



Environmental Analysis Program

Pomona College
Biogeochemistry Laboratory

SOP-L414

Soil Carbon and Nitrogen Analysis by Flash EA-IRMS

Standard Operating Procedure

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1. Purpose

This SOP describes the complete workflow for determining $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and elemental concentrations (%C, %N) in soil and sediment samples by combustion on the Thermo Scientific Flash IRMS

Elemental Analyzer (EA) coupled to the Delta V IRMS through the ConFlo IV interface (Werner & Brand, 2001). It covers sample preparation, sequence design, the analytical run, and post-analysis data correction including blank correction, linearity correction, scale normalization, and drift correction (Coplen, 2011; Paul et al., 2007).

For general instrument operation, startup/shutdown, gas checks, tuning, and pre-analysis tests, see [SOP-L208](#).

2. Scope

- **Applies to:** Soil, sediment, and plant tissue samples analyzed for $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, %C, and %N by combustion EA-IRMS in NC (dual reactor) mode.
- **Does not apply to:** Sulfur isotope analysis (NCS mode; requires dedicated reactor and Sulfinert column), GasBench applications (see [SOP-L415](#)), TC/EA applications ($\delta^2\text{H}$, $\delta^{18}\text{O}$), or liquid samples.
- **Location:** Sample preparation in SGM Rm 133; analysis in the Oxtoby Isotope Lab, SGM Rm 135.

3. Responsibilities

Role	Responsibility
Lab PI	Approve methods, oversee data quality
Lab Manager	Prepare/verify Isodat methods; maintain reactors
Certified User	All steps from sample prep through data reduction
Student Researcher	May perform sample prep and data reduction under supervision

4. Definitions

- **Blank correction:** Subtraction of the isotopic and elemental contribution of an empty tin capsule from each sample signal; necessary because capsule tin contributes a small amount of C and N (Paul et al., 2007).
- **$\delta^{13}\text{C}$:** Stable carbon isotope ratio expressed in per mil (‰) relative to Vienna Pee Dee Belemnite (VPDB): $\delta^{13}\text{C} = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000$ where $R = {}^{13}\text{C}/{}^{12}\text{C}$ (Coplen, 2011).
- **$\delta^{15}\text{N}$:** Stable nitrogen isotope ratio expressed in per mil (‰) relative to atmospheric N_2 (AIR): $\delta^{15}\text{N} = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000$ where $R = {}^{15}\text{N}/{}^{14}\text{N}$ (Coplen, 2011).
- **Drift correction:** Adjustment for systematic temporal change in measured δ values during a sequence, estimated from bracketing reference materials (Paul et al., 2007).
- **Flash EA:** The Thermo Scientific Flash IRMS Elemental Analyzer peripheral; combusts samples at 1020 °C in an O_2 pulse and separates N_2 and CO_2 chromatographically before delivery to the IRMS via the ConFlo IV.
- **Isodat:** ThermoFisher software used to design analytical sequences, control the instrument, and export raw isotope data.

- **Linearity correction:** Adjustment applied to account for the dependence of measured δ values on peak signal amplitude.
- **Reference material (RM):** A substance with a certified or consensus δ value used to anchor measured values to the VPDB or AIR scale (Werner & Brand, 2001).
- **Scale normalization:** Two-point (or multi-point) correction that maps measured δ values to the international isotope-ratio scale using certified RMs spanning the expected sample range (Paul et al., 2007).
- **Tin capsule:** 5 x 9 mm pressed-tin container used to encapsulate solid samples; tin ignites in the O₂ pulse, raising combustion temperature briefly to ensure complete combustion.

5. Safety and Hazards

See [SOP-L208, Section 4](#) for the full hazard list.



Application-Specific Hazards

- **Tin capsule handling:** Tweezers must be clean and dry; oils from fingers contaminate capsules and introduce C/N blanks.
- **Compressed gases:** He carrier and O₂ combustion gas; see SOP-L208 for gas handling requirements.

5.1. Required PPE

- ☐ Safety glasses
- ☐ Lab coat
- ☐ Nitrile gloves (sample prep and capsule loading)

5.2. Required Training

- [SOP-L002 Laboratory Safety](#)
- [SOP-L208 IRMS General Instrument Use](#) (must be a certified IRMS User before independent operation)

6. Equipment and Materials

Item	Location	Notes
Tin capsules (5 x 9 mm)	SGM Rm 133	Ultra-clean; handle with tweezers only
Micro-balance (0.001 mg)	SGM Rm 133	Tare and zero before each capsule
Tweezers (stainless)	SGM Rm 133	Clean with EtOH before use
Soil / sediment samples (dried, ground to <150 μ m)	SGM Rm 133	See SOP-L116
Flash IRMS EA	SGM Rm 135	NC dual-reactor mode
Tin capsule storage plate	SGM Rm 133	Labeled 96-well plate

Item	Location	Notes
Certified reference materials	SGM Rm 133	e.g., USGS40, USGS41a, IAEA-N1
Lab logbook	SGM Rm 135	

7. Estimated Time

Task	Time
Sample weighing (per capsule)	3–5 min
Sequence setup in Isodat	20–30 min
Analytical run (per sample)	~7 min (N ₂ + CO ₂ in series)
Data reduction (R script)	30–60 min

8. Procedure

8.1. Sample Preparation

1. Dry samples at 60 °C to constant mass per [SOP-L105](#).
2. Grind and homogenize to <150 µm per [SOP-L116](#).
3. For carbonate removal (inorganic C), acidify with 1 M HCl, re-dry, and record the treatment in the lab notebook before proceeding.
4. Clean the micro-balance pan and zero the balance.
5. Using clean tweezers, place a tin capsule on the balance. Tare.
6. Weigh 10–30 mg of ground sample into the capsule (adjust to keep the N₂ and CO₂ peaks within the instrument's linear range).
7. Fold and crimp the capsule tightly, removing all air.
8. Record the capsule mass in the lab notebook and in the sequence spreadsheet.

8.2. Sequence Design

1. Open Isodat Sequence Editor and load the NC dual-reactor method.
2. Build the sequence in this order:
 - 3–5 conditioning samples (similar matrix to unknowns)
 - 1 blank capsule
 - Bracketing RMs (at least 2 certified materials, e.g., USGS40 + USGS41a for $\delta^{13}\text{C}$; IAEA-N1 + IAEA-N2 for $\delta^{15}\text{N}$)
 - Unknowns, with 1 RM per 10 samples
 - Check standard at end
 - 1 blank capsule at end
3. Save the sequence file as `YYYYMMDD_Project_SeqID_User.seq`.

8.3. Analytical Run

1. Complete the pre-analysis checklist in SOP-L208.
2. Load the capsule plate into the Flash EA autosampler carousel.
3. Press **Start** in Isodat.
4. Monitor the first 5 injections for expected peak shape and amplitude.

8.4. Post-Analysis Data Correction

Apply corrections in this order using the R script in the project repository:

```
library(tidyverse)

# 1. Import raw Isodat export (.csv)
raw <- read_csv("data/raw/YYYYMMDD_Project_SeqID_User_raw.csv")

# 2. Blank correction
#   Subtract mean blank d13C and blank area from each sample

# 3. Linearity correction
#   Regress RM d-values on peak amplitude; apply slope correction

# 4. Scale normalization (two-point)
#   Use two certified RMs to anchor measured values to VPDB / AIR

# 5. Drift correction
#   Regress RM residuals on run order; apply time-based correction

# 6. QC evaluation
#   Check RM recoveries; flag samples outside ±0.3 permil
```

9. Quality Control and Acceptance Criteria

- Reference material $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ must recover within $\pm 0.2\text{‰}$ of certified values after all corrections.
- Blank capsules must produce peak areas below 0.5 Vs (N_2) and 1 Vs (CO_2).
- Replicate analyses of a check standard ($n \geq 3$) must show $\text{SD} \leq 0.15\text{‰}$.
- Flag and investigate any sequence where $>20\%$ of RMs fall outside acceptance criteria before reporting results.

10. Data Management

1. Export raw data from Isodat immediately after the run as .csv.
2. Save under `data/raw/YYYYMMDD_Project_SeqID_User_raw.csv` in the project repository.

3. Commit raw data and the R correction script per [SOP-L801](#).
4. Store all corrected data in `data/processed/`.

11. Waste Disposal

Waste Type	Disposal Method
Used tin capsules	Solid waste (non-hazardous)
Gloves, Kimwipes	Standard trash unless contaminated
Acidified soil residues	Acid waste per SOP-L006

12. References

- Coplen, T. B. (2011). Guidelines and recommended terms for expression of stable-isotope-ratio and gas-ratio measurement results. *Rapid Communications in Mass Spectrometry*, 25(17), 2538–2560. <https://doi.org/10.1002/rcm.5129>
- Paul, D., Skrzypek, G., & F'orizs, I. (2007). Normalization of measured stable isotopic compositions to isotope reference scales – a review. *Rapid Communications in Mass Spectrometry*, 21(18), 3006–3014. <https://doi.org/10.1002/rcm.3185>
- Werner, R. A., & Brand, W. A. (2001). Referencing strategies and techniques in stable isotope ratio analysis. *Rapid Communications in Mass Spectrometry*, 15(7), 501–519. <https://doi.org/10.1002/rcm.258>

13. Related SOPs

SOP	Title	Relationship
L002	Laboratory Safety	Required prerequisite
L208	IRMS General Instrument Use	Instrument-level procedures
L415	Carbonate Isotopes by GasBench-IRMS	Companion application SOP
L802	Quality Control and Quality Assurance	General QC framework

14. Training Record

Before performing soil CN analysis independently, personnel must:

1. Read this SOP and [SOP-L208](#) in full
2. Be a certified IRMS User (SOP-L208, Training Record)
3. Demonstrate capsule preparation under supervision (5 capsules, masses within ± 0.05 mg of target)
4. Perform a supervised analytical run with acceptable QC results
5. Complete at least one supervised data reduction session
6. Be signed off by the lab manager or PI