest level that still allows flash combustion.