SOIL METHODS

Field Sampling and Laboratory Analysis for Soils and Sediments

Environmental Analysis Program
Teaching and Research Laboratories, Seeley G Mudd
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1. PURPOSE

To provide standardized methods for sampling, measuring, and analyzing key soil physical, chemical, and biological properties to assess soil health, fertility, nutrient cycling, and carbon dynamics.

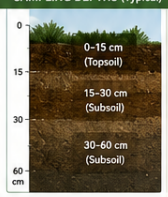
2. KEY SOIL PROPERTIES AND INDICATORS

CARBON	NITROGEN	FERTILITY	MICROBIAL PROCESSES	ORGANIC MATTER	N CYCLE	TEXTURE	BULK DENSITY
Total Organic Carbon (TOC)	Total N, NH_4^+, NO_3^-, Available N	pH, EC, P, K, Ca, Mg, S, CEC	Biomass C & N, Enzyme Activity, Respiration	OM Content, OM Fractionation, C:N Ratio	Mineralization, Nitrification, Denitrification	Sand, Silt, Clay Percentages	Mass/Volume (g cm^{-3})

3. SAMPLING GUIDELINES

- Define objectives and sampling design.
- Avoid unusual areas (manure piles, erosion spots, field edges).
- Use clean tools; avoid contamination.
- Take composite samples (5–10 cores per plot).
- Record GPS, depth, land use, vegetation, and management.
- Air-dry samples in shade; remove debris and roots; pass through 2 mm sieve unless stated otherwise.

SAMPLING DEPTHS (Typical)



TOOLS

4. SAMPLE PREPARATION

Air dry (shade) → Remove debris & roots → Break clods (gently) → Sieve (2 mm) → Store in clean, labeled bags

5. MEASUREMENT AND ANALYSIS PROCEDURES

5.1 SOIL CARBON & ORGANIC MATTER

Total Organic Carbon (TOC)

- Dry combustion (CN analyzer) or Walkley–Black (wet oxidation)

Organic Matter (OM)

- OM (%) = TOC (%) × 1.724 (Van Bemmelen factor)

5.2 TOTAL NITROGEN

- Kjeldahl digestion followed by distillation and titration or CN analyzer

5.3 AVAILABLE NITROGEN

- NH_4^+–N: Extract with 2 M KCl, analyze colorimetrically (phenate method)
- NO_3^-–N: Extract with 2 M KCl, analyze colorimetrically (cadmium reduction method)

5.4 SOIL FERTILITY

- pH: 1:2.5 soil:water (pH meter)
- EC: 1:5 soil:water (EC meter)
- Available P: Olsen (alkaline) or Bray (acidic), measure colorimetrically
- Exchangeable K, Ca, Mg: Extract with 1 M NH_4OAc, analyze by AAS/ICP-OES
- CEC: 1 M NH_4OAc (pH 7), measure by distillation or calculation

5.5 MICROBIAL PROCESSES

Microbial Biomass C (MBC)

- Fumigation–Extraction (0.5 M K_2SO_4), analyze TOC

Microbial Biomass N (MBN)

- Fumigation–Extraction, analyze Total N

Enzyme Activities (examples)

- Dehydrogenase, Phosphatase, Urease – colorimetric assays

Soil Respiration

- Incubation method, measure CO_2 evolution

5.6 NITROGEN CYCLE PROCESSES

Mineralization

- Incubate soil, measure NH_4^+ and NO_3^- over time

Nitrification

- Incubate, measure NO_3^- production

Denitrification

- Anaerobic incubation, measure N_2O or N_2 (gas chromatography)

5.7 SOIL TEXTURE (HYDROMETER METHOD)

Disperse soil with $Na_4P_2O_7$ → Shake and mix → Measure hydrometer reading at 40 s (sand) → Measure reading at 2 h (clay) → Calculate % Sand, % Silt, % Clay

5.8 BULK DENSITY

1. Collect core using undisturbed core ring → 2. Trim ends flush → 3. Oven dry at 105 °C to constant weight → 4. Bulk Density (BD) = Oven dry mass (g) ÷ Core volume (cm^3)

6. CALCULATIONS

Organic Matter (%) = TOC (%) × 1.724

$$\text{Bulk Density (g cm}^{-3}) = \frac{\text{Oven dry mass (g)}}{\text{Core volume (cm}^3)}$$

Porosity (%) = [1 − (BD / PD)] × 100
(PD = Particle Density, ~2.65 g cm^{-3})

Carbon Stock (Mg C ha^{-1}) =
SOC (%) × BD (g cm^{-3}) × Depth (cm) × 100

Nitrogen Stock (Mg N ha^{-1}) =
Total N (%) × BD × Depth × 100

7. QUALITY CONTROL

- Use clean, calibrated instruments.
- Run blanks, standards, duplicates.
- Follow standard protocols.
- Record all data and conditions.
- Store samples properly.

8. SAMPLE STORAGE

- Air-dried samples: in labeled bags, at room temperature.
- Moisture-sensitive samples (NH_4^+, microbial): refrigerate at 4 °C.
- Long-term storage: freeze at −20 °C for microbial / enzyme samples.

9. DATA RECORDING

Record the following:

- Sample ID, location, depth
- Date, time, sampler
- Soil type, land use, management
- All observations and results

10. TYPICAL UNITS

- TOC, TN, OM: %
- Available N, P, K: mg kg^{-1}
- EC: dS m^{-1}
- pH: unitless
- BD: g cm^{-3}
- Enzyme activity: µg product g^{-1} soil h^{-1}
- Microbial biomass: mg kg^{-1}
- Carbon/Nitrogen stock: Mg ha^{-1}

11. INTERPRETATION GUIDELINES (GENERAL)

Property	Low	Medium	High
Organic Carbon (%)	< 0.75	0.75 – 1.50	> 1.50
Total Nitrogen (%)	< 0.07	0.07 – 0.15	> 0.15
Available P (mg kg^{-1})	< 10	10 – 20	> 20
Available K (mg kg^{-1})	< 100	100 – 200	> 200
pH	< 5.5	5.5 – 7.5	> 7.5
Bulk Density (g cm^{-3})	> 1.60	1.20 – 1.60	< 1.20

12. SAFETY AND ENVIRONMENT

- Wear PPE: gloves, lab coat, safety glasses.
- Handle chemicals with care; read SDS.
- Dispose of wastes properly.
- Minimize environmental impact.

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Pomona College
Claremont, California

SOIL METHODS

Soil Methods

Field sampling and laboratory analysis for soils and sediments

EA Program – Biogeochemistry Lab, Pomona College

Compiled: June 26, 2026

SOP Number	**Title**
SOP-F400	Soil Sampling and Processing
SOP-F401	Soil Texture Determination by Feel (Ribbon Method)
SOP-F402	Soil Bulk Density – Core Method
SOP-F403	Soil Infiltration Rate – Ring Infiltrometer Method
SOP-F404	Soil Temperature Profiles
SOP-F405	Soil Color Determination (Munsell)
SOP-F406	Soil Respiration Chambers (Field Static Chamber Method)
SOP-F407	Soil Profile Description
SOP-L400	Soil Moisture – Gravimetric Method
SOP-L401	Soil Texture Analysis – Hydrometer Method
SOP-L402	Soil pH and Salinity
SOP-L403	Soil Nitrate and Ammonium – KCl Extraction
SOP-L404	Soil Nitrate Quick Test – Test Strip Method
SOP-L405	Soil Extractable Phosphorus
SOP-L406	Soil Organic Matter – Loss on Ignition
SOP-L407	Total Carbon – Shimadzu TOC-L with SSM-5000A
SOP-L408	Soil Respiration – Solvita CO˜2˜ Burst Test
SOP-L409	Microbial Biomass Carbon – Chloroform Fumigation-Extraction
SOP-L410	Hot-Plate Acid Digestion of Soils for Trace Metal Analysis
SOP-L411	Microwave-Assisted Acid Digestion of Soils for Trace Metal Analysis
SOP-L411	Nitrification Potential – Shaken Soil Slurry Method
SOP-L413	Compost Maturity – Solvita CO˜2˜/NH˜3˜ Test
SOP-L414	Soil Carbon and Nitrogen Analysis by Flash EA-IRMS
SOP-L415	Carbonate Isotope Analysis by GasBench II-IRMS
SOP-L416	Cation Exchange Capacity (CEC)
SOP-L417	Soil Aggregate Stability – Wet Sieving Method
SOP-L418	Water-Extractable Dissolved Organic Carbon (Soil)
SOP-L419	Soil Color – Munsell Lab Confirmation
SOP-L420	Soil Labile Amino-Nitrogen – Solvita SLAN Test
SOP-L475	Flash EA Soil Combustion for IRMS Analysis

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F400

Soil Sampling and Processing

Standard Operating Procedure

SOP Number	SOP-F400
Revision	1.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes the procedures for collecting, processing, and storing soil samples for laboratory analysis. It covers field sampling using a soil auger, sample homogenization, sieving, and subdivision for biological assays, carbon analysis, and other soil properties (Food and Agriculture Organization of the United Nations, 2024; Robertson et al., 1999).

2. Scope

- **Applies to:** All field-based soil sampling for EA Program research, including photovoltaic panel studies, agricultural plots, and restoration sites
- **Does not apply to:** Deep soil coring (> 30 cm) requiring mechanized equipment; contaminated site sampling requiring chain-of-custody protocols
- **Location:** All EA Program field sites

3. Responsibilities

Role	Responsibility
Lab PI	Approve sampling plan, ensure training compliance
Field Lead	Coordinate sampling logistics, ensure protocol adherence
Trained Personnel	Collect and process samples according to this SOP
New Students	Complete training before independent sampling

4. Definitions

- **Bulk sample:** A large, mixed sample collected from multiple locations within a sampling unit and combined before analysis; represents the average composition of the unit rather than any single point.
- **Composite sample:** A sample formed by combining and mixing a defined number of subsamples from a plot or area; reduces analytical cost while improving spatial representativeness; the number and pattern of subsamples must be recorded (Robertson et al., 1999).
- **Core (soil core):** A cylindrical soil sample extracted with a push-probe or hammer corer; preserves the depth profile and allows depth-discrete sampling; also used for bulk density determination (see SOP-F402).
- **Fine earth fraction:** Soil material passing through a 2 mm sieve; sand, silt, and clay particles; the standard fraction for chemical analysis; gravel and coarser material are removed and their mass fraction recorded (Robertson et al., 1999).
- **Grab sample:** A single, point-in-time sample collected from one location; appropriate when spatial variability is low or when individual point values are required; contrasted with composite sampling.
- **Pedogenic horizon:** A layer of soil with distinct physical, chemical, or biological properties developed through soil-forming processes; designated by letter symbols (O, A, E, B, C, R); sampled discretely in profile descriptions (see SOP-F407).
- **Push-probe (soil probe):** A hollow stainless steel tube pushed into the soil by hand or with a slide hammer to collect an intact soil core; the standard tool for rapid depth-discrete sampling in fine-textured soils without large stones (Robertson et al., 1999).
- **Sampling depth increment:** A defined depth interval (e.g., 0–10 cm, 10–20 cm) from which soil is collected; depth increments must be consistent within a study for results to be comparable (Robertson et al., 1999).

- **Subsample:** One of the individual small samples taken from within a plot that are combined to form a composite; also refers to a representative aliquot drawn from a bulk sample for laboratory analysis.

5. Safety and Hazards

Hazards

- **Physical:** Repetitive strain from augering, sharp edges on sieving equipment, trip hazards at field sites.
- **Biological:** Soil-borne pathogens, tick and insect exposure at field sites.
- **Chemical:** Potential exposure to contaminated soils at some sites.

5.1. Required PPE

- ☐ Closed-toed shoes or field boots
- ☐ Nitrile or latex gloves
- ☐ Sun protection (hat, sunscreen)
- ☐ Safety glasses (when sieving)

Refer to SOP-F001 Field Safety for general field safety procedures.

6. Equipment and Materials

Item	Notes
Soil auger (Dutch or bucket)	Diameter 5–10 cm; clean between sites
Push-probe (soil core sampler)	For depth-discrete sampling
Rubber mallet or slide hammer	For driving probe into hard soils
Stainless steel bowl or plastic bucket	For compositing subsamples
Stainless steel sieve (2 mm)	For fine earth fraction
Ziploc bags or Whirl-Pak bags (labeled)	For each sample
Permanent markers and labels	Waterproof; include site, depth, date
Cooler with ice	For biological subsamples (enzyme assays, MBC, microbial DNA)
Field notebook	Per SOP-F003
GPS unit	Per SOP-F006
Isopropyl alcohol and paper towels	For decontaminating tools between sites

7. Sampling Design

Before sampling, confirm the sampling design with the PI and reference SOP-F005 Random Sampling Design for plot layout and randomization (Robertson et al., 1999). Record the following in

the field notebook:

- Site name and GPS coordinates
- Sampling date and personnel
- Target depth(s) or horizon(s)
- Number of subsamples per composite
- Auger/probe diameter
- Any deviations from the sampling plan

8. Procedure

8.1. Field Collection

1. Navigate to the sampling location per SOP-F006.
2. Record the GPS coordinates of each sample location.
3. Inspect the surface for hazards. Clear surface litter only if required by the protocol; record whether litter was cleared.
4. Insert the auger or push-probe vertically to the target depth. For depth-increment sampling:
   - Collect the 0–10 cm increment first. Remove and place in a labeled sample container.
   - Re-insert the auger and collect the 10–20 cm increment. Place in a separate container.
   - Repeat for additional depth increments as required by the sampling plan.
5. For composite samples, collect subsamples at each designated subplot location (typically 5–10 per plot) and combine in the bowl or bucket before homogenizing.
6. Record the following for each sample:
   - Sample ID per the project labeling scheme
   - GPS coordinates
   - Depth increment
   - Observed soil conditions (color, texture, moisture, visible roots, stoniness)
   - Any anomalies (disturbance, compaction, visible contamination)

8.2. Field Homogenization

7. Break up clods and remove surface litter, roots greater than 5 mm, visible rocks, and obvious debris by hand (Robertson et al., 1999).
8. Mix the soil thoroughly in the bowl or bucket until the color and texture appear uniform.
9. If the sample is to be composited across subsamples, combine all subsamples before mixing.

8.3. Sieving (Field or Lab)

Sieving can be performed in the field (when fresh soil is required for biological assays) or in the lab after air-drying (for chemical analysis) (Food and Agriculture Organization of the United Nations, 2024; Robertson et al., 1999):

1. Pass the soil through a 2 mm stainless steel sieve.

2. Weigh and record the mass of coarse fragments (> 2 mm) retained if the project protocol requires correction for coarse fragment content.
3. The fine earth fraction (< 2 mm) is the standard fraction for all chemical and biological analyses.

8.4. Sample Subdivision for Multiple Analyses

Divide the sieved fine earth fraction into the required subsamples before further drying or freezing (Robertson et al., 1999):

Subsample type	Treatment	Storage
Microbial biomass C/N	Field-moist; process within 24 h	Cooler 4 °C then lab
Enzyme assays	Field-moist; process within 24 h or freeze	Cooler/freezer (-20 °C)
Mineral N (KCl)	Field-moist or frozen; extract within 48 h	Cooler 4 °C
Soil moisture (gravimetric)	Field-moist	Sealed bag
Carbon/nitrogen (EA-IRMS)	Air-dry, then grind	Room temperature
pH, texture, other chemical	Air-dry	Room temperature

Biological Subsamples

Do not freeze biological subsamples unless the analysis protocol explicitly permits it.

8.5. Tool Decontamination Between Sites

Wipe the auger, probe, and bowl with isopropyl alcohol and let dry before moving to the next sampling location. This prevents cross-contamination of soils between sites and is particularly important for sites with invasive species or contamination concerns (Food and Agriculture Organization of the United Nations, 2024).

9. Quality Control

- Collect field duplicates at a rate of 1 per 10 samples to assess sampling variability (Robertson et al., 1999).
- Record all deviations from the sampling plan in the field notebook.
- Verify auger depth calibration at the start of each sampling day.
- Ensure sieve integrity (no torn or damaged mesh) before processing.

10. Waste Disposal

Waste Type	Disposal Method
Used gloves	Standard trash
Decontamination rinse water	Pour on ground away from sampling plots
Coarse fragments	Return to field site or dispose in trash

11. References

- Robertson et al. (1999)
- Food and Agriculture Organization of the United Nations (2024)

Food and Agriculture Organization of the United Nations. (2024). *Standard operating procedure for soil microbial biomass (Carbon): Chloroform fumigation-extraction method.* FAO. https://www.fao.org/soils-portal/soil-survey/soil-analysis/en/

Robertson, G. P., Coleman, D. C., Bledsoe, C. S., & Sollins, P. (1999). *Standard soil methods for long-term ecological research.* Oxford University Press.

12. Related SOPs

SOP	Title	Relationship
F001	Field Safety	Required prerequisite
F005	Random Sampling Design	Plot layout and randomization
F006	GPS and Site Navigation	Sample location coordinates
F007	Field Decontamination	Tool decontamination between sites
F402	Soil Bulk Density	Companion core-based method
F407	Soil Profile Description	Horizon-based sampling context
L400	Soil Moisture	Required for dry-weight correction

13. Training Record

Before sampling independently, personnel must:

1. Read this SOP in full
2. Complete SOP-F001 Field Safety training
3. Participate in a supervised sampling session
4. Demonstrate proper auger technique, homogenization, and sieving
5. Be signed off by the field lead or PI

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F401

Soil Texture Determination by Feel (Ribbon Method)

Standard Operating Procedure

SOP Number	SOP-F401
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Author(s)	Marc Los Huertos
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1. Purpose

This SOP describes the field determination of soil texture using the texture-by-feel (ribbon) method. This rapid, equipment-free technique classifies soil into one of 12 USDA textural classes based on how a moistened soil sample behaves when formed into a ball, extruded into a ribbon, and rubbed between the fingers (Soil Survey Division Staff, 2017; Thien, 1979). Field texturing provides an immediate estimate of sand, silt, and clay content without laboratory analysis.

2. Scope

- **Applies to:** Field characterization of mineral soils at all EA Program sites; preliminary texture assessment for soil sampling campaigns
- **Does not apply to:** Soils with very high organic matter content (>20%, e.g., peat); quantitative particle size analysis (use the hydrometer method, SOP-L401); soils with large gravel or rock fragment content (assess the fine fraction only)
- **Location:** All EA Program field sites; can also be performed on samples brought back to the lab

3. Responsibilities

Role	Responsibility
Lab PI	Approve protocol, ensure training compliance
Field Lead	Coordinate sampling, verify observer calibration
Trained Personnel	Perform texture assessments, record results
New Students	Complete training and calibration exercise before independent use

4. Definitions

- **Clay:** The finest soil particle size class, with equivalent spherical diameter less than 0.002 mm; imparts stickiness, plasticity, and cohesion to the moist soil; forms a long, flexible ribbon when extruded between thumb and forefinger.
- **Loam:** A textural class with roughly balanced proportions of sand, silt, and clay; exhibits moderate ribbon length (25–50 mm), moderate grittiness, and moderate stickiness; the most common agricultural soil texture.
- **Plasticity:** The ability of moist soil to be molded and deformed without cracking or crumbling; highest in clay-rich soils; assessed by rolling soil into a thread between the palms.

- **Ribbon:** The flat, continuous strip extruded when moist soil is pushed between the thumb and forefinger; ribbon length is the primary diagnostic criterion in the field texture-by-feel method; longer ribbons indicate higher clay content.
- **Sand:** Soil mineral particles with equivalent spherical diameter 0.05–2.0 mm; feels gritty between the fingers; does not form a ribbon; individual grains are visible to the naked eye.
- **Silt:** Soil mineral particles with equivalent spherical diameter 0.002–0.05 mm; feels smooth and floury when dry; slippery and slightly plastic when moist; imparts a silky texture to the soil.
- **Texture class:** A USDA classification category (e.g., sandy loam, silt clay loam, clay) based on the relative percentages of sand, silt, and clay; determined from the USDA textural triangle and confirmed by laboratory hydrometer analysis (see SOP-L401).
- **Water content (field):** The moisture status of the soil at the time of testing; texture-by-feel must be performed on soil adjusted to a defined moist or wet state to produce consistent results across operators.

5. Safety and Hazards

Hazards

- **Biological:** Soil-borne pathogens; wash hands thoroughly after handling soil
- **Chemical:** At contaminated sites, soil may contain heavy metals or other hazardous materials; wear gloves
- **Physical:** Standard field hazards (ticks, snakes, heat exposure)

5.1. Required PPE

- ☐ Closed-toed shoes or field boots
- ☐ Nitrile gloves (recommended, especially at unknown or contaminated sites)
- ☐ Sun protection (hat, sunscreen)
- ☐ Hand sanitizer or soap and water for post-handling cleanup

5.2. Required Training

- SOP-F001 Field Safety

6. Background

6.1. Soil Particle Size Fractions

Soil texture is defined by the relative proportions of three mineral particle size fractions (Soil Survey Division Staff, 2017):

Fraction	Diameter	Feel
Sand	2.0–0.05 mm	Gritty; individual grains visible and felt between fingers
Silt	0.05–0.002 mm	Smooth, floury, or silky; like rubbing flour or talc
Clay	< 0.002 mm	Sticky when wet; plastic and moldable; stains fingers

6.2. The USDA Textural Triangle

The 12 USDA textural classes are defined by the percentages of sand, silt, and clay. The texture-by-feel method estimates which class a soil belongs to without measuring exact percentages.

6.3. Why Field Texture Matters

- Determines water-holding capacity, drainage, and aeration
- Guides sampling strategy (e.g., different sample weights for carbon analysis; see SOP-L406)
- Informs the soil texture correction factor for nutrient extractions (see SOP-L403)
- Rapid enough to characterize many points across a landscape in a single field day

7. Required Materials

Item	Notes
Water bottle with squeeze top	For wetting soil incrementally
Small ruler (cm)	For measuring ribbon length
Field data sheet	Printed from Appendix A
Clipboard and pencil	Mechanical pencil preferred
GPS unit or smartphone	For recording sample locations
Trowel or soil knife	For collecting subsurface samples
Sealable plastic bags	If bringing samples back for confirmation
Paper towels or rag	For cleaning hands between samples

No specialized laboratory equipment is required.

8. Estimated Time

Task	Time
Per sample (experienced observer)	2–5 minutes
Per sample (new observer)	5–10 minutes
Calibration exercise (training)	30–45 minutes with known-texture standards

9. Procedure

9.1. Collect the Sample

1. Select a representative location and record GPS coordinates
2. Clear surface litter and organic debris
3. Collect approximately a golf-ball-sized amount of mineral soil from the target depth (typically 0–15 cm)
4. Remove visible roots, gravel, and organic fragments

9.2. Moisten the Sample

1. Place the soil in your palm
2. Add water drop by drop from the squeeze bottle while kneading the soil
3. Continue adding water and kneading until the soil reaches the consistency of modeling clay or putty
4. The soil should be uniformly moist but not dripping wet
5. Crush all aggregates between your fingers during kneading

! Getting the Moisture Right

This is the most critical step. If the soil is too dry, it will crumble and you'll underestimate clay content. If too wet, it will be slippery and you'll overestimate clay. The target is a smooth, pliable consistency similar to play dough. This may take 2–3 minutes of kneading.

9.3. Test 1: Form a Ball

1. Try to form the moistened soil into a ball (bolus) by squeezing and rolling in your palm
2. **If the soil will not form a ball**, it is **Sand**. Record and move to the next sample.
3. If a ball forms, proceed to Test 2

9.4. Test 2: Ribbon Test

1. Place the ball between your thumb and forefinger
2. Gently push the soil forward with your thumb, squeezing it into a flat ribbon about 2 mm thick and 1 cm wide
3. Let the ribbon extend over your forefinger, unsupported, until it breaks from its own weight
4. Measure the ribbon length with a ruler before it breaks

5. Repeat 3–5 times and use the longest consistent ribbon

Ribbon length determines clay content:

Ribbon Length	Clay Content	Category
No ribbon forms	$< 10\%$	Low clay
< 2.5 cm (< 1 inch)	10–20%	Low-moderate clay
2.5–5 cm (1–2 inches)	20–35%	Moderate clay
> 5 cm (> 2 inches)	$> 35\%$	High clay

9.5. Test 3: Feel Test (Grittiness and Smoothness)

1. Take a pea-sized piece of the moistened soil
2. Place it in your palm and add excess water to make a muddy slurry
3. Rub the slurry between your thumb and forefinger using a circular motion
4. Classify the dominant feel:

Feel	Sensation	Dominant Fraction
Gritty	Like rubbing sugar or fine sand between fingers; individual grains felt and possibly heard	Sand-dominated
Smooth	Like rubbing flour or talc; silky, no grains felt	Silt-dominated
Neither	Neither gritty nor silky dominates; sticky	Sand and silt balanced

9.6. Determine Textural Class

Combine the ribbon length and feel test results using the following decision matrix (Thien, 1979):

	Gritty Feel	Smooth Feel	Neither Gritty nor Smooth
No ribbon	Sand	Silt	Loam
< 2.5 cm	Sandy loam	Silt loam	Loam
2.5–5 cm	Sandy clay loam	Silty clay loam	Clay loam
> 5 cm	Sandy clay	Silty clay	Clay

Additional Clues

- **Loamy sand** falls between sand and sandy loam: barely forms a ball, very gritty, ribbon <1 cm
- **Silt** (pure): very smooth, almost soapy when wet, forms a weak ball but no ribbon

- When in doubt between two classes, record both (e.g., "sandy loam / loam") and note the uncertainty

9.7. Record Results

Record the textural class, ribbon length, feel, and any notes on the field data sheet (Appendix A). Photograph the ribbon if possible for documentation.

10. Supplementary Test: Jar Sedimentation

For a semi-quantitative confirmation of field texture, you can perform a jar test on the same soil:

1. Fill a clear, straight-sided jar (mason jar or pasta sauce jar) one-third full with soil
2. Fill the jar with water, leaving 2 cm of headspace
3. Add a pinch of non-foaming dish soap (dispersing agent)
4. Cap tightly and shake vigorously for 2 minutes
5. Set the jar on a level surface and do not disturb
6. After 1 minute, mark the settled layer on the jar (this is **sand**)
7. After 2 hours, mark the next layer (this is **silt**)
8. After 24–48 hours, the remaining suspended material has settled (this is **clay**); the water above may still be slightly turbid
9. Measure the thickness of each layer and calculate approximate percentages

> **Note**
>
> The jar test provides a rough visual estimate only. For precise particle size distribution, use the hydrometer method (SOP-L401).

11. Quality Control

- **Calibration:** Before independent fieldwork, each observer must practice on at least 5 soils of known texture (confirmed by hydrometer analysis). Results should match the confirmed texture class for at least 4 of 5 samples.
- **Inter-observer agreement:** When multiple observers are surveying, have each observer independently classify at least 10% of the same samples. Agreement should be within one textural class. Inter-observer variability is a well-documented limitation of texture-by-feel; training and calibration reduce but do not eliminate systematic differences among observers (Salley et al., 2018).
- **Documentation:** Record ribbon length to the nearest 0.5 cm, not just the textural class, so results can be reviewed later.
- **Confirmation:** For critical projects, bring duplicate samples back to the lab for hydrometer analysis on at least 10% of field-textured samples.

When texture-by-feel results will inform quantitative analyses (e.g., pedotransfer functions for water-holding capacity), confirm at least 10% of field-textured samples with hydrometer analysis (SOP-L401). The accuracy of the feel method varies by textural class: sandy and clayey soils are identified most reliably, while loams and silt loams are misclassified more often (Salley et al., 2018).

12. Data Management

Enter results from field sheets into the project spreadsheet within 48 hours. Required fields:

- Date, site, plot ID, observer
- GPS coordinates
- Sample depth (cm)
- Ribbon length (cm)
- Feel (gritty, smooth, or neither)
- Textural class
- Notes (e.g., high gravel content, high organic matter, very dry)

13. References

Salley, S. W., Herrick, J. E., Holmes, B. M., Karl, J. W., Levi, M. R., McCord, S. E., Waal, C. van der, & Edwards, B. R. P. (2018). A comparison of soil texture-by-feel estimates: Implications for the citizen scientist. *Soil Science Society of America Journal*, *82*(6), 1526–1537. https://doi.org/10.2136/sssaj2018.04.0137

Soil Survey Division Staff. (2017). *Soil survey manual*. Government Printing Office. https://www.nrcs.usda.gov/resources/guides-and-instructions/soil-survey-manual

Thien, S. J. (1979). A flow diagram for teaching texture-by-feel analysis. *Journal of Agronomic Education*, *8*, 54–55. https://doi.org/10.2134/jae.1979.0054

14. Training Record

Before performing field texture assessments independently, personnel must:

1. Read this SOP in full
2. Complete a calibration exercise on at least 5 soils of known texture
3. Demonstrate the moistening, ball, ribbon, and feel tests under supervision
4. Achieve agreement within one textural class on 4 of 5 known samples
5. Be signed off by the field lead or PI

15. Appendix A: Soil Texture Field Data Sheet

Print this sheet and use it in the field.

Site: _____ **Date:** _____

Observer: _____ **Project:** _____

Sample #	GPS / Location	Depth (cm)	Ball? (Y/N)	Ribbon (cm)	Feel (G/S/N)	Texture Class	Notes
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							
13							
14							
15							

Feel key: G = Gritty, S = Smooth, N = Neither

Texture class options: Sand, Loamy sand, Sandy loam, Loam, Silt loam, Silt, Sandy clay loam, Clay loam, Silty clay loam, Sandy clay, Silty clay, Clay

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F402

Soil Bulk Density – Core Method

Standard Operating Procedure

SOP Number	SOP-F402
Revision	0.2
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the determination of soil bulk density using the volumetric core method. Bulk density (ρ_b) is the mass of oven-dried soil per unit volume of field-moist soil, expressed in g/cm^3. It is used to convert gravimetric measurements (e.g., nutrient concentrations in mg/kg) to volumetric or areal bases (e.g., kg/ha), to assess soil compaction, to calculate total porosity, and to evaluate management effects on soil physical quality. The core method is the most widely used direct measurement of bulk density and is described in the SSSA Methods of Soil Analysis (Blake & Hartge, 1986; Grossman & Reinsch, 2002).

2. Scope

- **Applies to:** Mineral soils at all EA Program field sites, including agricultural plots, photovoltaic panel study sites, and restoration sites. Suitable for depths of 0–30 cm using hand-driven cores.
- **Does not apply to:** Soils with abundant coarse fragments (greater than approximately 20% by volume of gravel, cobbles, or stones), which require the excavation or clod method. Also not applicable to organic soils (histosols) or very loose sandy soils where core insertion causes compaction of the sample.
- **Location:** All EA Program field sites; laboratory drying and weighing in SGM 133

3. Responsibilities

Role	Responsibility
Lab PI	Approve sampling plan, ensure training compliance
Field Lead	Coordinate sampling logistics, verify equipment
Trained Personnel	Collect cores and process samples per this SOP
New Students	Complete training before independent sampling

4. Definitions

- **Bulk density (ρ_b):** The mass of oven-dry soil per unit volume of field-moist soil, including pore space; expressed in g cm^{-3} or Mg m^{-3}; typically ranges from 1.0–1.8 g cm^{-3} for mineral soils and 0.1–0.5 g cm^{-3} for organic soils.
- **Coarse fragment correction:** An adjustment to bulk density calculations that removes the volume and mass of rock fragments (greater than 2 mm) from the core sample; required in stony or gravelly soils where coarse fragments occupy significant core volume.
- **Core sampler:** A thin-walled, beveled steel cylinder of known volume pushed into the soil to extract an intact sample for bulk density determination; internal dimensions must be measured and recorded for each sampler.

- **Dry bulk density:** The mass of oven-dry soil divided by the total volume of the sample including pore space; the standard reported value for bulk density; calculated after drying at 105 °C to constant mass.
- **Porosity (ϕ):** The fraction of total soil volume occupied by pores (air + water): $\phi = 1 - \rho_b/\rho_p$ where ρ_p is particle density (assumed 2.65 g cm^{-3} for mineral soils); porosity decreases as bulk density increases.
- **Soil carbon stock:** The mass of organic carbon per unit land area (Mg C ha^{-1}) calculated from bulk density, sampling depth, and organic carbon concentration; bulk density measurement is the primary source of uncertainty in carbon stock estimates.
- **Soil compaction:** An increase in bulk density caused by mechanical pressure (tillage, vehicle traffic, trampling); reduces porosity, impedes root growth, and decreases infiltration rates.

5. Safety and Hazards

Hazards

- **Physical:** Driving the core sampler with a mallet or hammer poses a risk of hand and finger injury. Trowels and soil knives are sharp. Repetitive hammering can cause strain.
- **Biological:** Soil-borne pathogens; wash hands thoroughly after handling soil. Tick and insect exposure at field sites.
- **Environmental:** Standard field hazards (heat, uneven terrain, sun exposure).

5.1. Required PPE

- ☐ Closed-toed shoes or field boots
- ☐ Nitrile or leather work gloves
- ☐ Safety glasses (when driving the sampler)
- ☐ Sun protection (hat, sunscreen)

5.2. Related SOPs

Refer to SOP-F001 Field Safety for general field safety procedures, including transportation, weather hazards, and emergency response.

6. Equipment and Materials

6.1. Field Equipment

Item	Notes
Bulk density core rings	Known internal volume; typical dimensions: 5 cm height x 5 cm diameter (approximately 98 cm^3) or 7.5 cm x 7.5 cm

Item	Notes
Core sampler (drive head)	Fits over the core ring; beveled cutting edge
Rubber mallet or dead-blow hammer	For driving the sampler
Driving cap or block	Wooden or rubber block to protect the sampler from mallet strikes
Flat-blade trowel or soil knife	For excavating around the core and trimming ends
Straight-edged blade or spatula	For trimming flush with ring ends
Plastic caps or aluminum foil	Two per core, to seal both ends after trimming
Pre-labeled ziplock bags	For transporting sealed cores
Cooler with ice packs	If gravimetric moisture will be determined separately
Ruler or depth gauge	For measuring insertion depth
Field notebook	For recording site, depth, and core ID
GPS unit or smartphone	For recording sampling coordinates

6.2. Laboratory Equipment

Item	Notes
Drying oven	Set to 105 C; see SOP-L105
Analytical balance	0.01 g resolution minimum; SOP-L102
Desiccator	For cooling dried samples before weighing
Pre-weighed aluminum drying tins	Large enough to hold the full core volume
Calipers or ruler	For verifying core ring dimensions

7. Procedure

7.1. Pre-Sampling Preparation

1. Measure and record the internal dimensions of each core ring (inside diameter and height) using calipers. Calculate the volume:

$$V = \pi \left(\frac{d}{2}\right)^2 \times h$$

where d = inside diameter (cm) and h = height (cm).

2. Pre-label core rings or bags with site name, plot ID, depth, and replicate number
3. Pre-weigh aluminum drying tins and record the tare mass
4. Clean core rings and the sampler with DI water before use
5. File a field work planning form per SOP-F001

7.2. Field Sampling

1. Navigate to the predetermined sampling location and record GPS coordinates
2. Clear surface litter, loose organic debris, and any vegetation from a small area (approximately 15 x 15 cm) at the sampling point without disturbing the mineral soil surface

> **Important**
>
> Do not compress or disturb the soil surface before inserting the core. The goal is to capture the soil at its natural field density.

3. Assemble the core ring inside the drive head. Ensure the beveled cutting edge faces downward.
4. Place the assembled sampler vertically on the soil surface
5. Position the driving cap or wooden block on top of the sampler
6. Drive the sampler into the soil using the mallet, applying steady, even blows. Drive until the top of the core ring is flush with, or slightly below, the soil surface.
7. Do not over-drive the sampler. If the ring is driven more than 1 cm past flush, the sample may be compacted and should be discarded. Over-driving and under-driving the core introduce bias in opposite directions; the magnitude of the bias depends on soil type and moisture status (Grossman & Reinsch, 2002; Page-Dumroese et al., 1999).
8. Carefully excavate the soil around the sampler using the trowel, leaving a pedestal of soil beneath and around the core
9. Slide the trowel or soil knife beneath the bottom of the core to sever it from the underlying soil
10. Lift the sampler and core out of the ground

7.3. Trimming and Capping

1. Remove the core ring from the drive head
2. Using a straight-edged blade, trim the soil flush with the top and bottom of the ring. Remove any material that extends beyond the ring face. Be careful not to smear or compress the soil.
3. If large roots or rocks protrude from the core face, note this in the field notebook. If coarse fragments constitute a substantial fraction of the core, record this and consider whether the core method is appropriate for this site.
4. Cap both ends of the ring with plastic caps or wrap tightly with aluminum foil
5. Place the capped core in a labeled ziplock bag
6. Store in the cooler for transport to the laboratory

> **Tip**
>
> Collect a minimum of three replicate cores per plot or treatment to account for spatial variability. Five replicates are preferred for statistical robustness, especially in heterogeneous soils or when detecting small differences between treatments.

7.4. Backfilling

Backfill the sampling hole with surrounding soil to minimize site disturbance and prevent tripping hazards.

7.5. Laboratory Processing

1. Remove the plastic caps or foil from the core
2. Transfer the entire soil core from the ring into a pre-weighed aluminum drying tin. Use a spatula or trowel to ensure all soil is transferred; scrape the inside of the ring if necessary.
3. Record the mass of the tin plus field-moist soil (this can also be used to calculate gravimetric moisture if a separate subsample is not taken)
4. Place the tin in the drying oven at 105 C for a minimum of 24 hours (until constant mass is achieved). See SOP-L105.
5. Remove the tin from the oven and cool in a desiccator for 20–30 minutes
6. Weigh the tin plus oven-dried soil and record the mass

8. Quality Control and Acceptance Criteria

8.1. Calculations

Bulk density (g/cm^3):

$$\rho_b = \frac{M_{\text{dry}}}{V_{\text{core}}}$$

where M_{dry} = mass of oven-dried soil (g) and V_{core} = internal volume of the core ring (cm^3).

Gravimetric moisture content (if determined from the same core):

$$\theta_g = \frac{M_{\text{wet}} - M_{\text{dry}}}{M_{\text{dry}}}$$

where M_{wet} = mass of field-moist soil (g).

Total porosity (fraction):

$$\phi = 1 - \frac{\rho_b}{\rho_s}$$

where ρ_s is the particle density, typically assumed to be 2.65 g/cm^3 for mineral soils. The assumed particle density of 2.65 g/cm^3 is appropriate for most mineral soils but should be verified for soils with high organic matter (where it may be lower) or heavy mineral content (Blake & Hartge, 1986).

8.2. Expected Ranges

Typical bulk density values for mineral soils:

Soil Type	Expected ρ_b (g/cm^3)
Well-aggregated forest or prairie topsoil	0.8 – 1.2
Cultivated agricultural topsoil	1.1 – 1.5
Compacted or traffic-affected soil	1.5 – 1.8
Sandy subsoil	1.4 – 1.8
Clay-rich subsoil	1.1 – 1.6

Values outside the range of 0.5 – 2.0 g/cm^3 for mineral soils should be investigated for sampling or measurement error.

Variability is typically higher in surface soils than in subsoils because of biological activity and tillage effects (Al-Shammary et al., 2018). For carbon stock calculations, consistent sampling depth and core diameter are critical because small changes in bulk density propagate into large errors at the landscape scale (**walter2016?**).

8.3. QC Requirements

- Collect a minimum of three replicate cores per plot or treatment
- Calculate the coefficient of variation (CV) among replicates. A CV greater than 10% suggests high spatial variability or possible sampling problems (compaction, lost soil during extraction). Consider collecting additional replicates.
- Verify core ring dimensions at the start of each sampling campaign. If the ring is dented or deformed, replace it.
- Dry samples to constant mass. If the mass changes by more than 0.1 g between successive weighings (at least 4 hours apart), continue drying.

9. Waste Disposal

Waste Type	Disposal Method
Dried soil	Return to field site if uncontaminated, or standard trash
Used aluminum tins	Recycle or standard trash
Used gloves	Standard trash

10. References

Al-Shammary, A. A. G., Kouzani, A. Z., Kaynak, A., Khoo, S. Y., Norton, M., & Gates, W. (2018). Soil bulk density estimation methods: A review. *Pedosphere*, *28*(4), 581–596. https://doi.org/10.1016/S1002-0160(18)60034-7

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Grossman, R. B., & Reinsch, T. G. (2002). Bulk density and linear extensibility. In J. H. Dane & G. C. Topp (Eds.), *Methods of soil analysis, part 4: Physical methods* (pp. 201–228). Soil Science Society of America. https://doi.org/10.2136/sssabookser5.4.c9

Page-Dumroese, D. S., Jurgensen, M. F., Brown, R. E., & Mroz, G. D. (1999). Comparison of methods for determining bulk densities of rocky forest soils. *Soil Science Society of America Journal*, *63*(2), 379–383. https://doi.org/10.2136/sssaj1999.03615995006300020016x

11. Training Record

Before collecting bulk density cores independently, personnel must:

1. Read this SOP in full
2. Complete SOP-F001 Field Safety training
3. Complete SOP-F400 Soil Sampling training
4. Observe a trained operator perform core collection, trimming, and laboratory processing
5. Demonstrate proper core driving technique, trimming to flush, and capping without compacting the sample
6. Process at least three replicate cores through drying and weighing with a CV below 10%
7. Be signed off by the field lead or PI

12. Appendix A: Example Calculation

```
# Bulk density calculation from core method
# Core ring dimensions
diameter_cm <- 5.0
height_cm <- 5.0
volume_cm3 <- pi * (diameter_cm / 2)^2 * height_cm

# Masses
tin_g <- 15.32             # Tare mass of aluminum tin
tin_wet_g <- 112.45        # Tin + field-moist soil
tin_dry_g <- 95.78         # Tin + oven-dried soil (105 C, 24 h)

# Calculations
mass_dry_g <- tin_dry_g - tin_g
mass_wet_g <- tin_wet_g - tin_g

bulk_density <- mass_dry_g / volume_cm3
grav_moisture <- (mass_wet_g - mass_dry_g) / mass_dry_g
porosity <- 1 - (bulk_density / 2.65)
```

```
cat(sprintf("Core volume:          %.2f cm3\n", volume_cm3))
cat(sprintf("Oven-dry soil mass:   %.2f g\n", mass_dry_g))
cat(sprintf("Bulk density:         %.2f g/cm3\n", bulk_density))
cat(sprintf("Gravimetric moisture: %.3f g/g\n", grav_moisture))
cat(sprintf("Total porosity:       %.2f\n", porosity))
```

13. Appendix B: Coarse Fragment Correction

If the core contains gravel or rock fragments larger than 2 mm, the bulk density of the fine-earth fraction (less than 2 mm) can be calculated by removing the coarse fragments from the oven-dried sample, weighing them separately, and estimating their volume:

$$\rho_{b,\text{fine}} = \frac{M_{\text{dry}} - M_{\text{cf}}}{V_{\text{core}} - V_{\text{cf}}}$$

where M_{cf} = mass of coarse fragments (g) and V_{cf} = volume of coarse fragments (cm^3), estimated by water displacement or by dividing M_{cf} by an assumed rock density (typically 2.65 g/cm^3). The water-displacement method for estimating coarse-fragment volume is preferred when fragment shapes are irregular (Page-Dumroese et al., 1999).

Record the coarse fragment mass fraction and report whether the bulk density value refers to the whole soil or the fine-earth fraction.

14. Appendix C: Troubleshooting

Symptom	Probable Cause	Corrective Action
Core compacted during insertion	Soil too dry and hard; excessive force	Moisten the surface slightly (note in field notebook); do not hammer past flush
Soil falls out of the bottom	Loose sandy soil; insufficient cohesion	Try sampling when soil is slightly moist; use a core sampler with a retaining basket
Large roots fill the core	Heavily rooted location	Move to a nearby location; note the root density
CV among replicates exceeds 10%	High spatial variability; compaction artifacts	Collect additional replicates; inspect technique
Bulk density above 2.0 g/cm^3	Measurement error or rock fragment in core	Re-check masses and volume; inspect for gravel
Bulk density below 0.5 g/cm^3	Organic layer sampled; incomplete core	Verify that mineral soil was sampled; check for lost soil

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F403

Soil Infiltration Rate – Ring Infiltrometer Method

Standard Operating Procedure

SOP Number	SOP-F403
Revision	0.2
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the field measurement of soil infiltration rate using single-ring and double-ring infiltrometers. Infiltration rate is the velocity at which water enters the soil surface, typically expressed in cm/h or mm/h. It is a key parameter for evaluating stormwater management, irrigation efficiency, soil compaction, hydrologic modeling, and land use impacts on soil physical properties. The double-ring method follows ASTM D3385 (ASTM International, 2018); the single-ring method follows Bouwer (1986). Infiltration measurements are also essential for calibrating hydrologic models and for evaluating the effects of soil sealing in urban environments (Hillel, 1998; Reynolds et al., 2002).

2. Scope

- **Applies to:** Field measurement of infiltration rate on mineral soils at all EA Program sites, including agricultural plots, photovoltaic panel study sites, restoration areas, and urban soils
- **Does not apply to:** Soils with very low hydraulic conductivity (less than 1 x 10^{-5} cm/s; see ASTM D5093 for sealed-inner-ring method); rocky or gravelly soils that prevent ring insertion; laboratory column permeability tests
- **Location:** All EA Program field sites

3. Responsibilities

Role	Responsibility
Lab PI	Approve sampling plan, ensure training compliance
Field Lead	Coordinate logistics, select test locations
Trained Personnel	Conduct infiltration tests per this SOP
New Students	Complete training before independent testing

4. Definitions

- **Double-ring infiltrometer:** A measurement apparatus consisting of two concentric steel rings driven into the soil surface; the inner ring provides the measurement zone; the outer ring creates a buffer that minimizes lateral flow from the inner ring, enforcing predominantly vertical infiltration.
- **Final infiltration rate (steady-state rate):** The constant rate at which water enters the soil after the soil profile is saturated and the infiltration rate has stabilized; approximates the saturated hydraulic conductivity (K_{sat}) of the surface soil.

- **Green-Ampt model:** A physically based infiltration model that describes the rate of water entry into soil as a function of saturated hydraulic conductivity, matric suction at the wetting front, and soil moisture deficit; used to interpret falling-head infiltration data.
- **Infiltration rate:** The volumetric flux of water entering the soil surface per unit area per unit time, expressed in mm h^{-1} or cm h^{-1}; decreases from an initially high rate in dry soil to a lower, stable final rate as the soil wets.
- **Macropore flow:** Preferential movement of water through large continuous pores (earthworm burrows, root channels, structural cracks); produces non-uniform wetting and anomalously high initial infiltration rates that do not reflect bulk soil hydraulic conductivity.
- **Matric potential:** The energy status of soil water due to capillary and adsorptive forces; expressed in kPa or cm H_2O; drives water movement from wet to dry zones during infiltration.
- **Saturated hydraulic conductivity (K_{sat}):** The proportionality constant in Darcy's law relating water flux to hydraulic gradient under saturated conditions; the primary soil hydraulic property controlling drainage, runoff, and deep percolation; approximated by the steady-state infiltration rate.
- **Single-ring infiltrometer:** A simplified apparatus using one ring driven into the soil; easier to deploy than double-ring but subject to lateral flow error; appropriate for screening or comparative measurements when absolute K_{sat} is not required.

5. Safety and Hazards

Hazards

- **Physical:** Driving metal rings into the soil with a sledge or mallet poses a risk of hand, finger, and foot injury. The rings have sharp edges.
- **Ergonomic:** Carrying water containers (20 L jugs) and rings to remote field sites can cause strain.
- **Environmental:** Standard field hazards (heat exposure, uneven terrain, insects).

5.1. Required PPE

☐ Closed-toed shoes or field boots (steel-toed preferred when driving rings)
☐ Leather work gloves (when handling rings and sledge)
☐ Safety glasses (when driving rings)
☐ Sun protection (hat, sunscreen)

5.2. Related SOPs

Refer to SOP-F001 Field Safety for general field safety procedures.

6. Equipment and Materials

6.1. Method A: Double-Ring Infiltrometer (ASTM D3385)

Item	Notes
Inner ring	Minimum 30 cm (12 in) diameter, 20 cm tall; 14-gauge or heavier steel
Outer ring	Minimum 60 cm (24 in) diameter, 20 cm tall; same gauge as inner ring
Sledge hammer (3–5 kg)	For driving rings
Driving cap or wooden plank	Placed on top of the ring to distribute hammer blows evenly and prevent ring deformation
Carpenter's level	For checking ring verticality
Ruler or graduated staff (mm)	For measuring water level in the inner ring
Stopwatch or timer	For recording time intervals
Water supply	40–80 L per test; carry in jerrycans or connect to a portable tank
Bucket or pitcher	For filling rings without disturbing the soil surface
Plastic sheeting or burlap	Placed inside the ring during initial filling to prevent soil disturbance
Mariotte bottle (optional)	For maintaining constant head automatically
Field data sheet	Printed from Appendix A
Clipboard and pencil	Mechanical pencil preferred
GPS unit or smartphone	For recording test locations
Tape measure	For measuring ring insertion depth

6.2. Method B: Single-Ring Infiltrometer

Item	Notes
Single ring	30 cm diameter minimum (larger is better; 90 cm preferred for accuracy); 20 cm tall
All other equipment	Same as Method A, minus the outer ring

7. Procedure

7.1. Site Selection and Preparation

1. Select test locations based on the project sampling plan. Avoid locations with visible animal burrows, large root channels, cracks, or other macropore features unless the objective is specifically to characterize preferential flow.
2. Record GPS coordinates and site description
3. Clear surface litter and loose organic debris from a 1 x 1 m area without disturbing the mineral soil surface
4. Note antecedent conditions in the field notebook: recent rainfall, soil moisture (qualitative: dry, moist, wet), and surface condition (bare, vegetated, crusted)

> **Tip**
>
> Collect a companion bulk density core (SOP-F402) and a texture-by-feel assessment (SOP-F401) at each infiltration test location. These measurements help interpret the infiltration results.

7.2. Ring Installation – Double-Ring Method

1. Center the inner ring at the test location
2. Place the driving cap or wooden plank on top of the ring
3. Drive the inner ring vertically into the soil to a depth of approximately 5–10 cm (2–4 in) using even blows from the sledge. Avoid tilting the ring; check periodically with the level.
4. Measure and record the insertion depth on at least two sides
5. Center the outer ring around the inner ring. Drive it to approximately 10–15 cm (4–6 in), deeper than the inner ring, to prevent test water from escaping beneath the outer ring.
6. Check that the inner ring is still centered and level

> **Important**
>
> Do not drive the rings deeper than necessary. Over-driving compresses the soil along the ring walls and creates an artificial seal that reduces measured infiltration rates. If the soil cracks or separates from the ring wall, abandon the test location and start at a new spot.

7.3. Ring Installation – Single-Ring Method

1. Place the single ring at the test location
2. Drive to approximately 5 cm depth using the same technique as above
3. For best accuracy, use the largest diameter ring available (90 cm or larger reduces lateral flow error) (Bouwer, 1986). Single-ring measurements tend to overestimate true vertical infiltration because lateral divergence below the ring cannot be eliminated; the magnitude of overestimation decreases as ring diameter increases (Gregory et al., 2006).

7.4. Initial Filling

1. Place a sheet of plastic or burlap on the soil surface inside the inner ring (and inside the annular space for the double-ring method) to cushion the water and prevent soil disturbance during filling
2. Gently pour water into the outer ring (annular space) first, then into the inner ring, to approximately 10–15 cm depth
3. Carefully remove the plastic or burlap
4. For the double-ring method, maintain the water level in the outer ring at approximately the same height as the inner ring throughout the test. This eliminates the lateral pressure gradient and forces flow beneath the inner ring to be vertical.

7.5. Measuring Infiltration Rate

Falling-head method (most common for EA Program):

1. Record the initial water level in the inner ring and the start time
2. Allow the water to infiltrate. Record the water level at regular time intervals. Suggested intervals:

Elapsed Time	Recording Interval
0–30 minutes	Every 5 minutes
30–60 minutes	Every 10 minutes
60–120 minutes	Every 15 minutes
120+ minutes	Every 30 minutes

3. When the water level drops to approximately 3 cm above the soil surface, refill to the original level and record the new starting level and time. Record each refill event.
4. Maintain the water level in the outer ring by refilling as needed.
5. Continue the test until the infiltration rate reaches a steady state (three or more consecutive readings with less than 10% change). This is the steady-state or "final" infiltration rate.
6. For most soils, steady state is reached within 1–3 hours. Clayey soils may require 4–6 hours or longer.

Constant-head method (Mariotte bottle):

1. Set up the Mariotte bottle to maintain a constant water depth (typically 5–10 cm) in the inner ring
2. Record the volume of water consumed from the bottle at regular time intervals
3. Calculate the infiltration rate from the volume consumed, the ring area, and the time interval
4. Continue until steady state is reached

7.6. Ending the Test

1. Record the final water level, time, and cumulative number of refills
2. Remove the rings by rocking gently and pulling upward
3. Backfill the holes and restore the site
4. Record any observations about the wetting front depth, root channels, or other features visible after ring removal

8. Quality Control and Acceptance Criteria

8.1. Calculations

Infiltration rate for each time interval (falling-head method):

$$f = \frac{\Delta h}{\Delta t}$$

where f = infiltration rate (cm/h), Δh = drop in water level (cm) during the interval, and Δt = duration of the interval (h). If the ring was refilled during the interval, add the refill depth to Δh.

Cumulative infiltration:

$$F = \sum_{i=1}^{n} f_i \times \Delta t_i$$

where F = cumulative infiltration (cm) and n = number of intervals.

Steady-state infiltration rate (f_c) is the final, approximately constant rate, typically reported as the mean of the last three or more consistent readings.

8.2. Expected Ranges

Steady-state infiltration rates vary widely with soil texture and structure:

Soil Type / Condition	Expected f_c (cm/h)
Coarse sand, well-structured	5 – 25+
Sandy loam	2.5 – 7.5
Loam, well-structured	1.0 – 5.0
Silt loam	0.5 – 2.5
Clay loam	0.1 – 1.0
Compacted or crusted soil	less than 0.5

Values outside the range of 0.01–50 cm/h should be investigated for measurement error or unusual soil conditions.

Rates measured at EA Program sites in the Pomona/Claremont area should be interpreted in the context of the local aridic moisture regime, where antecedent moisture is often very low and hydrophobicity may develop under prolonged dry conditions (Hillel, 1998). Urban and peri-urban soils may show infiltration rates well below their textural expectation due to compaction from foot traffic and construction (**lai2010?**).

8.3. QC Requirements

- Conduct a minimum of three replicate tests per plot or treatment to capture spatial variability
- Record antecedent soil moisture and recent precipitation for each test; these strongly affect initial rates
- Verify that the outer ring water level is maintained throughout the test (double-ring method)
- Inspect ring installation for gaps between the ring wall and the soil; if gaps are visible, the test is invalid

- Ring insertion depth and ponded water depth both influence measured infiltration rate; standardize these dimensions across replicates and sites to ensure comparability (Gregory et al., 2006).
- Plot the infiltration rate versus time curve for each test. A monotonically decreasing curve approaching a constant value is expected. Erratic increases may indicate macropore activation or ring leakage.

9. Waste Disposal

Waste Type	Disposal Method
Test water	Allowed to infiltrate on site; no disposal needed
Used plastic sheeting	Standard trash
Used gloves	Standard trash

10. References

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11. Training Record

Before conducting infiltration tests independently, personnel must:

1. Read this SOP in full
2. Complete SOP-F001 Field Safety training
3. Observe a trained operator perform a complete double-ring infiltration test from ring installation through steady state
4. Demonstrate proper ring driving technique without ring deformation or soil cracking
5. Conduct at least one complete test under supervision and produce an infiltration curve that reaches steady state
6. Be signed off by the field lead or PI

12. Appendix A: Infiltration Test Field Data Sheet

Print this sheet and use it in the field.

Site: _____ **Plot ID:** _____ **Date:** _____

Observer: _____ **GPS:** _____

Method: Double-ring / Single-ring (circle one)

Inner ring diameter: _____ cm **Outer ring diameter:** _____ cm (if double-ring)

Ring insertion depth: Inner _____ cm, Outer _____ cm

Antecedent conditions: Last rain _____ days ago, _____ mm; Soil moisture: dry / moist / wet

Surface condition: bare / vegetated / crusted / mulched

Initial water depth in inner ring: _____ cm

Reading #	Clock Time	Elapsed (min)	Water Level (cm)	Refill? (Y/N)	Level After Refill (cm)	Interval Δh (cm)	Interval Rate (cm/h)	Notes
0		0		–	–	–	–	Start
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
13								
14								
15								

Steady-state rate (f_c): _____ cm/h (mean of last ____ readings)

Total test duration: _____ minutes

Additional notes: _____

13. Appendix B: Example Calculation and Plotting

```r
library(tidyverse)

# Example data from a falling-head double-ring test
# Ring inner diameter = 30 cm
ring_diam_cm <- 30
ring_area_cm2 <- pi * (ring_diam_cm / 2)^2

infiltration <- tibble(
  elapsed_min = c(0, 5, 10, 15, 20, 30, 45, 60, 90, 120, 150, 180),
  water_level_cm = c(15.0, 13.8, 12.9, 12.1, 11.4, 10.2, 8.8, 7.6,
                     5.8, 4.2, 2.8, 1.5)
)

# Calculate interval infiltration rates
infiltration <- infiltration %>%
  mutate(
    delta_h_cm = -diff(c(NA, water_level_cm)),
    delta_t_h = c(NA, diff(elapsed_min)) / 60,
    rate_cm_h = delta_h_cm / delta_t_h,
    cumulative_cm = cumsum(replace_na(delta_h_cm, 0))
  )

# Steady-state rate (last 3 intervals)
f_c <- mean(tail(infiltration$rate_cm_h[!is.na(infiltration$rate_cm_h)], 3))
cat(sprintf("Steady-state infiltration rate: %.2f cm/h\n", f_c))

# Plot infiltration rate vs time
ggplot(infiltration %>% filter(!is.na(rate_cm_h)),
       aes(x = elapsed_min, y = rate_cm_h)) +
  geom_point(size = 3) +
  geom_line() +
  geom_hline(yintercept = f_c, linetype = "dashed", color = "blue") +
  annotate("text", x = max(infiltration$elapsed_min) * 0.7,
           y = f_c + 0.3,
           label = sprintf("f[c] == %.2f~cm/h", f_c),
           parse = TRUE, color = "blue") +
  labs(
    x = "Elapsed time (min)",
    y = "Infiltration rate (cm/h)",
    title = "Double-Ring Infiltration Test"
  ) +
  theme_minimal()
```

14. Appendix C: Troubleshooting

Symptom	Probable Cause	Corrective Action
Water leaks around the outside of the ring	Insufficient insertion depth; gap between ring wall and soil	Drive ring deeper; pack bentonite or moist soil around the outside base
Infiltration rate increases mid-test	Macropore activation; preferential flow path opened	Note in field notebook; this may be a real feature of the soil. If clearly an artifact (ring shifted), restart.
Very slow infiltration (less than 0.1 cm/h)	Dense clay; surface crust; compacted layer	Continue test for at least 4–6 hours to confirm steady state; consider ASTM D5093 sealed-ring method
Rate never stabilizes	Heterogeneous soil; macropores draining and refilling	Extend test duration; collect additional replicates; report the range rather than a single value
Ring deformed during installation	Rocks or roots; excessive force	Replace with a new ring at a nearby location
Soil disturbed inside the ring	Overly aggressive filling; ring bounced during driving	Restart at a new location; always use plastic sheeting during initial fill
Single-ring rate much higher than expected	Lateral flow beneath the ring	Use a larger-diameter ring (90 cm+) or switch to the double-ring method

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F404

Soil Temperature Profiles

Standard Operating Procedure

SOP Number	SOP-F404
Revision	0.3
Author(s)	Marc Los Huertos, Artemis Cruz
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes procedures for measuring soil temperature at multiple depths to construct soil temperature profiles. Soil temperature influences biological activity (microbial respiration, root growth, seed germination), chemical reaction rates (nutrient mineralization, decomposition), and physical processes (evaporation, frost heave) (Ham, 2002; Hillel, 1998). Temperature profiles are especially relevant for photovoltaic panel studies, where shading alters the soil thermal regime.

The amplitude and phase lag of diurnal and seasonal temperature waves attenuate with depth according to the thermal diffusivity of the soil profile, making multi-depth measurement essential for characterizing subsurface energy budgets (Hillel, 1998). Soil temperature also governs rates of microbial respiration and nitrogen mineralization, with Q_{10} values typically ranging from 2 to 3 for the biologically active zone (Onwuka & Mang, 2018). When continuous monitoring is not feasible, air-temperature-based models can be used to estimate soil temperature, but these require site-specific calibration (Zheng et al., 1993).

2. Scope

- **Applies to:** Spot measurements using handheld probe thermometers and continuous monitoring using datalogger-based thermocouple or thermistor systems at all EA Program field sites
- **Does not apply to:** Soil heat flux measurements (requires heat flux plates); fire-related soil heating studies
- **Location:** All EA Program field sites, including photovoltaic panel study areas, Bernard Field Station, and restoration sites

3. Responsibilities

Role	Responsibility
Lab PI	Approve monitoring plan, ensure training compliance
Field Lead	Coordinate installation, assign measurement schedules
Trained Personnel	Install sensors, collect data, maintain equipment
New Students	Complete training before independent measurement

4. Definitions

- **Diurnal temperature variation:** The cycle of soil temperature change over a 24-hour period driven by solar radiation and radiative cooling; amplitude decreases with depth and is nearly zero below about 50 cm in most soils.
- **Horizon temperature:** The temperature measured at a defined soil depth corresponding to a specific genetic horizon (e.g., A, B, C); used to characterize the thermal environment of the root zone and microbial communities.
- **Soil temperature:** The thermal energy state of the soil, expressed in °C; controls rates of microbial activity, organic matter decomposition, plant root growth, and soil gas fluxes.
- **Temperature gradient:** The rate of change in temperature with depth (°C cm^{-1}); drives heat flux between soil layers and between the soil surface and the atmosphere.
- **Temperature profile:** A set of soil temperature measurements taken at multiple depths simultaneously or sequentially at a single location; used to characterize heat storage, heat flux, and seasonal thermal dynamics.
- **Thermistor:** A temperature sensor whose electrical resistance changes predictably with temperature; commonly used in data loggers for continuous soil temperature monitoring; requires calibration against a traceable reference thermometer.
- **Thermocouple:** A temperature sensor composed of two dissimilar metals joined at a measuring junction; produces a small voltage proportional to the temperature difference between the junction and a reference; used in handheld soil thermometers.

5. Safety and Hazards

Hazards

- **Physical:** Sharp probe tips, rebar stakes, hammering pilot holes in hard soil
- **Biological:** Ticks, snakes, and other field hazards (see SOP-F001)
- **Electrical:** Datalogger batteries and wiring (low voltage, minimal risk)
- **Environmental:** Heat exposure during summer measurements in southern California

5.1. Required PPE

- ☐ Closed-toed shoes or field boots
- ☐ Gloves (when hammering pilot holes or handling rebar)
- ☐ Sun protection (hat, sunscreen)
- ☐ Safety glasses (when hammering)

5.2. Required Training

- SOP-F001 Field Safety

6. Summary of Methods

Two approaches are described in this SOP. Select based on your project requirements. Both methods are described in the SSSA Methods of Soil Analysis (Ham, 2002). The handheld method follows the GLOBE soil temperature protocol (**globe2014soil?**), which has been widely adopted for teaching and citizen-science applications.

Method	Best For	Temporal Resolution	Cost
Handheld probe	Spot measurements, surveys across many points	Single time point per visit	Low
Datalogger system	Continuous monitoring, diurnal and seasonal patterns	Minutes to hours (automated)	High

6.1. Standard Measurement Depths

Depth	Rationale
2 cm	Near-surface; responds rapidly to air temperature and solar radiation
5 cm	Root zone surface; standard GLOBE protocol depth
10 cm	Upper root zone; dampened diurnal signal
20 cm	Mid root zone; minimal diurnal variation
50 cm	Deep reference; seasonal variation only

Choosing Depths

For most EA Program projects, 5 cm and 10 cm are the minimum. Add 2 cm for surface energy balance studies and 20 cm or 50 cm for deep profile characterization.

7. Required Materials

7.1. Method A: Handheld Probe

Item	Notes
Thermocouple (Type T or Type K) or Resistance Temperature Detector (RTD) probes	Probe length must exceed deepest measurement depth + 2 cm. These probes carry a systematic measurement error; raw readings must be corrected using the probe-specific formula (see Data Correction under Data Management).
Electronic reader for probes	For the RTD probes, use either of the Omega HH804 readers. For the Type T probes, pair probe 1 with cable 1 and reader T819, and probe 3 with cable 2 and reader T299. The cable and reader both affect the reading, and the correction formulas are valid only for these specific equipment pairings.
Permanent marker, tape, or heat-shrink band	For marking measurement depths along the probe (see Prepare Depth Guide Marks)
Nail or awl	Same diameter as thermometer probe, for making pilot holes in hard soil
Hammer	For driving the nail into compacted soil
Field data sheet	Printed from Appendix A
Clipboard and pencil	Mechanical pencil preferred
GPS unit or smartphone	For recording measurement locations
Timer or watch	For tracking equilibration time

7.2. Method B: Datalogger System

Item	Notes
Datalogger	Campbell Scientific CR1000, CR300, or equivalent
Thermocouple probes	Type T or Type E; one per measurement depth
Thermistor probes (alternative)	Campbell Scientific 109SS or equivalent
Averaging thermocouple probe (optional)	Campbell Scientific TCAV-L for energy balance studies
Soil temperature profiler (optional)	Hukseflux STP01 or METER TEROS 06
Signal cable	Sufficient length from sensors to datalogger
Weatherproof enclosure	For datalogger and battery
12V battery and solar panel	For continuous power
PVC conduit	For protecting cable runs
Shovel or soil knife	For sensor installation
Laptop with datalogger software	For programming and data download

8. Estimated Time

Task	Time
Handheld: per measurement point (all depths)	10–15 minutes (including equilibration)
Datalogger: initial installation	2–4 hours per station
Datalogger: data download visit	15–30 minutes
Datalogger: sensor replacement	30–60 minutes

9. Procedure: Method A – Handheld Probe

9.1. Prepare Depth Guide Marks

Before going to the field, mark each measurement depth directly on the probe. The temperature sensor is at the tip of the probe, so each mark is placed at the target depth measured from the tip. In the field, push the probe in until the mark for the target depth is level with the soil surface.

Target Depth	Mark Distance from Tip
5 cm	5 cm
10 cm	10 cm
20 cm	20 cm

Use a permanent marker, a wrap of tape, or a heat-shrink band at each depth. Verify the marks against a ruler before each field season, since tape and marker can shift or wear off with use.

9.2. Select Measurement Locations

1. Navigate to the predetermined sampling location and record GPS coordinates
2. Select a representative spot with undisturbed soil surface
3. Gently brush away surface litter without disturbing the mineral soil

9.3. Take Measurements

1. If the soil is hard, use the nail and hammer to make a pilot hole to the target depth. Make the hole the same diameter as the probe to ensure good thermal contact.
2. Insert the probe and gently push until the mark for the target depth is level with the soil surface
3. Wait at least 2 minutes for the probe to equilibrate with the soil. Equilibration time depends on the thermal conductivity of the soil and the thermal mass of the probe; dry sandy soils may require 5 minutes or longer (Ham & Senock, 1992).
4. Read the temperature every 1 minute until two consecutive readings are within 0.5 C of each other
5. Record the stabilized temperature, depth, time, and location on the data sheet
6. Remove the probe and repeat at the next depth in the same hole (work from shallowest to deepest)

7. Move to the next measurement location

> **Equilibration Is Essential**
>
> Do not rush readings. An un-equilibrated probe will read too warm in cool soil and too cool in warm soil. Two minutes is the minimum; 3–5 minutes may be needed in dry or very cold soil.

9.4. Timing

- For comparable data across sites, complete all measurements within 1 hour of local solar noon (approximately 12:00–13:00 PST)
- For diurnal profiles, measure at consistent intervals (e.g., every 2–3 hours over a 24-hour period)
- Record the exact time of each measurement

10. Procedure: Method B – Datalogger System

10.1. Sensor Installation

1. Select a representative location and record GPS coordinates
2. Dig a narrow trench or pit to the deepest sensor depth on one side of the measurement point (do not disturb the soil column directly above the sensors)
3. Insert sensors horizontally into the undisturbed soil wall at each target depth, pressing firmly to ensure good thermal contact
4. Backfill the trench carefully, maintaining the original soil layering as much as possible
5. Route cables through PVC conduit to the datalogger enclosure
6. Mark the sensor locations with discreet, flush-to-ground markers

> **Settling Period**
>
> Soil disturbed during installation needs time to return to representative thermal conditions. Allow a minimum of 48 hours before using data for analysis. One to two weeks is ideal for deep sensors.

10.2. Datalogger Programming

1. Connect all sensors to the datalogger following the wiring diagram for your sensor type
2. Program the datalogger to scan sensors at the desired interval (typically every 1–5 minutes)
3. Configure storage to record averages at 15-minute or 30-minute intervals
4. Set the datalogger clock to local standard time (do not use daylight saving time)
5. Verify readings against a calibrated handheld thermometer at 5 cm depth

10.3. Data Download

1. Visit the station at regular intervals (weekly to monthly, depending on battery and storage capacity)
2. Connect a laptop and download data using the manufacturer's software
3. Check for gaps, sensor failures, or anomalous readings in the field
4. Transfer data to the project database within 48 hours

11. Calibration

11.1. Handheld Probes

1. Prepare an ice-water bath (crushed ice + deionized water, stirred); this should read 0.0 C
2. Insert the probe and allow to equilibrate for 3 minutes
3. Record the reading; it should be within 0.5 C of 0.0 C
4. If outside tolerance, adjust or replace the thermometer
5. Calibrate before each field season and after any suspected damage

The ice-point calibration is a single-point check; for research requiring higher accuracy, a two-point calibration (ice-water and warm-water bath at approximately 40 C) is recommended (Ham, 2002).

Type K Probes

Type K probes require a multi-point calibration to build their error-correction formula. Measure the reading at a minimum of three temperatures: an ice bath (approximately 0 C), room temperature, and a heating plate at approximately 50 C. Use a reference thermometer at each point and fit the correction from the paired readings (see Data Correction).

11.2. Datalogger Sensors

1. Before installation, place all sensors in a well-stirred water bath
2. Record readings from all sensors simultaneously
3. All sensors should agree within 0.3 C
4. Document any offsets and apply corrections in post-processing if needed

12. Quality Control

- Calibrate all thermometers and sensors before each field season
- For handheld measurements, take readings at a minimum of 3 replicate locations per plot
- Record the time of every measurement (soil temperature changes throughout the day)
- For datalogger systems, inspect data weekly for drift, gaps, and anomalous values
- Include a surface air temperature measurement alongside soil temperatures for context
- Photograph each measurement location at the start of each season

13. Data Management

13.1. Handheld Data

Enter data from field sheets into the project spreadsheet within 48 hours. Required fields:

- Date, time (local standard time), site, plot ID, observer
- Measurement depth (cm)
- Temperature (C)
- Surface condition (bare soil, litter, vegetated, shaded)
- Notes (e.g., soil was saturated, recent rain)

13.2. Data Correction

The handheld probes carry a systematic measurement error, so raw readings must be corrected before analysis. Apply the calibration formula matched to the specific probe, cable, and reader combination; the corrections below are valid only for the equipment pairings listed. Let T_{raw} be the reading in °C and T_{corr} the corrected temperature in °C.

RTD Probe 1

$$T_{\text{corr}} = 1.160\,T_{\text{raw}} - 13.23$$

RTD Probe 2

$$T_{\text{corr}} = 1.167\,T_{\text{raw}} - 14.609$$

Type T Probe 1 (Cable 1, reader T819)

$$T_{\text{corr}} = 1.028\,T_{\text{raw}} - 8.797$$

Type T Probe 3 (Cable 2, reader T299)

$$T_{\text{corr}} = 1.004\,T_{\text{raw}} - 8.121$$

Record which probe, cable, and reader were used for each measurement so the correct formula can be applied during processing. Type K probes must be calibrated and corrected using a formula derived from their own three-point calibration (see Calibration).

13.3. Datalogger Data

- Download raw files and archive unmodified copies
- Process data into a standardized format: timestamp, site, depth, temperature
- Flag and document any corrections (sensor offsets, gap-filling)
- Store processed data in the project database with metadata

14. Waste and Site Impact

- Minimize soil disturbance during installation; backfill trenches carefully
- Remove all temporary equipment, flagging, and markers after the study
- Leave permanent sensors in place for long-term monitoring unless the study is complete
- Pack out all trash and supplies

15. References

Ham, J. M. (2002). Soil temperature and heat flux. In J. H. Dane & G. C. Topp (Eds.), *Methods of soil analysis, part 4: Physical methods* (pp. 1181–1233). Soil Science Society of America. https://doi.org/10.2136/sssabookser5.4.c50

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16. Training Record

Before measuring soil temperature independently, personnel must:

1. Read this SOP and SOP-F001 Field Safety in full
2. Calibrate a handheld probe using an ice-water bath
3. Demonstrate proper use of depth marks and equilibration timing
4. For datalogger work: demonstrate sensor installation, datalogger programming, and data download under supervision
5. Be signed off by the field lead or PI

17. Appendix A: Soil Temperature Field Data Sheet

Print this sheet and use it in the field.

Site: _____ **Plot ID:** _____ **Date:** _____

Observer: _____ **Instrument ID:** _____

Surface condition: _____ **Weather:** _____

Air temperature (C): _____ **Time of air temp reading:** _____

Location #	GPS / Description	Time	2 cm (C)	5 cm (C)	10 cm (C)	20 cm (C)	50 cm (C)	Notes
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								

Calibration check (ice bath reading): _____ C **Time:** _____

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F405

Soil Color Determination (Munsell)

Standard Operating Procedure

SOP Number	SOP-F405
Revision	0.2
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes the field determination of soil color using the Munsell Soil Color Charts. Soil color is one of the most readily observable soil properties and provides information about organic matter content, drainage and aeration status, mineralogy, and degree of weathering (Munsell Color, 2009; Schoeneberger et al., 2012). Lower values (darker soils) generally reflect higher organic matter content, especially in A horizons, while high chroma indicates well-drained, oxidized conditions and low chroma suggests prolonged saturation or reducing environments (Lynn & Pearson, 1993; Shields et al., 1966). Red and yellow hues are controlled primarily by iron oxide mineralogy: hematite produces redder hues (5YR and redder), while goethite produces yellowish-brown hues (7.5YR–10YR) (Schwertmann, 1993; Torrent & Schwertmann, 1983). Consistent color determination is essential for soil profile descriptions (SOP-F407), wetland delineation, and site characterization.

2. Scope

- **Applies to:** Field determination of soil color for all EA Program soil sampling campaigns, profile descriptions, and site characterizations
- **Does not apply to:** Instrumental color measurement (colorimeters or spectrophotometers); rock or sediment color classification using the Rock Color Chart; Munsell readings on dried, ground samples in the lab (see SOP-L419)
- **Location:** All EA Program field sites; can also be performed on fresh samples in the lab

3. Responsibilities

Role	Responsibility
PI / Field Lead	Ensure Munsell charts are available and in good condition
Trained Personnel	Determine soil color per this SOP
New Students	Complete training and inter-observer calibration exercise

4. Definitions

- **Chroma:** The colorimetric purity or saturation of a color; the third component of Munsell notation (e.g., the 4 in 10YR 4/4); low chroma (2 or less) in subsurface horizons indicates gleying and periodic waterlogging.
- **Gleying:** A reduction process in waterlogged soils that depletes iron and produces low-chroma (gray, blue-gray, or green-gray) matrix colors; a diagnostic indicator of hydric soil conditions.
- **Hue:** The dominant spectral color in Munsell notation (e.g., 10YR, 7.5YR, 2.5Y, Gley 1); the first element of a Munsell designation; corresponds to the dominant wavelength of reflected light.
- **Matrix color:** The dominant color occupying more than 50% of the exposed soil face; recorded first in the Munsell notation before mottles or redoximorphic features.
- **Mottle:** A spot or blotch of color contrasting with the matrix color; described by abundance (few, common, many), size (fine, medium, coarse), and contrast (faint, distinct, prominent).
- **Munsell color system:** A standardized three-dimensional color notation (Hue Value/Chroma) used universally in soil science for objective color description; readings must be made in consistent natural or reference lighting conditions.
- **Redoximorphic features:** Color patterns caused by reduction and oxidation of iron and manganese under fluctuating saturation; include masses (concentrations of iron oxides) and depletions (zones of low chroma); diagnostic for hydric soil identification and wetland delineation.
- **Value:** The lightness or darkness of a color in Munsell notation; ranges from 0 (black) to 10 (white); the second component of Munsell notation (e.g., the 4 in 10YR 4/4).

5. Safety and Hazards

Hazards

- **Biological:** Soil-borne pathogens; wash hands after handling soil.
- **Physical:** Standard field hazards (ticks, snakes, heat exposure).
- **Chemical:** At contaminated sites, soil may contain heavy metals or other hazardous materials; wear gloves.

5.1. Required PPE

☐ Closed-toed shoes or field boots
☐ Nitrile gloves (recommended)
☐ Sun protection (hat, sunscreen)

5.2. Emergency Procedures

Refer to SOP-F001 Field Safety.

6. Equipment and Materials

Item	Notes
Munsell Soil Color Charts (current edition)	Handle with clean, dry hands; protect from rain and direct soil contact
Trowel or soil knife	For exposing fresh soil surfaces
Squeeze bottle with DI water	For moistening dry samples
Paper towels	For cleaning hands between samples
Field notebook	For recording colors

7. Background: The Munsell System

Munsell color notation has three components (Munsell Color, 2009; Soil Survey Staff, 2022):

- **Hue:** The dominant spectral color (e.g., 10YR, 2.5Y, 5YR). Pages in the chart book are arranged by hue.
- **Value:** Lightness, from 0 (absolute black) to 10 (absolute white). Vertical axis on each page.
- **Chroma:** Color intensity or saturation, from 0 (neutral gray) outward. Horizontal axis on each page.

A complete Munsell notation is written as: **Hue Value/Chroma** (e.g., 10YR 4/3). The corresponding color name (e.g., "brown" or "dark yellowish brown") is listed on each chip.

8. Procedure

8.1. Sample Preparation

1. Expose a fresh soil surface by breaking a ped or cutting with a trowel. Do not use smeared or scraped surfaces, as smearing compresses the soil and alters the apparent color.
2. Determine color on **moist** soil. If the soil is dry, add a few drops of DI water to a small subsample and allow it to absorb for 30 seconds before reading.
3. If both moist and dry colors are needed (e.g., for profile descriptions), read the moist color first, then allow a portion to air-dry and read the dry color.

8.2. Reading the Color

1. Work in natural daylight, ideally under an overcast sky or in open shade. Avoid direct sunlight on the soil or the chart, as glare distorts color perception. Do not read colors under artificial light or at twilight.
2. Hold the soil sample behind the chart page apertures so that the soil is visible through the holes.
3. Compare the soil to the color chips (Schoeneberger et al., 2012). Start with the hue page that appears closest, then move to the value row that matches, then slide along the chroma axis.
4. Select the chip that most closely matches the soil. If the soil falls between two chips, record the notation of the nearest chip and note "between X and Y" in the field notebook.
5. Record the full Munsell notation and the color name:
   - Example: **10YR 3/2 (very dark grayish brown)**
6. If the soil contains mottles or redoximorphic features, record the matrix color and then the mottle color(s) separately, noting the abundance (few, common, many), size (fine, medium, coarse), and contrast (faint, distinct, prominent).

8.3. Special Cases

Organic soils (O horizons, peat): Use the Gley pages or the 10YR page with very low value and chroma. Very dark colors (value 2 or less) are common.

Gleyed soils: Use the Gley 1 and Gley 2 pages for soils with chroma of 2 or less and hues bluer than 5Y. Gleyed colors indicate prolonged saturation and reducing conditions.

Mottled soils: Record:

- Matrix color (dominant color occupying more than 50% of the surface)
- Mottle colors (each distinct color)
- For each mottle: abundance, size, and contrast

Abundance	Definition
Few	$< 2\%$ of exposed surface
Common	2–20% of exposed surface

Abundance	Definition
Many	> 20% of exposed surface

Size	Definition
Fine	< 5 mm diameter
Medium	5–15 mm diameter
Coarse	> 15 mm diameter

Contrast	Definition
Faint	Hue, value, and chroma differ by 1 unit or less from matrix
Distinct	Differ by 2–3 units in value or chroma
Prominent	Differ by more than 3 units

Redoximorphic features are diagnostic for hydric soil identification and wetland delineation; consistent recording of mottle properties is critical for regulatory applications (Soil Survey Division Staff, 2017).

9. Quality Control

- At the start of each field season, have two or more observers independently read the same soil samples to assess inter-observer agreement. Readings should agree within one chip (one unit of value or chroma) in at least 80% of comparisons.
- Replace Munsell charts when color chips are faded, soiled, or damaged. Faded chips produce systematic errors.
- Record lighting conditions (overcast, open shade, sunny) in the field notebook; lighting affects color perception.
- Always specify whether the reading was on moist or dry soil.

Digital colorimeters and smartphone-based color apps are increasingly used as supplements to visual Munsell matching; however, instrument readings and visual assessments can disagree, especially at low chroma and low value, so visual Munsell determination remains the standard for USDA soil survey and regulatory work (Viscarra Rossel et al., 2006).

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11. Related SOPs

SOP	Title	Relationship
F001	Field Safety	Required prerequisite
F400	Soil Sampling	Soil collection for color reading
F407	Soil Profile Description	Color is part of profile description
L419	Soil Color (Munsell – Lab)	Lab confirmation of field color

12. Training Record

Before determining soil color independently, personnel must:

1. Read this SOP in full
2. Complete an inter-observer calibration exercise with a trained operator, achieving agreement within one chip on at least 8 of 10 test samples
3. Be signed off by the PI or field lead

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F406

Soil Respiration Chambers (Field Static Chamber Method)

Standard Operating Procedure

SOP Number	SOP-F406
Revision	0.2
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes the deployment and operation of static (closed, non-flow-through) chambers for manual measurement of soil CO_2 efflux in the field. Static chambers are a simple, low-cost method for quantifying soil respiration across treatments, land uses, or seasonal gradients when the Picarro CRDS automated system (SOP-F504) is not available or when additional spatial replication is needed. The method captures headspace gas samples at timed intervals, which are analyzed for CO_2 concentration using a portable infrared gas analyzer (IRGA), syringe samples returned to the

lab, or soda-lime traps (Hutchinson & Mosier, 1981; Livingston & Hutchinson, 1995; Parkin & Venterea, 2012).

The static chamber method remains one of the most widely used approaches for point-scale soil CO_2 flux measurement because of its simplicity and low cost, though it is subject to known biases from headspace concentration buildup and pressure artifacts (Pumpanen et al., 2004; Rochette & Eriksen-Hamel, 2005). At a global scale, soil respiration represents one of the largest fluxes in the terrestrial carbon cycle, and small changes can have significant implications for atmospheric CO_2 concentrations (Davidson et al., 2000; Raich & Schlesinger, 1992).

2. Scope

- **Applies to:** Manual static chamber measurements of soil CO_2 efflux at EA Program field sites; applicable to grasslands, agricultural plots, restoration sites, and forest soils
- **Does not apply to:** Automated dynamic chambers with the Picarro CRDS analyzer (see SOP-F504); eddy covariance or gradient flux methods; aquatic CO_2 flux measurements; N_2O or CH_4 flux measurements requiring GC or CRDS analysis (though the same chambers can be used with different analytical endpoints)
- **Location:** All EA Program terrestrial field sites

3. Responsibilities

Role	Responsibility
PI / Field Lead	Approve chamber design and deployment plan
Trained Personnel	Install collars, deploy chambers, collect gas samples
New Students	Complete training before independent use

4. Definitions

- **Basal soil respiration:** CO_2 efflux from undisturbed soil at ambient temperature and moisture without addition of labile substrate; reflects the combined metabolic activity of the soil microbial community and root respiration.
- **CO_2 efflux (soil respiration rate):** The flux of CO_2 from the soil surface to the atmosphere, expressed in µmol CO_2 m^{-2} s^{-1} or g C m^{-2} d^{-1}; the primary field measurement made with a closed or open dynamic chamber system.
- **Closed dynamic chamber:** A chamber placed over the soil surface in which headspace CO_2 concentration rises over time as CO_2 is released from the soil; flux is calculated from the rate of concentration increase, chamber volume, and collar area.
- **Collar:** A short ring of PVC or metal inserted several centimeters into the soil before measurement to create a gas-tight seal with the chamber; installed at least 24 hours before measurements to allow soil disturbance effects to dissipate.
- **Heterotrophic respiration:** CO_2 produced by soil microorganisms decomposing organic matter; one component of total soil respiration along with autotrophic (root and rhizosphere) respiration.

- **LI-COR analyzer (LI-8100A / LI-8150):** A portable infrared gas analyzer system used for automated soil CO_2 flux measurement; calculates flux from the exponential rise in headspace CO_2 concentration over a defined measurement period.
- **Q_{10} coefficient:** The factor by which soil respiration rate increases for a 10 °C rise in temperature; typically 1.5–3.5 for mineral soils; used to temperature-normalize measurements made under different field conditions.
- **Rhizosphere priming:** Enhanced decomposition of soil organic matter stimulated by root exudates; inflates field respiration measurements relative to bare-soil or root-exclusion treatments.

5. Safety and Hazards

Hazards

- **Physical:** Driving collars into soil with a mallet poses impact injury risk. Carrying chambers and equipment over uneven terrain.
- **Heat:** Southern California field sites can exceed 40 C. Metal chambers become extremely hot in direct sunlight; handle with gloves.
- **Chemical:** Soda-lime ($NaOH/Ca(OH)_2$) is corrosive and causes burns on skin and eye contact. Concentrated NaOH solutions used for soda-lime traps are hazardous. Handle with gloves and safety glasses.

5.1. Required PPE

- ☐ Closed-toed shoes or field boots
- ☐ Nitrile or leather gloves
- ☐ Safety glasses (when handling soda-lime or driving collars)
- ☐ Sun protection (hat, sunscreen)

5.2. Related SOPs

Refer to SOP-F001 Field Safety for general field safety procedures.

6. Equipment and Materials

Item	Notes
PVC or metal collars	Installed in the soil at least 24 hours before measurement
Chamber lids	Opaque (dark) for total respiration; clear for net CO_2 exchange
Rubber mallet	For installing collars
Sealing material	Closed-cell foam gasket or water trough on collar rim

Item	Notes
Syringes (30–60 mL)	For headspace gas sampling (syringe method)
Evacuated vials (Exetainer)	For storing gas samples for lab analysis
Portable IRGA (optional)	For real-time CO_2 concentration readings
Soda-lime (optional)	For absorption-based CO_2 measurement
Timer or stopwatch	For tracking deployment intervals
Thermometer or soil temp probe	For concurrent soil temperature
Soil moisture probe (optional)	TDR or capacitance sensor
Ruler or tape measure	For measuring headspace height
Field data sheet	For recording deployment times and readings
GPS unit or smartphone	For recording chamber locations

7. Procedure

7.1. Collar Installation

1. Install PVC or metal collars into the soil to a depth of approximately 5 cm at least 24 hours (preferably 48 hours) before the first measurement to allow the soil to recover from disturbance.
2. Do not sever major roots during installation; if roots are encountered, reposition slightly.
3. Measure and record the collar inside diameter and the height of the collar above the soil surface on at least two sides. These dimensions are needed to calculate headspace volume.

7.2. Chamber Deployment

1. Record the start time, soil temperature (at 5 cm depth), and soil moisture at each collar location.
2. Place the chamber lid on the collar, ensuring a gas-tight seal (foam gasket compressed or water trough filled).
3. If using the syringe method, collect a headspace sample immediately after sealing (time zero), then at regular intervals (e.g., 10, 20, 30, and 40 minutes). The choice of deployment interval (typically 20–45 minutes) represents a trade-off: shorter deployments reduce the concentration-feedback bias that suppresses diffusion as headspace CO_2 rises, but longer deployments provide a more detectable concentration change in low-flux soils (Hutchinson & Mosier, 1981; Pumpanen et al., 2004).
4. If using a portable IRGA, connect the chamber to the analyzer and record CO_2 concentration continuously or at 1-minute intervals.
5. If using soda-lime traps, weigh the pre-dried soda-lime, place it inside the sealed chamber, and retrieve it after a fixed deployment period (typically 24 hours). Re-dry and reweigh to determine CO_2 absorbed.

7.3. Ending the Measurement

1. Remove the chamber lid at the end of the measurement period.

2. Record the end time.
3. Transfer syringe samples to evacuated vials (Exetainers) for transport to the lab if not using IRGA.

8. Quality Control and Acceptance Criteria

8.1. Flux Calculation

Linear method (syringe or IRGA):

$$F = \frac{\Delta C}{\Delta t} \times \frac{V}{A} \times \frac{P}{RT}$$

where F = CO_2 flux (micromol m^{-2} s^{-1}), $\Delta C/\Delta t$ = rate of change in headspace CO_2 concentration (ppm s^{-1}), V = headspace volume (m^3), A = collar area (m^2), P = atmospheric pressure (Pa), R = ideal gas constant (8.314 J mol^{-1} K^{-1}), and T = air temperature (K).

Soda-lime method:

$$F = \frac{(M_f - M_i) \times 1.69}{A \times t}$$

where M_f and M_i are the final and initial soda-lime masses (g), 1.69 is the stoichiometric correction factor, A = collar area (m^2), and t = deployment time (hours).

8.2. QC Requirements

- Deploy at least three replicate chambers per treatment or land use.
- Inspect collar seals before each deployment. Soil settling or animal disturbance can break the seal.
- Record soil temperature and moisture alongside every flux measurement; these are the primary drivers of soil respiration. Soil respiration typically increases exponentially with temperature, with Q_{10} values of 2–3 (Bond-Lamberty & Thomson, 2010; Raich & Schlesinger, 1992). Reporting concurrent soil temperature and moisture allows flux values to be normalized or modeled across campaigns and sites.
- Reject any measurement where the R^2 of the linear regression (concentration vs. time) is below 0.90, unless the flux is near zero.
- Avoid measuring during or immediately after rainfall, as water pooling on the collar disrupts gas exchange.

9. Waste Disposal

Waste Type	Disposal Method
Used soda-lime	Allow to dry; dispose as solid waste (non-hazardous once fully carbonated)
Syringe needles	Sharps container
Used gloves	Standard trash

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11. Related SOPs

SOP	Title	Relationship
F001	Field Safety	Required prerequisite
F005	Random Sampling Design	Chamber placement design
F400	Soil Sampling	Companion soil samples
F504	Picarro GHG Analyzer	Automated alternative

SOP	Title	Relationship
L400	Soil Moisture	Moisture alongside flux
L408	Solvita CO_2 Burst	Lab respiration complement

12. Training Record

Before deploying soil respiration chambers independently, personnel must:

1. Read this SOP and SOP-F504 in full
2. Demonstrate proper collar installation and headspace volume measurement
3. Perform a supervised syringe sampling or IRGA measurement at one site
4. Calculate flux from field data and demonstrate agreement with the trainer's calculation
5. Be signed off by the PI or field lead

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F407

Soil Profile Description

Standard Operating Procedure

SOP Number	SOP-F407
Revision	0.2
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes the systematic description of soil profiles exposed in hand-dug pits or road cuts. A soil profile description documents the vertical sequence of horizons, their physical and morphological properties, and the relationships among them. Profile descriptions are fundamental to soil classification, land use assessment, understanding pedogenic processes, and interpreting laboratory analytical data in a landscape context (Schoeneberger et al., 2012; Soil Survey Staff, 2022). The systematic description of soil horizons is the foundation of pedology and provides the morphological evidence from which pedogenic processes (e.g., eluviation, illuviation, gleization) are inferred (Buol et al., 2011; Schaetzl & Anderson, 2015).

2. Scope

- **Applies to:** Field description of soil profiles at all EA Program sites, including research plots, restoration sites, and instructional field exercises
- **Does not apply to:** Auger-based sampling where no profile face is exposed (see SOP-F400); engineering soil classifications (Unified Soil Classification System); geotechnical borehole logging
- **Location:** All EA Program field sites where soil pits can be safely excavated

3. Responsibilities

Role	Responsibility
PI / Field Lead	Select pit locations; approve excavation; ensure safety
Trained Personnel	Excavate pits and describe profiles per this SOP
New Students	Complete training before independent profile description

4. Definitions

- **Consistence:** The resistance of soil to deformation or rupture; described at field moisture conditions (loose, very friable, friable, firm, very firm, extremely firm for moist soil) and separately for dry and wet conditions (sticky, plastic ratings).
- **Horizon:** A layer of soil approximately parallel to the land surface with distinct physical, chemical, or biological properties that differ from adjacent layers; the fundamental unit of soil profile description; designated by master symbols (O, A, E, B, C,

  R) and subordinate suffixes (e.g., Bt, Bhs).

- **Horizon boundary:** The contact between two soil horizons; described by distinctness (very abrupt, abrupt, clear, gradual, diffuse based on transition thickness) and topography (smooth, wavy, irregular, broken).
- **Master horizon:** A primary genetic horizon designation: O (organic surface), A (mineral surface with organic matter accumulation), E (eluviated), B (subsoil with illuvial accumulation or alteration), C (parent material), R (bedrock).
- **Ped:** A natural soil aggregate formed by soil-forming processes; described by type (granular, blocky, subangular blocky, prismatic, columnar, platy) and size class (very fine, fine, medium, coarse, very coarse).
- **Pedon:** The smallest three-dimensional body of soil that fully represents the range of properties of a given soil; typically 1–10 m^2 in surface area and extending to the C horizon or bedrock.
- **Soil profile:** A vertical cross-section of soil from the surface to the parent material or bedrock, exposing the sequence of genetic horizons; described in a pit or by augering.
- **Soil structure:** The arrangement and organization of soil particles into aggregates (peds); described by type (shape), class (size), and grade (distinctness of development: structureless, weak, moderate, strong).

- **Texture (field):** The relative proportions of sand, silt, and clay in the fine earth fraction (less than 2 mm); estimated in the field by the feel method (see SOP-F401) and confirmed by hydrometer analysis (see SOP-L401).

5. Safety and Hazards

Hazards

- **Physical:** Soil pits deeper than 50 cm can collapse, especially in sandy or dry soils. Pits deeper than 1.2 m (4 ft) require shoring or sloping per OSHA regulations (29 CFR 1926, Subpart P). Never enter an unshored pit deeper than 1.2 m.
- **Biological:** Soil-borne pathogens; ticks, snakes, and burrowing insects in riparian or wildland settings.
- **Environmental:** Heat exposure; dehydration during summer fieldwork.

5.1. Required PPE

- ☐ Closed-toed shoes or field boots
- ☐ Nitrile gloves
- ☐ Sun protection (hat, sunscreen)
- ☐ Safety glasses (when chopping through indurated horizons)
- ☐ Hard hat (if working in deep pits or near road cuts)

5.2. Emergency Procedures

Refer to SOP-F001 Field Safety. If a pit wall collapses while someone is inside, call 911 immediately. Do not attempt excavation without proper rescue training and equipment.

6. Equipment and Materials

Item	Notes
Sharpshooter shovel	For excavating the pit
Flat-edged spade or soil knife	For cleaning the profile face
Hand pick or mattock	For indurated or cemented horizons
Munsell Soil Color Charts	See SOP-F405
Squeeze bottle with DI water	For moistening soil for color and texture
Metric tape measure (2 m)	For measuring horizon depths
Clinometer or hand level	For measuring slope at the pit site
Compass	For recording aspect
10% HCl solution in dropper bottle	For testing for carbonates (effervescence)
Field notebook or profile description form	NRCS-style form preferred
Camera	For photographing the profile face

Item	Notes
Ruler or scale bar	Placed against the profile for photo scale
GPS unit	For recording pit coordinates per SOP-F006
Flagging tape	For marking horizon boundaries
Sample bags (labeled)	If collecting horizon samples for lab analysis

7. Procedure

7.1. Pit Excavation

1. Select the pit location based on the project objectives and landscape position. Avoid disturbed areas, utility lines, and locations near structures.
2. Excavate a pit approximately 50 cm wide and 80–120 cm deep (or to the parent material or restrictive layer, whichever is shallower). One vertical face should be oriented to receive even light (face the sun or open shade).
3. Pile excavated soil upslope and on a tarp to facilitate backfilling.
4. Clean the profile face with the flat-edged spade or soil knife to expose a fresh, undisturbed surface. Avoid smearing.

7.2. Identifying Horizon Boundaries

1. Examine the profile face for changes in color, texture, structure, roots, pores, and cementation. Each distinct zone is a horizon.
2. Mark horizon boundaries with flagging tape pins or nails.
3. Measure the depth of each boundary from the soil surface using the metric tape. Record the upper and lower boundary depth for each horizon (e.g., A: 0–18 cm; Bt: 18–45 cm).
4. Describe the boundary distinctness and topography:

Distinctness	Width of Transition
Abrupt	< 2 cm
Clear	2–5 cm
Gradual	5–15 cm
Diffuse	> 15 cm

Topography	Description
Smooth	Nearly a plane
Wavy	Pockets wider than deep
Irregular	Pockets deeper than wide
Broken	Discontinuous

Boundary distinctness reflects the rate and nature of pedogenic change between horizons; abrupt boundaries often indicate a lithologic discontinuity or a strong process boundary (e.g., plowing), while diffuse boundaries suggest gradual, ongoing translocation (Schaetzl & Anderson, 2015).

7.3. Describing Each Horizon

For each horizon, record the following properties:

Horizon designation: Use standard USDA master horizon symbols (O, A, E, B, C, R) with appropriate suffixes (t, k, w, g, etc.) (Soil Survey Division Staff, 2017). When assigning subordinate suffixes (e.g., **t** for clay accumulation, **k** for carbonate, **g** for gleying), verify the morphological evidence for each suffix against the criteria in Keys to Soil Taxonomy (Soil Survey Staff, 2022) to ensure that the designation supports formal classification.

Color: Determine moist and dry color using Munsell charts per SOP-F405. Record matrix color and any mottles.

Texture: Determine texture by feel per SOP-F401. Record the USDA textural class (e.g., sandy loam, clay loam).

Structure: Describe the type, size, and grade:

Type	Shape Description
Granular	Roughly spherical, porous
Blocky (angular)	Sharp-edged blocks
Blocky (subangular)	Rounded-edge blocks
Platy	Flat, plate-like
Prismatic	Vertical columns, flat tops
Columnar	Vertical columns, rounded tops
Massive	No visible structure (coherent mass)
Single grain	No structure (loose, individual particles)

Size: very fine, fine, medium, coarse, very coarse (ranges depend on structure type; consult the *Field Book for Describing and Sampling Soils*).

Grade: weak, moderate, strong.

Soil structure type and grade influence water movement, root penetration, and aeration. Strongly developed angular blocky structure in B horizons often correlates with high clay content and shrink-swell behavior, while granular structure in A horizons is associated with biological activity and high organic matter (Schaetzl & Anderson, 2015; Soil Survey Division Staff, 2017).

Consistence: Describe the resistance to rupture at the appropriate moisture state:

- Dry: loose, soft, slightly hard, hard, very hard, extremely hard
- Moist: loose, very friable, friable, firm, very firm, extremely firm
- Wet: nonsticky/nonplastic to very sticky/very plastic

Roots: Record abundance (few, common, many) and size (very fine, fine, medium, coarse).

Pores: Record abundance and size.

Effervescence: Apply 1–2 drops of 10% HCl to the horizon face and record the reaction:

Effervescence	Description
None	No visible bubbles
Slight	Few bubbles, barely audible
Strong	Obvious bubbles, clearly audible
Violent	Vigorous bubbling, foam

Other features: Record any clay films, coatings, concretions, cementation, or biological features (krotovinas, worm casts).

7.4. Site Description

Record the following site-level information:

- GPS coordinates per SOP-F006
- Slope (percent) and aspect (degrees)
- Landscape position (summit, shoulder, backslope, footslope, toeslope)
- Parent material (if identifiable: alluvium, colluvium, residuum, loess, etc.). At EA Program sites in the Pomona/Claremont area, parent materials are commonly alluvium derived from the San Gabriel Mountains or residuum from granitic bedrock, and argillic horizons (Bt) are frequently developed in older geomorphic surfaces (Buol et al., 2011).
- Vegetation and land use
- Drainage class (excessively drained to very poorly drained)
- Depth to water table or bedrock (if encountered)
- Evidence of erosion or deposition

7.5. Photography

1. Place a ruler or scale bar vertically against the profile face.
2. Photograph the entire profile with horizon labels visible.
3. Take close-up photos of features of interest (mottles, clay films, cementation, root channels).
4. Record photo numbers in the field notebook.

7.6. Backfilling

Backfill the pit promptly after description and sampling. Replace soil in reverse order (subsoil first, topsoil last). Compact gently to prevent subsidence. Restore surface litter.

8. Quality Control

- Use the NRCS *Field Book for Describing and Sampling Soils* as the authoritative reference for all terminology and classification.
- Have a second observer verify at least the horizon designations and colors for profiles used in research publications.

- Photograph every profile with a scale bar as a permanent record.
- Cross-check field texture estimates with laboratory hydrometer results (SOP-L401) for at least one horizon per profile.

9. References

Buol, S. W., Southard, R. J., Graham, R. C., & McDaniel, P. A. (2011). *Soil genesis and classification* (6th ed.). Wiley-Blackwell.

Schaetzl, R. J., & Anderson, S. (2015). *Soils: Genesis and geomorphology* (2nd ed.). Cambridge University Press. https://doi.org/10.1017/CBO9780511815560

Schoeneberger, P. J., Wysocki, D. A., Benham, E. C., & Soil Survey Staff. (2012). *Field book for describing and sampling soils* (3.0). USDA NRCS, National Soil Survey Center. https://www.nrcs.usda.gov/resources/guides-and-instructions/field-book-for-describing-and-sampling-soils

Soil Survey Division Staff. (2017). *Soil survey manual*. Government Printing Office. https://www.nrcs.usda.gov/resources/guides-and-instructions/soil-survey-manual

Soil Survey Staff. (2022). *Keys to soil taxonomy* (13th ed.). USDA Natural Resources Conservation Service. https://www.nrcs.usda.gov/resources/guides-and-instructions/keys-to-soil-taxonomy

10. Related SOPs

SOP	Title	Relationship
F001	Field Safety	Required prerequisite
F400	Soil Sampling	Horizon samples for lab analysis
F401	Texture by Feel	Field texture for each horizon
F402	Soil Bulk Density	Core samples from horizons
F405	Soil Color (Munsell)	Color determination per horizon
L401	Texture (Hydrometer)	Lab confirmation of field texture

11. Training Record

Before describing soil profiles independently, personnel must:

1. Read this SOP, SOP-F405, and SOP-F401 in full
2. Describe at least two supervised soil profiles with results reviewed by the PI or a trained pedologist
3. Demonstrate correct use of the Munsell charts, texture by feel, HCl effervescence test, and structure terminology
4. Be signed off by the PI or field lead

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L400

Soil Moisture – Gravimetric Method

Standard Operating Procedure

SOP Number	SOP-L400
Revision	0.3
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes the gravimetric determination of soil moisture content. Gravimetric moisture is a fundamental soil property required for expressing analytical results on a dry-weight basis (e.g., microbial biomass carbon, nutrient concentrations) and for characterizing site conditions (Gardner, 1986).

2. Scope

- **Applies to:** All soil samples collected per SOP-F400 Soil Sampling and Processing that require moisture correction or moisture characterization.
- **Does not apply to:** Volumetric soil moisture measured with in-situ probes (TDR, capacitance sensors); soil water potential measurements.
- **Location:** EA Program laboratory.

3. Definitions

- **Air-dry moisture content:** The moisture content of soil equilibrated with ambient laboratory air (typically 20–40 °C, 30–60% relative humidity); generally a few percent and varies with humidity (Gardner, 1986).
- **Constant mass:** The criterion for complete drying: successive weighings separated by at least 2 hours of additional oven time agree within 0.01 g.
- **Dry-weight correction factor (f):** The ratio of moist soil mass to oven-dry soil mass for the same sample; used to convert results expressed per gram of moist soil to per gram of oven-dry soil: $f = (W_{\text{moist}} - W_{\text{container}})/(W_{\text{dry}} - W_{\text{container}})$.
- **Field-moist soil:** Soil at the moisture content it had when collected from the field; the reference state for biological and enzyme activity measurements (Robertson et al., 1999).
- **Gravimetric moisture content (θ_g):** Mass of water per mass of oven-dry soil: $\theta_g = (M_{\text{wet}} - M_{\text{dry}})/M_{\text{dry}}$; expressed as g g^{-1} or as a percentage (Gardner, 1986).
- **Oven-dry soil:** Soil dried at 105 °C to constant mass; the standard reference state for expressing soil chemical and physical properties (Gardner, 1986).
- **Soil moisture:** The amount of water contained in the pore spaces of soil; may be expressed as gravimetric content (θ_g, g g^{-1}), volumetric content (θ_v, m^3 m^{-3}), or as a percentage (Gardner, 1986).
- **Volumetric moisture content (θ_v):** Volume of water per volume of soil: $\theta_v = \theta_g \times \rho_b/\rho_w$ where ρ_b is bulk density and ρ_w is the density of water (1.0 g cm^{-3}).

4. Safety and Hazards

Hazards

- **Thermal:** Drying ovens operate at 105 °C. Use heat-resistant gloves and tongs when removing samples.
- **Physical:** Hot aluminum tins retain heat for several minutes after removal from the oven. Do not handle bare-handed.

4.1. Required PPE

☐ Safety glasses
☐ Laboratory coat
☐ Heat-resistant gloves (when handling hot tins)

5. Equipment and Materials

5.1. Equipment

Item	Notes
Drying oven	Regulated at 105 ±2 °C; verify with a calibrated thermometer (Gardner, 1986)
Analytical balance	Resolution 0.01 g
Desiccator	With active color-indicating silica gel
Crucible tongs	For safe removal of hot tins

5.2. Consumables

Item	Notes
Aluminum tins or ceramic crucibles	Pre-labeled with permanent marker or engraved
Spatula	For transferring soil to tins

6. Estimated Time

Task	Time Required
Sample preparation and weighing	5–10 minutes per batch
Oven drying	24 hours at 105 °C
Cooling and re-weighing	30–45 minutes per batch (including cooling)
Total per batch	Approximately 25 hours (mostly unattended)

7. Procedure

7.1. Preparation

1. Print copies of the Soil Moisture Datasheet (Appendix A) for recording measurements.
2. Label aluminum tins or ceramic crucibles with a permanent marker or engraving tool.
3. Pre-dry the empty containers at 105 °C for at least 1 hour to remove any residual moisture (Gardner, 1986).
4. Cool the containers in a desiccator to room temperature.
5. Weigh each empty container to the nearest 0.01 g and record as Tin Weight on the datasheet.

7.2. Sample Weighing

1. Using a spatula, transfer approximately 10–20 g of field-moist soil into a pre-weighed container.

2. Record the mass of the container plus moist soil to the nearest 0.01 g as Wet Weight on the datasheet.
3. Avoid compacting the soil; a loose arrangement promotes even drying.

> **Tip**
>
> Process moisture subsamples at the same time as other subsamples (e.g., for microbial biomass or respiration) to ensure the moisture value accurately reflects the condition of the soil used in those analyses (Robertson et al., 1999).

7.3. Drying

1. Place the uncovered containers in the drying oven set to 105 °C (Gardner, 1986).
2. Dry for a minimum of 24 hours.
3. After 24 hours, remove one or two containers, cool in the desiccator, and re-weigh. If the mass has decreased by more than 0.01 g compared to a check 2 hours earlier, return all samples to the oven for an additional 4 hours and re-check.
4. Drying is complete when the mass is constant (change of less than 0.01 g between successive weighings).

7.4. Cooling and Final Weighing

1. Transfer all containers from the oven to a desiccator.
2. Allow to cool to room temperature (approximately 20–30 minutes).
3. Weigh each container plus dry soil to the nearest 0.01 g and record as Dry Weight on the datasheet.

7.5. Calculations

Gravimetric soil moisture content (%):

$$\theta_g(\%) = \left(\frac{W_{\text{moist}} - W_{\text{dry}}}{W_{\text{dry}} - W_{\text{container}}} \right) \times 100$$

where:

- W_{moist} = mass of container + moist soil (g)
- W_{dry} = mass of container + oven-dried soil (g)
- $W_{\text{container}}$ = mass of empty container (g)

Dry-weight correction factor (for converting field-moist analytical results to a dry-weight basis):

$$f = \frac{W_{\text{moist}} - W_{\text{container}}}{W_{\text{dry}} - W_{\text{container}}}$$

To convert a result expressed per gram of moist soil to per gram of dry soil, multiply by f (Gardner, 1986).

8. Quality Control

- Run analytical duplicates for at least 10% of samples; the coefficient of variation between duplicates should be less than 5% (Robertson et al., 1999).
- Verify oven temperature with a calibrated thermometer at the start of each drying cycle.
- Ensure the desiccator contains active (indicating) desiccant; replace or regenerate when the indicator changes color.
- Re-weigh a subset of samples after an additional 2 hours of drying to confirm mass constancy (Gardner, 1986).

9. Waste Disposal

Waste Type	Disposal Method
Oven-dried soil	Standard trash or return to field site
Aluminum tins	Clean and reuse, or recycle

10. References

- Gardner (1986)
- Robertson et al. (1999)

Gardner, W. H. (1986). Water content. In A. Klute (Ed.), *Methods of soil analysis, part 1: Physical and mineralogical methods* (2nd ed., pp. 493–544). Soil Science Society of America.

Robertson, G. P., Coleman, D. C., Bledsoe, C. S., & Sollins, P. (1999). *Standard soil methods for long-term ecological research.* Oxford University Press.

11. Training Record

Before performing this procedure independently, personnel must:

1. Read this SOP in full
2. Demonstrate safe operation of the drying oven
3. Demonstrate proper use of the analytical balance and desiccator
4. Perform the procedure under supervision with acceptable duplicate precision
5. Be signed off by the PI or lab manager

12. Appendix A: Soil Moisture Datasheet

Print this page and use it to record measurements in the lab.

Project / Soil Location: _____

Sampling Date (YYMMDD): _____

Researcher Name: _____

Oven Temperature (°C): _____

Date of Entry (YYMMDD): _____

Soil ID	Soil Tin #	Tin Weight (g)	Wet Weight (Tin + Wet Soil) (g)	Dry Weight (Tin + Dry Soil) (g)
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L401

Soil Texture Analysis – Hydrometer Method

Standard Operating Procedure

SOP Number	SOP-L401
Revision	0.1
Author(s)	Marc Los Huertos, Isaac Medina
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the determination of soil particle size distribution using the hydrometer method (ASTM D422). Soil texture (the relative proportions of sand, silt, and clay) is a fundamental physical property that controls water-holding capacity, drainage, nutrient retention, and soil structure. The hydrometer method measures the density of a soil-water suspension at timed intervals as progressively finer particles settle, allowing calculation of the percent sand, silt, and clay and assignment of a USDA textural class.

For a rapid field estimate of texture without laboratory equipment, see SOP-F401 Texture by Feel.

2. Scope

- **Applies to:** Mineral soils with less than approximately 20% coarse fragments (greater than 2 mm). Soils with high organic matter or carbonates require pre-treatment (see Section 6.4).
- **Does not apply to:** Pure gravel, organic soils (histosols), or soils where only a textural class estimate is needed (use SOP-F401 instead)
- **Location:** EA Program laboratory, SGM 133

3. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, ensure training compliance
Lab Manager	Maintain equipment, prepare HMP solution
Trained Personnel	Perform the analysis over multiple sessions
New Students	Complete training before independent use

4. Definitions

- **Clay:** Soil mineral particles with an equivalent spherical diameter less than 0.002 mm (2 µm); slow-settling; comprises the finest soil texture fraction.
- **Dispersion:** The process of separating soil aggregates into individual particles by chemical treatment (sodium hexametaphosphate) and mechanical agitation; essential for accurate hydrometer analysis.

- **ASTM 151H hydrometer:** A standardized glass hydrometer calibrated in g L^{-1} for use in soil texture analysis; reads the density of the suspension at the center of the hydrometer bulb.
- **Sand:** Soil mineral particles with an equivalent spherical diameter of 0.05–2.0 mm; collected on a 0.05 mm sieve after sedimentation.
- **Sedimentation:** The settling of soil particles in suspension under gravity; particle settling velocity follows Stokes' Law: $v = 2gr^2(\rho_s - \rho_l)/(9\eta)$, where r is particle radius, ρ_s is particle density, ρ_l is liquid density, and η is dynamic viscosity.
- **Silt:** Soil mineral particles with an equivalent spherical diameter of 0.002–0.05 mm; intermediate settling rate; calculated as silt = 100% - sand% - clay%.
- **Stokes' Law:** The physical law governing particle sedimentation velocity in a viscous fluid; the basis for hydrometer texture analysis; assumes spherical particles of uniform density (2.65 g cm^{-3}).
- **Texture class:** A USDA textural classification (e.g., sandy loam, clay loam, silty clay) based on the relative percentages of sand, silt, and clay; determined from the USDA textural triangle.

5. Safety and Hazards

Hazards

- **Chemical:** Sodium hexametaphosphate (HMP) is a mild skin and eye irritant. Hydrogen peroxide (30%) is a strong oxidizer and causes burns on contact. Hydrochloric acid (1 N) is corrosive.
- **Physical:** The milkshake mixer creates a vigorous vortex; keep loose clothing and hair clear. Hot plates and drying ovens pose burn risk.

5.1. Required PPE

- ☐ Safety glasses (chemical-safety goggles when using H_2O_2 or HCl)
- ☐ Lab coat
- ☐ Nitrile or latex gloves
- ☐ Hearing protection not required but recommended near the mixer

5.2. Emergency Procedures

For H_2O_2 or HCl splashes, flush with water for at least 15 minutes. For HMP powder inhalation, move to fresh air. Refer to SOP-L003 Hazardous Materials Handling.

6. Equipment and Materials

6.1. Equipment

Item	Notes
No. 10 sieve (2 mm mesh)	For removing gravel
No. 200 sieve (0.075 mm mesh)	For sand fraction recovery
Milkshake mixer and dispersion cup	For mechanical dispersion
151H hydrometer	ASTM standard
1 L sedimentation cylinder with No. 13 stopper	Glass or polycarbonate
Stopwatch or timer	For recording settling times
Thermometer (0.5 C resolution)	For temperature corrections
Analytical balance (0.01 g)	See SOP-L102
Drying oven (110 C)	See SOP-L105
Hot plate	For pre-treatment heating
250 mL and 400 mL beakers	For pre-treatment and weighing
Soil tins (pre-weighed)	For moisture determination
Mortar and pestle	For breaking up air-dried aggregates
Squeeze bottle (DI water)	For quantitative transfers

6.2. Reagents

Reagent	Preparation / Notes
Sodium hexametaphosphate (HMP)	40 g/L solution in DI water; prepare fresh weekly
Hydrogen peroxide, 30% (CAS 7722-84-1)	For organic matter removal (pre-treatment)
Hydrochloric acid, 1 N (CAS 7647-01-0)	For carbonate removal (pre-treatment)
Distilled or deionized water	From *SOP-L11*

7. Procedure

The analysis spans multiple lab sessions. Plan accordingly:

Session	Tasks	Time Required
1	Air-dry 200 g of soil	24+ hours (passive)
2	Sieve through No. 10; subsample for moisture	1–2 hours
3	Calculate DW_e; pre-treat and/or disperse	2–3 hours + 16 h soak
4	Conduct hydrometer readings	24+ hours (intermittent)
5	Recover sand on No. 200 sieve; complete data entry	1–2 hours

7.1. Air-Drying the Soil

1. Spread approximately 200 g of field-moist soil in a labeled tin or beaker

2. Allow to equilibrate with laboratory air for 24 hours or more. Very wet soils may require several days.
3. Break up large clods periodically to promote even drying

7.2. Sieving Through the No. 10 Sieve

Soil is defined as particles less than 2 mm. Gravel must be removed before analysis but quantified.

1. Homogenize the air-dried soil by thorough mixing
2. Weigh approximately 65 g (silt/clay soils) or 120 g (sandy soils) of air-dried soil and record the mass
3. Using a mortar and pestle, gently break up aggregates so that individual particles pass the No. 10 sieve. Do not crush primary mineral grains.
4. Sieve the soil through the No. 10 sieve (2 mm)
5. Weigh and record the mass of soil that passed through the sieve
6. Calculate the percent passing:

$$\text{Percent passing No. 10} = \frac{M_{\text{passed}}}{M_{\text{total}}} \times 100$$

7.3. Determining Hygroscopic Moisture Content

Air-dried soil still contains hygroscopic water adsorbed to clay surfaces, salts, and organic matter. This must be measured and corrected for.

1. Select a soil tin; record the tin number and tare mass
2. Transfer a 10–15 g subsample of the air-dried, sieved soil to the tin and record the air-dried mass (tin + soil)
3. Dry in the oven at 110 C for approximately 24 hours
4. Cool in a desiccator and record the oven-dried mass (tin + soil)
5. Calculate hygroscopic moisture:

$$\text{Moisture}_H = \frac{M_{\text{air-dry w/ tin}} - M_{\text{oven-dry w/ tin}}}{M_{\text{air-dry w/ tin}} - M_{\text{tin}}}$$

> **Note**
>
> The standard drying temperature of 105–110 C is somewhat arbitrary. Clay minerals may retain 10–15% water (dry basis) at 400 C. Organic components also release volatiles over a range of temperatures. The key is to specify and consistently use the same temperature.

7.4. Calculating Oven-Dry Equivalent Mass (DW_e)

The target oven-dry equivalent mass for the hydrometer test is 50 g for fine-textured soils or 100 g for coarse-textured soils (greater than approximately 70% sand).

Since air-dried soil contains hygroscopic water, the actual mass of air-dried soil to weigh out is:

$$DW_{\text{air}} = \frac{DW_e}{1 - \text{Moisture}_H}$$

where $DW_e = 50$ g or 100 g.

7.5. Pre-Treatment (If Required)

Pre-treatment is necessary for soils high in organic matter, carbonates, iron oxides, or soluble salts. If the soil is low in all of these, proceed directly to Section 6.6.

1. Transfer DW_{air} g of soil to a 400 mL beaker
2. **Carbonate removal:** Add 50 mL DI water and sufficient 1 N HCl to reduce the pH to 3.0–4.0. Stir and allow to equilibrate for 10 minutes until effervescence ceases.
3. **Organic matter removal:** Carefully add 30% H_2O_2 a few milliliters at a time, allowing frothing to subside between additions. If necessary, acidify the suspension with a few drops of 1 N HCl (H_2O_2 oxidation requires an acidic medium). When frothing subsides, heat to 90 C and continue adding H_2O_2 until no further reaction occurs. Rinse down the beaker walls and continue heating until excess H_2O_2 is consumed.
4. **Iron oxide removal (if needed):** Add 150 mL citrate-bicarbonate buffer. Add 3 g sodium hydrosulfite ($Na_2S_2O_4$) gradually (may froth). Heat in a water bath at 80 C for 20 minutes.
5. **Soluble salt removal (if needed):** Transfer soil to a centrifuge tube, add 100 mL DI water, and centrifuge at 1,500 rpm for 10 minutes. Decant the supernatant. Repeat until the supernatant is clear.

> ⚠ Warning
>
> Handle 30% H_2O_2 in the fume hood. It is a strong oxidizer that causes skin burns on contact. Add it slowly to avoid violent frothing.

7.6. Dispersing the Soil Sample

1. Weigh DW_{air} g of pre-treated (or untreated) soil into the dispersion cup and record the mass to 0.1 g
2. Add 125 mL of 40 g/L sodium hexametaphosphate solution
3. Stir until the slurry is thoroughly wetted
4. Cover and soak for at least 16 hours (overnight)
5. Add distilled water to the first indentation mark on the dispersion cup
6. Attach the cup to the milkshake mixer and mix for 5 minutes (sandy soils) or 15 minutes (fine-textured soils)

7.7. Conducting the Hydrometer Test

Blank preparation:

1. Prepare a blank cylinder: add 125 mL of HMP solution and fill to the 1,000 mL mark with distilled water
2. Record the blank hydrometer reading and temperature. If the zero mark is below the meniscus surface, record the correction as a negative number. Read at the top of the meniscus (the suspension is opaque).

Sample measurement:

1. Transfer the dispersed soil suspension to a sedimentation cylinder, using a squeeze bottle to rinse all material from the dispersion cup
2. Fill the cylinder to the 1,000 mL mark with distilled water
3. Stopper the cylinder (No. 13) and invert 180 degrees and back in a steady 2-second cycle for one full minute. Vigorously agitate during the first two inversions to loosen settled material.
4. Set the cylinder upright and start the timer immediately
5. At 20 seconds elapsed, slowly lower the hydrometer into the suspension (spinning gently to dislodge air bubbles) and read at 40 seconds. Remove, rinse, and dry the hydrometer.
6. Repeat the inversion and 40-second reading two more times (three total readings at 40 s) for accuracy
7. After the final 40-second reading, lower a thermometer into the suspension and record the temperature

> **!** Important
>
> The hydrometer must be removed, rinsed, and dried between every reading. Particles accumulate on the glass surface and progressively bias subsequent readings if not cleaned.

8. Continue taking hydrometer and temperature readings at the following schedule:

Reading	Time After Inversion	Temperature?	Blank Reading?
0	Before inverting	Yes	Yes
1	40 seconds (x3)	Yes (after)	–
2	2 minutes	–	–
3	5 minutes	Yes	Yes
4	15 minutes	–	–
5	30 minutes	–	–
6	60 minutes	Yes	Yes
7	2 hours	Yes	Yes
8	3 hours (optional)	Yes	Yes
9	6–8 hours (optional)	Yes	Yes
10	24 hours	Yes	Yes

9. Place the cylinder in a stable, undisturbed location between readings

7.8. Recovering Sand on the No. 200 Sieve

The hydrometer method does not resolve sand subfractions well. Sieve analysis of the sand fraction provides this detail.

1. After the final hydrometer reading, pour the cylinder contents through a No. 200 sieve (0.075 mm)
2. Using tap water (with a sediment trap in the sink), wash the material on the sieve until the rinse water is clear
3. Select a soil tin; record the tin number and tare mass
4. Carefully wash the retained sand from the sieve into the tin using a squeeze bottle. Do not use a brush or metal tool on the sieve mesh.
5. Dry at 110 C for 24 hours
6. Cool and weigh the tin plus dried sand; record the mass

> **Tip**
>
> The No. 200 sieve screens are delicate. Forcing particles through or scrubbing with a brush can stretch or distort the mesh. Always use a gentle water stream or squeeze bottle.

8. Quality Control and Acceptance Criteria

8.1. Calculations

The key calculated quantities for each hydrometer reading are:

Corrected hydrometer reading:

$$R_c = R_{\text{actual}} - R_{\text{blank}}$$

Effective particle diameter (D_e, mm) at each reading time is calculated from Stokes' Law, accounting for temperature-corrected viscosity and density of the suspension fluid.

Percent finer at each reading:

$$PF = \frac{R_c \times a}{DW_e} \times 100$$

where a is a correction factor based on the specific gravity of soil particles (typically 2.65 for mineral soils).

Textural class is determined by plotting the calculated percent sand, silt, and clay on the USDA textural triangle.

8.2. Bias and Interference Sources

Source	Effect	Correction
Temperature and viscosity	Cold water increases viscosity, slowing settling	Temperature correction factor applied to each reading
Temperature and density	Water density varies with temperature	Density correction in Stokes' Law
Hygroscopic moisture	Air-dried soil mass overestimates oven-dry mass	DW_e correction (Section 6.4)
Incomplete dispersion	Aggregates settle as single large particles; underestimates clay	Use HMP + mechanical mixing; pre-treat if needed
Meniscus reading	Opaque suspension prevents reading at bottom of meniscus	Read at top of meniscus; apply meniscus correction
Flocculation	Ca^{2+} and Al^{3+} bridge clay particles	HMP displaces polyvalent cations; Na^+ hydrates clay surfaces

8.3. QC Requirements

- Run a duplicate analysis on at least 10% of samples
- Duplicate results should agree within one textural class
- Include a blank cylinder (HMP + water, no soil) with every batch
- Record all temperatures; temperature drift greater than 2 C during the reading series indicates an unstable environment
- Cross-check a subset of hydrometer results against field texture-by- feel estimates (SOP-F401)

9. Waste Disposal

Waste Type	Disposal Method
Soil suspension (no pre-treatment chemicals)	Settle, decant water, dispose of soil in standard trash
HMP solution (spent)	Drain with copious water (low hazard)
H_2O_2 waste (from pre-treatment)	Dilute and drain, or collect for EH&S if concentrated
HCl waste (from pre-treatment)	Neutralize with $NaHCO_3$ and drain, or collect per SOP-L003
Sieve washings	Use a sediment trap; dispose of trapped soil in standard trash

10. References

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- Soil Science Division Staff. (2017). *Soil Survey Manual*. USDA Handbook No. 18. USDA-NRCS.

11. Training Record

Before performing hydrometer analysis independently, personnel must:

1. Read this SOP in full
2. Complete SOP-L002 Laboratory Safety, SOP-L003 Hazardous Materials Handling, and SOP-L400 Soil Moisture training
3. Observe a trained operator perform the full multi-session analysis
4. Demonstrate proper soil dispersion, hydrometer reading technique, and temperature recording under supervision
5. Complete one full analysis (all sessions) with acceptable duplicate precision
6. Be signed off by the PI or lab manager

12. Appendix A: Example Calculations in R

```r
library(tidyverse)

# --- Hygroscopic moisture correction ---
tin_mass_g <- 15.32
air_dry_w_tin_g <- 27.45
oven_dry_w_tin_g <- 27.18

moisture_H <- (air_dry_w_tin_g - oven_dry_w_tin_g) /
              (air_dry_w_tin_g - tin_mass_g)

cat(sprintf("Hygroscopic moisture: %.4f g/g\n", moisture_H))

# --- Oven-dry equivalent mass ---
DW_e <- 50  # g; use 100 for sandy soils
```

```r
DW_air <- DW_e / (1 - moisture_H)

cat(sprintf("Air-dried soil to weigh: %.2f g\n", DW_air))

# --- Hydrometer readings ---
# Example data: time (min), hydrometer reading, temp (C), blank reading
readings <- tibble(
  time_min = c(0.67, 2, 5, 15, 30, 60, 120, 1440),
  R_actual = c(38, 32, 27, 22, 19, 16, 13, 8),
  temp_C = c(23, 23, 23, 22, 22, 22, 22, 21),
  R_blank = c(5, 5, 5, 5, 5, 5, 5, 5)
)

# Corrected readings
readings <- readings %>%
  mutate(
    R_corrected = R_actual - R_blank,
    # Simplified percent finer (a = 1 for Gs = 2.65)
    percent_finer = (R_corrected / DW_e) * 100
  )

# Sand, silt, clay from key readings
# Percent sand: 100 - PF at 40 sec (0.67 min)
# Percent clay: PF at 1440 min (24 h)
pct_sand <- 100 - readings$percent_finer[readings$time_min == 0.67]
pct_clay <- readings$percent_finer[readings$time_min == 1440]
pct_silt <- 100 - pct_sand - pct_clay

cat(sprintf("\nSand: %.1f%%  Silt: %.1f%%  Clay: %.1f%%\n",
            pct_sand, pct_silt, pct_clay))

# Plot particle size distribution curve
ggplot(readings, aes(x = time_min, y = percent_finer)) +
  geom_point(size = 3) +
  geom_line() +
  scale_x_log10() +
  labs(
    x = "Settling time (minutes, log scale)",
    y = "Percent finer (%)",
    title = "Hydrometer Particle Size Distribution"
  ) +
  theme_minimal()
```

13. Appendix B: USDA Textural Classes

Once percent sand, silt, and clay are determined, assign the textural class using the USDA textural triangle. The 12 classes are:

Class	Sand (%)	Silt (%)	Clay (%)
Sand	85–100	0–15	0–10
Loamy sand	70–90	0–30	0–15
Sandy loam	43–85	0–50	0–20
Loam	23–52	28–50	7–27
Silt loam	0–50	50–88	0–27
Silt	0–20	80–100	0–12
Sandy clay loam	45–80	0–28	20–35
Clay loam	20–45	15–53	27–40
Silty clay loam	0–20	40–73	27–40
Sandy clay	45–65	0–20	35–55
Silty clay	0–20	40–60	40–60
Clay	0–45	0–40	40–100

14. Appendix C: Troubleshooting

Symptom	Probable Cause	Corrective Action
Clay content seems too low	Incomplete dispersion	Extend soaking time; increase mixer time; verify HMP concentration
Readings inconsistent at 40 s	Insufficient mixing; uneven settling	Ensure vigorous, consistent inversions; repeat three times
Temperature drifts more than 2 C	Lab temperature unstable	Move cylinder away from heat sources; consider a water bath
Blank reading changes over time	HMP solution degrading	Prepare fresh HMP solution weekly
Sand fraction sums do not match hydrometer	Soil lost during sieve transfer	Rinse quantitatively; check for cracks in the sieve
Percent finer exceeds 100% or is negative	Data entry or calculation error	Double-check raw readings, blank correction, and DW_e

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L402

Soil pH and Salinity

Standard Operating Procedure

SOP Number	SOP-L402
Revision	0.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the measurement of soil pH and electrical conductivity (EC, as a proxy for salinity) on soil-water and soil-$CaCl_2$ extracts. Soil pH controls nutrient availability, metal solubility, microbial activity, and the fate of contaminants. Soil salinity (estimated from EC) affects plant growth, soil structure, and is a primary criterion for classifying saline, sodic, and saline-sodic soils. The methods follow Thomas (1996) for pH and Rhoades (1996) for EC, as described in the SSSA *Methods of Soil Analysis, Part 3* (Rhoades, 1996; Thomas, 1996).

For meter operation, calibration, and electrode maintenance, see SOP-L300 pH and Conductivity – Bench-Top Measurements.

2. Scope

- **Applies to:** Air-dried, sieved (less than 2 mm) mineral soils from all EA Program sites; compost and potting media (with appropriate extraction ratios noted in Section 6)
- **Does not apply to:** In situ field pH measurements with portable probes (see SOP-F200); water sample pH and conductivity (see SOP-L300); saturated paste extraction for formal salinity classification of irrigation water
- **Location:** EA Program laboratory, SGM 133

3. Definitions

- **Cation exchange capacity (CEC):** The total capacity of a soil to hold exchangeable cations; closely related to soil pH buffering capacity (see SOP-L416).
- **Electrical conductivity (EC):** A measure of a soil extract's ability to conduct electricity, proportional to total dissolved ion concentration; expressed in dS m^{-1} or mS cm^{-1}; used as a surrogate for soluble salt content.
- **Paste extract (saturated paste):** A soil-water mixture prepared at the saturation moisture content; the USDA standard extract for measuring soil salinity.
- **pH:** The negative base-10 logarithm of the hydrogen ion activity; soil pH is measured in a defined soil-to-water or soil-to-$CaCl_2$ suspension and controls nutrient availability and microbial activity.
- **Salinity:** The total concentration of dissolved salts in soil solution; high salinity reduces plant-available water and can be toxic to salt-sensitive crops; typically characterized by EC of the saturation extract (EC_e).

- **Soil-to-water ratio:** The ratio of soil mass to water volume used in pH and EC measurements (e.g., 1:1, 1:2, 1:5); must be reported alongside the result because pH and EC values differ between ratios.

4. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, ensure training compliance
Lab Manager	Maintain meter and electrodes, stock reagents
Trained Personnel	Prepare extracts, measure and record results
New Students	Complete training before independent use

5. Safety and Hazards

Hazards

- **Chemical:** 0.01 M $CaCl_2$ is a mild irritant. pH buffer solutions and conductivity standards are low-hazard but should not contact eyes.
- **Physical:** No significant physical hazards beyond standard glassware handling.

5.1. Required PPE

- ☐ Safety glasses
- ☐ Lab coat
- ☐ Nitrile gloves

5.2. Emergency Procedures

For buffer or chemical splashes, flush with water. Refer to SOP-L002 Laboratory Safety.

6. Equipment and Materials

6.1. Equipment

Item	Notes
pH/conductivity meter	Calibrated per SOP-L300
pH combination electrode	Glass bulb with refillable KCl junction preferred
Conductivity cell	With automatic temperature compensation
Analytical balance (0.01 g)	See SOP-L102

Item	Notes
Beakers (50–100 mL)	Clean; one per sample plus blanks
Graduated cylinder or dispenser	For volumetric delivery of water or $CaCl_2$
Glass stirring rods	One per sample or rinsed between samples
Timer	For equilibration period

6.2. Reagents

Reagent	Preparation / Notes
Deionized water (Type I or II)	From *SOP-L11*
0.01 M $CaCl_2$ solution	Dissolve 1.47 g $CaCl_2$-$2H_2O$ in 1 L DI water
pH buffer solutions (4.01, 7.00, 10.01)	For pH meter calibration
Conductivity standard (1413 uS/cm)	For conductivity meter calibration

7. Procedure

7.1. Sample Preparation

1. Air-dry the soil at room temperature until constant mass (typically 24–48 hours)
2. Pass the dried soil through a 2 mm sieve. See SOP-F400 Soil Sampling for details on sieving and homogenization.
3. Store the sieved soil in a labeled bag or container at room temperature

7.2. Selecting the Extraction Ratio

Two standard soil-to-solution ratios are used. The choice depends on the project objectives and the need for comparability with published data:

Ratio	Method	When to Use
1:1	10 g soil : 10 mL water (or $CaCl_2$)	Most common in agronomic soil testing; closest to field conditions
1:5	10 g soil : 50 mL water (or $CaCl_2$)	Useful for saline soils, compost, or when more extract volume is needed for subsequent analyses

> **Note**
>
> Soil pH measured in 0.01 M $CaCl_2$ is typically 0.3–0.5 units lower than pH measured in DI water because the salt displaces H^+ and Al^{3+} from exchange sites. $CaCl_2$ pH is less sensitive to seasonal variation in soil salt content and is preferred for monitoring studies where consistency over time matters (Thomas, 1996). Record the extraction medium (water or $CaCl_2$) and ratio with every result.

7.3. Preparing the Soil Slurry (1:1 Method)

1. Weigh 10.0 g of air-dried, sieved soil into a clean beaker
2. Add 10 mL of DI water (for pH_{water} and EC) or 10 mL of 0.01 M $CaCl_2$ (for pH_{CaCl2})
3. Stir vigorously with a glass rod for 30 seconds
4. Allow to stand for 10 minutes
5. Stir briefly again before measurement

7.4. Preparing the Soil Extract (1:5 Method)

1. Weigh 10.0 g of air-dried, sieved soil into a clean beaker
2. Add 50 mL of DI water (or 50 mL of 0.01 M $CaCl_2$)
3. Stir vigorously for 30 seconds
4. Allow to stand for 30 minutes, stirring occasionally
5. For EC measurement on the 1:5 extract, allow the soil to settle and insert the conductivity cell into the clear supernatant. For pH, insert the electrode into the slurry while still partially suspended.

7.5. Measuring Electrical Conductivity

> **! Important**
>
> Always measure EC before pH on the same aliquot. The pH electrode's KCl reference junction leaks electrolyte into the sample and will artificially elevate the EC reading (APHA et al., 2012).

1. Calibrate the conductivity meter per SOP-L300, Section 6.3
2. Rinse the conductivity cell with DI water and blot dry
3. Insert the cell into the supernatant (1:5 method) or into the settled portion of the slurry (1:1 method). Tap gently to dislodge air bubbles.
4. Allow the reading to stabilize with automatic temperature compensation active
5. Record the specific conductance (uS/cm at 25 C), sample ID, and extraction ratio
6. Rinse the cell with DI water between samples

7.6. Measuring Soil pH

1. Calibrate the pH meter with a three-point calibration per SOP-L300, Section 6.2
2. Rinse the pH electrode with DI water and blot gently
3. Insert the electrode into the slurry so that the glass bulb and reference junction are fully immersed. For the 1:1 method, the electrode should contact the settled soil.
4. Allow the reading to stabilize (typically 30–60 seconds; may take 2–3 minutes for poorly buffered soils)
5. Record the pH value, extraction medium (water or $CaCl_2$), ratio, and sample ID
6. Rinse the electrode with DI water between samples

7.7. Converting 1:5 EC to Estimated Saturated Paste EC (EC_e)

The saturated paste extract (EC_e) is the standard for soil salinity classification, but the 1:5 extract is quicker to prepare. An approximate conversion can be made using the following relationship, though the factor varies with texture (Rhoades, 1996):

$$EC_e \approx EC_{1:5} \times f$$

where f is an empirical factor:

Soil Texture	f (approximate)
Sand	6 – 8
Loam	8 – 10
Clay	10 – 13

Warning

These conversion factors are approximate and site-specific. For formal salinity classification (e.g., land-use planning, irrigation suitability), prepare an actual saturated paste extract per USDA Handbook 60.

8. Quality Control and Acceptance Criteria

8.1. Interpretation of Soil pH

pH Range	Classification	Agronomic Significance
Less than 4.5	Extremely acidic	Toxic Al^{3+} levels; few crops tolerate
4.5 – 5.5	Strongly acidic	Low P availability; Mo deficiency; lime recommended
5.5 – 6.5	Moderately acidic	Suitable for most crops; some nutrient limitations
6.5 – 7.5	Neutral	Optimal for most crops and nutrient availability
7.5 – 8.5	Moderately alkaline	Common in arid soils; Fe, Mn, Zn may be limiting
Greater than 8.5	Strongly alkaline	Possible sodicity; Na toxicity

8.2. Interpretation of Soil Salinity (EC_e)

EC_e (dS/m)	Classification	Plant Response
0 – 2	Non-saline	No yield reduction for most crops

EC_e (dS/m)	Classification	Plant Response
2 – 4	Slightly saline	Sensitive crops affected
4 – 8	Moderately saline	Many crops affected
8 – 16	Strongly saline	Only tolerant crops survive
Greater than 16	Very strongly saline	Few plants survive

8.3. Saline, Sodic, and Saline-Sodic Classification

Class	EC_e (dS/m)	SAR / ESP	pH
Normal	Less than 4	SAR less than 13 / ESP less than 15	Variable
Saline	4 or greater	SAR less than 13 / ESP less than 15	Usually less than 8.5
Sodic	Less than 4	SAR 13 or greater / ESP 15 or greater	Usually greater than 8.5
Saline-sodic	4 or greater	SAR 13 or greater / ESP 15 or greater	Variable

SAR = sodium adsorption ratio; ESP = exchangeable sodium percentage. Full SAR/ESP determination requires cation analysis beyond the scope of this SOP.

8.4. QC Requirements

- Calibrate the pH meter before each measurement session (three-point). Acceptable slope: 90–102% of theoretical.
- Calibrate the conductivity meter before each session
- Re-read pH 7.00 buffer after every 10 samples; recalibrate if drift exceeds +/- 0.05 pH units
- Re-read the conductivity standard after every 10 samples; recalibrate if drift exceeds +/- 5%
- Run a DI water blank and a laboratory reference soil (if available) with each batch
- Run duplicate measurements on at least 10% of samples. Acceptable precision: +/- 0.1 pH units and +/- 5% relative percent difference for EC.
- Record extraction medium, ratio, calibration data, and QC results in the lab notebook

9. Waste Disposal

Waste Type	Disposal Method
Soil slurry waste (non-hazardous)	Standard trash once settled
$CaCl_2$ extract solutions	Drain with copious water (non-hazardous)
Used pH buffer solutions	Collect in waste container per SOP-L300
Used conductivity standard	Drain with copious water
DI water rinses	Drain

10. References

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11. Training Record

Before measuring soil pH and EC independently, personnel must:

1. Read this SOP and SOP-L300 in full
2. Complete SOP-L002 Laboratory Safety training
3. Demonstrate pH meter three-point calibration with acceptable slope
4. Demonstrate conductivity meter calibration
5. Prepare 1:1 and 1:5 extracts and measure a set of at least 5 practice soils under supervision with acceptable duplicate precision
6. Be signed off by the PI or lab manager

12. Appendix A: Example Calculation and Reporting

```r
library(tidyverse)

# Example data entry
soil_data <- tibble(
  sample_id = c("PV-01", "PV-02", "PV-03", "PV-04", "PV-05"),
  pH_water_1_1 = c(6.82, 7.45, 5.91, 8.12, 7.03),
  pH_CaCl2_1_1 = c(6.48, 7.10, 5.55, 7.78, 6.68),
  EC_1_5_uS_cm = c(245, 1820, 85, 3450, 310),
```

```
  texture = c("loam", "clay", "sand", "clay", "loam")
)

# Estimate EC_e from 1:5 EC using texture-based conversion factors
conversion <- c(sand = 7, loam = 9, clay = 11.5)

soil_data <- soil_data %>%
  mutate(
    EC_e_dS_m = (EC_1_5_uS_cm / 1000) * conversion[texture],
    salinity_class = case_when(
      EC_e_dS_m < 2 ~ "Non-saline",
      EC_e_dS_m < 4 ~ "Slightly saline",
      EC_e_dS_m < 8 ~ "Moderately saline",
      EC_e_dS_m < 16 ~ "Strongly saline",
      TRUE ~ "Very strongly saline"
    ),
    pH_class = case_when(
      pH_water_1_1 < 4.5 ~ "Extremely acidic",
      pH_water_1_1 < 5.5 ~ "Strongly acidic",
      pH_water_1_1 < 6.5 ~ "Moderately acidic",
      pH_water_1_1 < 7.5 ~ "Neutral",
      pH_water_1_1 < 8.5 ~ "Moderately alkaline",
      TRUE ~ "Strongly alkaline"
    )
  )

print(soil_data %>% select(sample_id, pH_water_1_1, pH_CaCl2_1_1,
                           EC_e_dS_m, salinity_class, pH_class))
```

13. Appendix B: Reagent Preparation

13.1. M $CaCl_2$ Solution

1. Weigh 1.47 g of $CaCl_2$-$2H_2O$ (MW 147.01) on the analytical balance
2. Dissolve in approximately 800 mL of DI water in a 1 L volumetric flask
3. Bring to 1 L with DI water and mix thoroughly
4. Label with concentration, preparation date, and preparer's initials
5. Store at room temperature in a capped polyethylene or borosilicate bottle. Stable indefinitely.

14. Appendix C: Troubleshooting

Symptom	Probable Cause	Corrective Action
pH reading drifts continuously	Poorly buffered soil; electrode junction fouled	Allow longer equilibration (up to 5 min); clean electrode per SOP-L300
pH in $CaCl_2$ higher than pH in water	Measurement error; contaminated $CaCl_2$ solution	Verify $CaCl_2$ concentration; recalibrate meter; re-measure
EC reading unexpectedly high	Fertilizer residue; saline soil; contaminated beaker	Verify with a blank; use acid-washed glassware (SOP-L101)
EC reading near zero	Electrode not fully immersed; air bubble in cell	Reposition cell; tap to dislodge bubbles
Large difference between 1:1 and 1:5 EC	Normal; dilution effect	Report both with the ratio clearly stated; convert to EC_e using appropriate factor
pH calibration slope below 90%	Aged electrode; dirty glass bulb	Soak in cleaning solution or 0.1 M HCl for 15–30 min; if slope does not recover, replace electrode

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L403

Soil Nitrate and Ammonium – KCl Extraction

Standard Operating Procedure

SOP Number	SOP-L403
Revision	0.4
Author(s)	Aaron Rogers
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes the extraction of inorganic nitrogen (nitrate, NO_3^-, and ammonium, NH_4^+) from soil using 2 M potassium chloride (KCl). The KCl extraction is the standard method for determining plant-available inorganic nitrogen in soils (Keeney & Nelson, 1982; Mulvaney, 1996). The high ionic strength of the KCl solution displaces NH_4^+ from cation exchange sites and brings NO_3^- into solution, producing a filtered extract suitable for colorimetric, flow-injection, or ion chromatographic analysis (Maynard et al., 2008).

2. Scope

- **Applies to:** Field-moist or air-dried soil samples collected per SOP-F400 Soil Sampling and Processing
- **Does not apply to:** Total nitrogen analysis (requires Kjeldahl or combustion digestion; see SOP-L407); dissolved nitrogen in water samples; semi-quantitative field screening (see SOP-L404)
- **Location:** EA Program laboratory, SGM 133

3. Responsibilities

Role	Responsibility
Lab PI	Approve protocol, ensure training compliance
Trained Personnel	Prepare extracts, record and report results
New Students	Complete training before independent use

3.1. Required Training

Students using this SOP should be trained on:

- SOP-F001 Field Safety
- SOP-L003 Hazardous Materials Handling
- SOP-L400 Soil Moisture

4. Definitions

- **Ammonium (NH_4^+):** The protonated form of ammonia; held on negatively charged cation exchange sites in soil; released into solution by the high ionic strength of the 2 M KCl extractant; the primary plant-available nitrogen pool in many soils.
- **Cation exchange capacity (CEC):** The total capacity of a soil to hold exchangeable positively charged ions; controls how much NH_4^+ can be stored and released; higher in clay-rich and organic-matter-rich soils.
- **KCl extraction:** Shaking soil with 2 mol L^{-1} potassium chloride solution at a defined soil-to-solution ratio (typically 1:10 w/v); the standard procedure for quantifying plant-available inorganic nitrogen in soils (Keeney & Nelson, 1982).
- **Mineral nitrogen (mineral N):** The sum of NH_4^+-N and NO_3^--N in the soil; the immediately plant-available nitrogen pool; expressed in mg N kg^{-1} dry soil.
- **Nitrate (NO_3^-):** The oxidized form of inorganic nitrogen; anion, so not held on cation exchange sites; highly mobile in soil solution; measured colorimetrically or by flow injection analysis after KCl extraction.
- **Soil-to-solution ratio:** The mass of soil (g) to volume of extractant (mL) used in the extraction; typically 1:10 for KCl extractions; must be consistent within and between studies for results to be comparable.

5. Safety and Hazards

Hazards

- **Chemical:** 2 M KCl is a mild irritant; avoid contact with eyes and skin. Review the SDS before first use.
- **Physical:** Reciprocating shaker can pinch fingers if containers are not secured properly.

5.1. Required PPE

- ☐ Nitrile gloves
- ☐ Safety glasses
- ☐ Lab coat
- ☐ Closed-toed shoes

6. Required Materials

6.1. Equipment

Item	Notes
Reciprocating shaker	For 2-hour extraction (Keeney & Nelson, 1982)
Magnetic stirrer with stir bar	For preparing KCl solution
Stir bar retriever	Magnetic wand

Item	Notes
Top-loading balance	0.1 g resolution
Graduated cylinder or volumetric flask	2 L capacity for KCl preparation
Repipet-type dispenser	For dispensing KCl solution (optional)
Drying oven	Set to 105 C (for moisture subsample)
Sorvall ST8R Centrifuge	Optional; see SOP-L109

6.2. Consumables

Item	Cat. No.	Notes
Whatman #42 filter paper		Ashless, 185 mm diameter
Filter funnels	09-855F	To fit filter paper
Test tubes or extraction bottles	S89476	50 mL capacity, with caps
Specimen containers or Nalgene bottles		For collecting filtered extracts
Aluminum weigh boats		For moisture subsamples
Pipette		For dispensing precise volumes

6.3. Reagents

Item	Cat. No.	Notes
Potassium chloride (KCl)	AA11595A1	ACS grade; 298.2 g per 2 L for 2 M solution
Distilled or deionized water		For preparing KCl solution

7. Estimated Time

Task	Time Required
KCl solution preparation	10 minutes active + 2 hours stirring
Moisture subsamples	5 minutes active + 24 hours drying
KCl extraction (per batch)	15 minutes setup + 2 hours shaking + 10 minutes settling
Filtering	20–30 minutes per batch

8. Procedure

8.1. Preparing 2 M KCl Solution

The 2 M KCl concentration is the standard for inorganic N extraction (Keeney & Nelson, 1982; Mulvaney, 1996). Lower concentrations (e.g., 1 M) may underestimate exchangeable NH_4^+ in

high-CEC soils (Maynard et al., 2008).

1. Weigh out 298.2 g KCl into a 2 L volumetric flask
2. Fill the flask approximately two-thirds full with distilled water
3. Place the flask on a magnetic stirrer and add a magnetic stir bar
4. Set the stirrer to low-medium speed and leave on for 4 hours until fully dissolved
5. Use a stir bar retriever to remove the magnetic stir bar
6. Fill the flask to the 2 L mark with distilled water and mix

> **Tip**
>
> Prepare the KCl solution the day before extraction. It can be stored at room temperature in a capped container indefinitely. Verify the solution with a conductivity meter if stored for more than 6 months.

8.2. Moisture Subsample

A companion gravimetric moisture determination is required to express results on an oven-dry soil basis (Keeney & Nelson, 1982).

1. Set aside 10 g of soil from each sample for moisture determination
2. Place each 10 g subsample into a labeled aluminum weigh boat
3. Dry at 105 C for 24 hours and record the dry weight
4. Calculate gravimetric moisture content per SOP-L400 Soil Moisture

8.3. KCl Extraction

The standard soil-to-solution ratio is approximately 1:5 to 1:10 (w/v) (Mulvaney, 1996). This SOP uses 7 g field-moist soil in 40 mL KCl, yielding an effective ratio of approximately 1:6 on an oven-dry basis for a typical soil at 15% gravimetric moisture.

1. Dispense 40 mL of 2 M KCl solution into labeled test tubes or extraction bottles
2. Add 7 g of field-moist soil to each tube (the total volume should reach approximately 50 mL). Record the exact mass of soil added to the nearest 0.01 g.
3. Cap the tubes securely
4. Place on the reciprocating shaker for 2 hours
5. Remove from the shaker and either:
   - Allow soil particles to settle for approximately 10 minutes (do not exceed 1 hour), or
   - Centrifuge for 3 minutes at 9,000 rpm (see SOP-L109: Sorvall ST8R Centrifuge)

> **Important**
>
> Record the exact mass of field-moist soil added to each tube. This value, together with the gravimetric moisture content, is needed to calculate results on an oven-dry basis (see Appendix

A).

8.4. Filtering

1. Set up filter funnels with Whatman #42 filter paper over labeled specimen containers or clean test tubes
2. Pour the settled or centrifuged solution into the funnels; do not fill above the filter paper line
3. Allow the solution to drain completely until only soil remains on the filter
4. Discard the used filter paper and soil

8.5. Extract Storage

1. Cap the filtered extract containers
2. If analysis will be completed within 48 hours, store at 4 C
3. If analysis will be delayed beyond 48 hours, freeze the extracts at -20 C until analysis (Keeney & Nelson, 1982)

! Important

Extracts should be analyzed as soon as possible. Prolonged storage, even frozen, can alter NH_4^+ and NO_3^- concentrations due to microbial activity or chemical transformations (Maynard et al., 2008).

9. Calculations

9.1. Converting Extract Concentration to Soil Concentration

The colorimetric or flow-injection analyzer reports the concentration of NO_3^--N or NH_4^+-N in the filtered extract (mg N/L). To convert this to mg N per kg oven-dry soil (ppm):

$$C_{\text{soil}}\ (\text{mg N kg}^{-1}) = \frac{C_{\text{extract}} \times V_{\text{KCl}}}{m_{\text{dry}}}$$

where:

- C_{extract} = concentration in the filtered extract (mg N/L)
- V_{KCl} = volume of KCl extractant added (L; typically 0.040 L)
- m_{dry} = oven-dry equivalent mass of soil extracted (kg), calculated as:

$$m_{\text{dry}} = \frac{m_{\text{moist}}}{1 + \theta_g}$$

where m_{moist} is the field-moist mass added (g, converted to kg) and θ_g is the gravimetric moisture content expressed as a decimal (g water / g oven-dry soil).

See Appendix A for a worked R code example.

9.2. Soil Texture Correction Factor (Optional)

When using the semi-quantitative quick test (SOP-L404), a texture-based correction factor may be applied. For the quantitative KCl extraction described here, no texture correction is needed because the exact soil mass and moisture content are measured directly.

Texture	Moist Soil Factor	Dry Soil Factor
Sand	2.3	2.6
Loam	2.0	2.5
Clay	1.7	2.2

These factors are retained here for reference when comparing quick-test results to KCl extraction results.

10. Quality Control

- Include a reagent blank (KCl solution only, no soil) in each extraction batch. The blank should read below the method detection limit for both NO_3^--N and NH_4^+-N.
- Run extraction duplicates for at least 10% of samples. Duplicate results should agree within 15% relative percent difference (RPD).
- Include a laboratory reference soil with known inorganic N content in each batch when available.
- Record the KCl solution preparation date and batch number.
- Verify that the analytical calibration curve for the colorimetric method has an $r^2 \geq 0.995$ before reporting results.
- Subtract the mean reagent blank concentration from all sample results if the blank reads above zero (Mulvaney, 1996).

11. Waste Disposal

Waste Type	Disposal Method
KCl extracts	Aqueous waste; drain with copious water if approved by EH&S, or collect for pickup
Used filter paper and soil	Standard trash
Unused KCl solution	Drain with water (non-hazardous at this concentration)

12. References

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13. Training Record

Before performing this procedure independently, personnel must:

1. Read this SOP in full
2. Complete SOP-L400 Soil Moisture training
3. Demonstrate competence preparing KCl solution
4. Perform the extraction and filtering procedure under supervision
5. Calculate results for one batch using the R code in Appendix A and verify against the instructor's values
6. Be signed off by the PI or lab manager

14. Appendix A: Calculation of Soil NO_3^--N and NH_4^+-N (R Code)

The following R code converts extract concentrations (mg N/L from the colorimetric analyzer) to soil concentrations (mg N/kg oven-dry soil, equivalent to ppm) for a batch of samples. It corrects for the exact mass of field-moist soil added and the gravimetric moisture content of each sample.

```
# ==============================================================================
# SOP-L403: KCl Extraction -- Convert extract concentration to soil ppm
# ==============================================================================

# ---- 1. Enter your data ---------------------------------------------------
# Replace the example values below with your actual data.
# Each vector must have the same length (one entry per sample).

sample_id <- c("S01", "S02", "S03", "S04", "S05")

# Mass of field-moist soil added to the extraction tube (g)
soil_moist_g <- c(7.03, 7.10, 6.98, 7.05, 7.12)

# Gravimetric moisture content (proportion, g water / g oven-dry soil)
```

```r
# From SOP-L400.  Example: 0.15 means 15% moisture.
theta_g <- c(0.152, 0.148, 0.161, 0.155, 0.143)

# Volume of 2 M KCl added (mL) -- typically 40 mL for all samples
vol_kcl_mL <- rep(40, length(sample_id))

# Extract concentrations from the colorimetric analyzer (mg N / L)
no3_extract_mg_L <- c(3.42, 5.18, 2.76, 4.01, 6.33)
nh4_extract_mg_L <- c(1.05, 0.87, 1.22, 0.93, 0.78)

# Reagent blank concentrations (mg N / L) -- mean of your blanks
blank_no3_mg_L <- 0.02
blank_nh4_mg_L <- 0.01

# ---- 2. Calculate oven-dry soil mass (kg) ----------------------------------
soil_dry_g  <- soil_moist_g / (1 + theta_g)
soil_dry_kg <- soil_dry_g / 1000

# ---- 3. Blank-correct extract concentrations --------------------------------
no3_corrected <- no3_extract_mg_L - blank_no3_mg_L
nh4_corrected <- nh4_extract_mg_L - blank_nh4_mg_L

# Ensure no negative values after blank correction
no3_corrected[no3_corrected < 0] <- 0
nh4_corrected[nh4_corrected < 0] <- 0

# ---- 4. Convert to mg N / kg oven-dry soil (ppm) ----------------------------
vol_kcl_L <- vol_kcl_mL / 1000

no3_ppm <- (no3_corrected * vol_kcl_L) / soil_dry_kg
nh4_ppm <- (nh4_corrected * vol_kcl_L) / soil_dry_kg

# ---- 5. Assemble results table ----------------------------------------------
results <- data.frame(
  Sample        = sample_id,
  Moist_g       = soil_moist_g,
  Moisture_pct  = round(theta_g * 100, 1),
  Dry_g         = round(soil_dry_g, 3),
  Vol_KCl_mL    = vol_kcl_mL,
  NO3_extract   = no3_extract_mg_L,
  NH4_extract   = nh4_extract_mg_L,
  NO3_ppm       = round(no3_ppm, 2),
  NH4_ppm       = round(nh4_ppm, 2),
  Total_inorgN  = round(no3_ppm + nh4_ppm, 2)
)

# ---- 6. Print results -------------------------------------------------------
```

```
cat("SOP-L403: KCl Extraction Results\n")
cat("================================\n\n")
print(results, row.names = FALSE)
cat("\n")
cat(sprintf("Reagent blank NO3-N: %.2f mg/L\n", blank_no3_mg_L))
cat(sprintf("Reagent blank NH4-N: %.2f mg/L\n", blank_nh4_mg_L))
cat("\nAll soil concentrations are mg N / kg oven-dry soil (ppm).\n")
```

14.1. Worked Example (Sample S01)

Using the example data above for sample S01:

- Field-moist soil added: 7.03 g
- Gravimetric moisture: 15.2% (0.152)
- Oven-dry equivalent: $7.03/(1 + 0.152) = 6.102$ g $= 0.006102$ kg
- KCl volume: 40 mL = 0.040 L
- NO_3^--N in extract: 3.42 mg/L (blank-corrected: 3.40 mg/L)
- Soil NO_3^--N: $(3.40 \times 0.040)/0.006102 = 22.29$ mg N/kg (ppm)
- NH_4^+-N in extract: 1.05 mg/L (blank-corrected: 1.04 mg/L)
- Soil NH_4^+-N: $(1.04 \times 0.040)/0.006102 = 6.82$ mg N/kg (ppm)
- Total inorganic N: $22.29 + 6.82 = 29.11$ mg N/kg (ppm)

15. Appendix B: Interpretation Guide

General interpretation thresholds for extractable inorganic N in agricultural and wildland soils. These are approximate and vary by crop, climate, and soil type (Hart et al., 1994; Mulvaney, 1996).

Extractable Inorganic N (mg N/kg)	Interpretation	Notes
Less than 10	Low	Nitrogen likely limiting plant growth
10–25	Moderate	Adequate for many crops and native vegetation
25–50	High	Sufficient N; fertilization generally not needed
Greater than 50	Very high	Excess N; potential leaching or denitrification concern

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L404

Soil Nitrate Quick Test – Test Strip Method

Standard Operating Procedure

SOP Number	SOP-L404
Revision	0.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes a rapid, semi-quantitative field or laboratory method for estimating soil nitrate (NO_3^-) concentration using colorimetric test strips in a calcium chloride ($CaCl_2$) extracting solution (Bronikowski & Thomason, 2022; Hartz, 2010). The method is useful as a screening tool for assessing soil nitrogen status, guiding fertilizer decisions, and identifying spatial patterns in nitrate availability across a site (Magdoff et al., 1984). It is not a substitute for the quantitative KCl extraction and colorimetric analysis described in SOP-L403 (Mulvaney, 1996), but provides rapid, low-cost results that can be obtained in the field or with minimal laboratory equipment.

2. Scope

- **Applies to:** Rapid estimation of soil nitrate in field-moist or air-dried mineral soils from all EA Program sites; on-site field screening during sampling campaigns
- **Does not apply to:** Quantitative reporting of soil nitrate for publication (use SOP-L403); ammonium determination (test strips do not measure NH_4^+); water sample nitrate (use SOP-L303 Hach Colorimetric)
- **Location:** Field sites or EA Program laboratory, SGM 133

3. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, ensure training compliance
Field Lead	Coordinate field testing logistics
Trained Personnel	Perform tests, record and report results
New Students	Complete training before independent use

4. Definitions

- **Calcium chloride ($CaCl_2$) extractant:** A dilute salt solution (approximately 0.01 M) used in the quick test to displace some exchangeable NO_3^- from soil without requiring the high-ionic-strength KCl used in quantitative extractions; minimizes matrix effects on the test strip reaction.
- **Colorimetric test strip:** A plastic strip impregnated with reagents that react with nitrate (or another target) to produce a visible color change; read visually against a printed color chart or with a reflectometer; semi-quantitative.
- **Detection range:** The span of concentrations over which a test strip or method produces a distinguishable response; for most nitrate strips, approximately 0–500 mg NO_3^--N L^{-1} in the extract; values outside this range require dilution or a quantitative method.
- **Matrix effect:** Interference from sample components (chloride, organic matter, pH) that alter the test strip reaction; minimized by using $CaCl_2$ extractant and diluting dark or strongly colored samples.

- **Semi-quantitative:** A measurement that provides an approximate concentration within broad categories (e.g., 0, 10, 25, 50, 100 mg/L) rather than a precise numerical value; appropriate for screening but not for publication or regulatory reporting.

5. Safety and Hazards

Hazards

- **Chemical:** Calcium chloride ($CaCl_2$) is a mild irritant to skin and eyes. The extracting solution is dilute (approximately 0.01 M) and poses minimal risk.
- **Biological:** Soil may contain pathogens. Wash hands after handling.

5.1. Required PPE

☐ Nitrile gloves
☐ Safety glasses
☐ Closed-toed shoes (if testing in the field)

5.2. Emergency Procedures

For $CaCl_2$ solution contact with eyes, flush with water for 15 minutes. Refer to SOP-L002 Laboratory Safety.

6. Equipment and Materials

6.1. Equipment

Item	Notes
Graduated centrifuge tubes (50 mL, e.g., Falcon tubes)	With volume markings at 30 and 40 mL
1-gallon jug or carboy	For preparing $CaCl_2$ extracting solution
Soil probe or auger	For field sampling
Bucket	For compositing and mixing field samples
Timer or watch	For tracking settling and reading times
Field notebook	For recording results

6.2. Consumables

Item	Notes
Nitrate test strips	Semi-quantitative colorimetric strips (e.g., Hach, EM Quant, or equivalent)
Calcium chloride ($CaCl_2$), anhydrous or dihydrate	For preparing extracting solution
Distilled or deionized water	For preparing extracting solution
Color comparison chart	Provided with test strips

7. Procedure

7.1. Preparing the Extracting Solution

1. Add 6 g (approximately 1 teaspoon) of anhydrous $CaCl_2$ to 1 gallon (3.78 L) of distilled water
2. Cap and shake until dissolved
3. This produces approximately 0.014 M $CaCl_2$, sufficient for approximately 125 tests
4. Label the container with the reagent name, concentration, and preparation date
5. Store at room temperature. The solution is stable indefinitely.

> **Note**
>
> $CaCl_2$ is used instead of plain water because the calcium ions displace nitrate from soil exchange sites, giving a more complete extraction (Gelderman & Beegle, 2015; Houba et al., 1986). The low concentration avoids interference with the test strip chemistry.

7.2. Collecting the Soil Sample

1. Collect soil from the target depth (typically 0–15 cm or 0–30 cm) using a probe or auger per SOP-F400 Soil Sampling
2. Composite multiple subsamples if a plot-level estimate is desired
3. Homogenize the soil in a bucket by hand-mixing
4. Remove visible roots and stones

7.3. Performing the Quick Test

1. Fill a graduated centrifuge tube with 30 mL of the $CaCl_2$ extracting solution
2. Add soil to the tube until the total volume reaches the 40 mL mark (approximately 10 mL of soil displaced)
3. Cap the tube securely
4. Shake vigorously until all soil is well mixed (approximately 30 seconds)
5. Allow the tube to stand upright for the soil particles to settle. Wait at least 10 minutes but no more than 1 hour.
6. Dip a nitrate test strip into the clearer supernatant near the top of the tube for 1 second. Do not stir up settled soil.

7. Remove the strip and hold horizontally
8. Compare the strip to the manufacturer's color chart at both 30 seconds and 60 seconds after dipping
9. Record the nitrate reading (ppm NO_3^--N or ppm NO_3^-, depending on the strip brand) and the reading time used

> **! Important**
>
> Read the strip at the times specified by the manufacturer. Reading too early or too late gives inaccurate results. If the strip brand reports in ppm NO_3^-, divide by 4.43 to convert to ppm NO_3^--N.

7.4. Applying the Soil Texture Correction Factor

The test strip reads the nitrate concentration in the extract solution, not in the soil. To estimate the nitrate concentration in the soil itself, apply a correction factor based on soil texture and moisture status (Gelderman & Beegle, 2015):

Texture	Moist Soil Factor	Dry Soil Factor
Sand	2.3	2.6
Loam	2.0	2.5
Clay	1.7	2.2

$$\text{Soil } NO_3^-\text{-N (ppm)} = \text{Strip reading (ppm)} \times \text{Correction factor}$$

Determine the soil texture using SOP-F401 Texture by Feel or SOP-L401 Hydrometer.

7.5. Recording Results

Record the following in the field notebook or data sheet for each test:

- Date, time, site, plot ID
- Sampling depth (cm)
- Soil condition (moist or dry)
- Estimated texture (sand, loam, or clay)
- Strip reading (ppm) and reading time (30 s or 60 s)
- Correction factor applied
- Corrected soil NO_3^--N (ppm)

8. Quality Control and Acceptance Criteria

8.1. Limitations

The test strip method is semi-quantitative. Results should be interpreted as approximate categories (low, moderate, high) rather than precise concentrations (Hartz, 2010; Schmidhalter, 2005). Key limitations include:

- Strip resolution is typically 10–25 ppm increments; fine differences are not detectable
- Color matching is subjective (ambient lighting affects perception)
- The method does not measure ammonium (NH_4^+); total inorganic N requires SOP-L403
- Soil moisture, organic matter, and temperature all influence extraction efficiency

8.2. Interpretation Guide

Corrected Soil NO_3^--N (ppm)	Interpretation	Agronomic Implication
Less than 10	Low	N likely limiting; fertilization may be warranted
10 – 25	Moderate	Adequate for many crops
25 – 50	High	Sufficient N; reduce or defer applications
Greater than 50	Very high	Excess N; potential leaching risk

These thresholds are general guidelines adapted from Magdoff et al. (1984) and Hartz (2010). Consult crop-specific guidelines and local extension recommendations.

8.3. QC Requirements

- Run a blank ($CaCl_2$ solution only, no soil) at the start of each testing session. The strip should read 0 ppm or below the lowest detection increment.
- Run a duplicate test on at least 1 in 10 samples. Duplicates should agree within one color-chart increment.
- Verify test strip expiration date before use. Expired strips may give unreliable color development.
- For critical decisions, confirm quick-test results with the quantitative KCl extraction method (SOP-L403)

9. Waste Disposal

Waste Type	Disposal Method
Soil slurry waste	Return to field site or standard trash
$CaCl_2$ solution	Drain (non-hazardous at this concentration)

Waste Type	Disposal Method
Used test strips	Standard trash
Used centrifuge tubes	Rinse and reuse, or standard trash

10. References

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11. Training Record

Before performing the nitrate quick test independently, personnel must:

1. Read this SOP in full
2. Complete SOP-F001 Field Safety training (if testing in the field) or SOP-L002 Laboratory Safety (if in the lab)
3. Prepare the $CaCl_2$ extracting solution under supervision
4. Perform at least three practice tests with acceptable blank and duplicate results
5. Demonstrate correct use of the color chart and correction factor
6. Be signed off by the PI, field lead, or lab manager

12. Appendix A: Example Calculation

```r
# Nitrate quick test correction example

strip_reading_ppm <- 15       # ppm NO3-N from test strip
soil_texture <- "loam"
soil_condition <- "moist"     # "moist" or "dry"

# Correction factors
factors <- data.frame(
  texture = c("sand", "loam", "clay"),
  moist = c(2.3, 2.0, 1.7),
  dry = c(2.6, 2.5, 2.2)
)

cf <- factors[factors$texture == soil_texture, soil_condition]

corrected_ppm <- strip_reading_ppm * cf

cat(sprintf("Strip reading:     %d ppm NO3-N\n", strip_reading_ppm))
cat(sprintf("Texture:           %s\n", soil_texture))
cat(sprintf("Condition:         %s\n", soil_condition))
cat(sprintf("Correction factor: %.1f\n", cf))
cat(sprintf("Corrected value:   %.0f ppm NO3-N\n", corrected_ppm))
```

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L405

Soil Extractable Phosphorus

Standard Operating Procedure

SOP Number	SOP-L405
Revision	0.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the extraction and colorimetric determination of plant-available (extractable) phosphorus in soils. Two extraction methods are presented: the Olsen method (0.5 M $NaHCO_3$ at pH 8.5) for neutral to alkaline soils, and the Bray-1 method (0.03 M NH_4F + 0.025 M HCl) for acidic soils (Bray & Kurtz, 1945; Olsen et al., 1954). Extractable P is quantified colorimetrically using the ascorbic acid/molybdate blue method (Kuo, 1996; Murphy & Riley, 1962).

Soil phosphorus availability is a key factor controlling plant productivity, and excess P in runoff is a primary driver of eutrophication in freshwater systems. Routine soil P testing guides fertilizer management and helps assess the environmental risk of P loss from agricultural and urban soils (Sharpley et al., 2003).

2. Scope

- **Applies to:** Air-dried, sieved (less than 2 mm) mineral soils from all EA Program field sites. The Olsen method is the default for southern California soils, which are typically neutral to alkaline. Use Bray-1 for soils with pH below 6.5.
- **Does not apply to:** Total phosphorus determination (requires acid digestion); dissolved reactive phosphorus in water samples (see SOP-L303 Hach Colorimetric); organic phosphorus fractionation
- **Location:** EA Program laboratory, SGM 133

3. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, ensure training compliance
Lab Manager	Prepare and standardize reagents, maintain spectrophotometer
Trained Personnel	Perform extractions and analyses, report results
New Students	Complete training before independent use

4. Definitions

- **Available phosphorus:** Phosphorus in soil solution and loosely sorbed fractions that can be taken up by plant roots; estimated by extraction with reagents that mimic root chemistry (Olsen bicarbonate in alkaline soils; Mehlich-3 or Bray in acid soils).
- **Bray P1 extraction:** An extraction using 0.025 M HCl + 0.03 M NH_4F; removes acid-soluble (Al- and Fe-bound) phosphorus; appropriate for acid and neutral soils (pH < 7.4) but not for calcareous soils.
- **Mehlich-3 extraction:** A multi-element extractant (0.2 M CH_3COOH + 0.25 M NH_4NO_3 + 0.015 M NH_4F + 0.013 M HNO_3 + 0.001 M EDTA); extracts plant-available P, K, Ca, Mg, and several micronutrients simultaneously; widely used in the eastern US and California.
- **Murphy-Riley method:** The standard colorimetric procedure for measuring dissolved reactive phosphorus; reacts phosphate with ammonium molybdate and ascorbic acid to form a blue phosphomolybdenum complex measured at 880 nm (Murphy & Riley, 1962).
- **Olsen extraction:** An extraction using 0.5 M $NaHCO_3$ (pH 8.5); removes Ca-phosphate forms; the standard method for calcareous and alkaline soils (pH > 7.0); results expressed as mg P kg^{-1}.
- **Sorption:** The attachment of phosphate ions to soil mineral surfaces (primarily Fe and Al oxides in acid soils; $CaCO_3$ in alkaline soils); limits plant availability and affects the choice of extraction method.

5. Safety and Hazards

Hazards

- **Chemical:** Ascorbic acid, ammonium molybdate, and antimony potassium tartrate are mild irritants. Sulfuric acid (used in the color reagent) is corrosive at concentrated strengths. Ammonium fluoride (NH_4F, Bray-1 extractant) is toxic and corrosive; handle in the fume hood. Sodium hydroxide (NaOH, for pH adjustment of Olsen reagent) is corrosive.
- **Physical:** Standard glassware handling risks.

5.1. Required PPE

☐ Safety glasses (splash goggles when handling H_2SO_4 or NH_4F)
☐ Lab coat
☐ Nitrile gloves

- ☐ Work in fume hood when handling NH_4F

5.2. Emergency Procedures

For acid or NH_4F splashes, flush with water for at least 15 minutes. For NH_4F skin contact, flush with water and apply 2.5% calcium gluconate gel if available. Refer to SOP-L003 Hazardous Materials Handling.

6. Equipment and Materials

6.1. Equipment

Item	Notes
Reciprocating or end-over-end shaker	For extraction
Spectrophotometer or colorimeter	880 nm wavelength; Hach DR3900 (SOP-L303) or equivalent
Analytical balance (0.01 g)	See SOP-L102
pH meter	For verifying extractant pH; see SOP-L300
Centrifuge or vacuum filtration apparatus	For clarifying extracts
Volumetric flasks (100 mL, 500 mL, 1 L)	Class A
Erlenmeyer flasks (125 or 250 mL)	One per sample plus blanks
Graduated cylinders (25, 50, 100 mL)	For measuring extractant volumes
Pipettes (1–10 mL)	Class A volumetric or calibrated micropipettes
Cuvettes (1 cm path length)	Glass or polystyrene, matched
Filter funnels and Whatman No. 42 filter paper	For clarifying extracts

6.2. Reagents – Olsen Extraction

Reagent	Preparation / Notes
Sodium bicarbonate ($NaHCO_3$), 0.5 M at pH 8.5	Dissolve 42.0 g $NaHCO_3$ in approximately 900 mL DI water. Adjust to pH 8.5 with 1 M NaOH. Bring to 1 L. Prepare fresh weekly.
Activated charcoal (P-free)	For decolorizing extracts; wash with 0.5 M $NaHCO_3$ before use to remove any residual P (Olsen et al., 1954)

6.3. Reagents – Bray-1 Extraction

Reagent	Preparation / Notes
Bray-1 extractant (0.03 M NH_4F + 0.025 M HCl)	Dissolve 1.11 g NH_4F in approximately 900 mL DI water. Add 2.1 mL concentrated HCl (12 M). Bring to 1 L. Prepare in fume hood. Store in polyethylene. (Bray & Kurtz, 1945)

6.4. Reagents – Ascorbic Acid Colorimetric Determination

Reagent	Preparation / Notes
Sulfuric acid (H_2SO_4), 5 N	Dilute 70 mL concentrated H_2SO_4 (18 M) into approximately 400 mL DI water; bring to 500 mL after cooling. **Add acid to water.**
Ammonium molybdate ($(NH_4)_6Mo_7O_{24}$-$4H_2O$), 4%	Dissolve 20 g in 500 mL DI water
Antimony potassium tartrate ($K(SbO)C_4H_4O_6$-$1/2H_2O$), 0.274%	Dissolve 0.274 g in 100 mL DI water
Ascorbic acid, 1.76%	Dissolve 1.76 g in 100 mL DI water. Prepare fresh daily.
Combined color reagent	Mix in order: 50 mL 5 N H_2SO_4 + 15 mL ammonium molybdate + 5 mL antimony potassium tartrate + 30 mL ascorbic acid. Prepare fresh for each batch. (Murphy & Riley, 1962)
Phosphorus stock standard (1000 mg/L P)	Dissolve 4.394 g anhydrous KH_2PO_4 (dried at 105 C) in 1 L DI water. Stable for months at 4 C.
Phosphorus working standards	Dilute stock to 0, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0 mg/L P in the appropriate extractant matrix ($NaHCO_3$ or Bray-1)

6.5. Reagents – General

Reagent	Notes
Deionized water (Type I or II)	From *SOP-L11*

7. Procedure

7.1. Sample Preparation

1. Air-dry the soil at room temperature until constant mass
2. Pass through a 2 mm sieve per SOP-F400 Soil Sampling
3. Determine gravimetric moisture content on a subsample per SOP-L400 to express results on a dry-weight basis

7.2. Selecting the Extraction Method

Soil pH	Recommended Extractant	Reference
6.5 or above	Olsen (0.5 M $NaHCO_3$, pH 8.5)	Olsen et al. (1954)
Below 6.5	Bray-1 (0.03 M NH_4F + 0.025 M HCl)	Bray & Kurtz (1945)

Determine soil pH per SOP-L402 before selecting the extractant. If comparing across sites with widely varying pH, consider using Mehlich-3 for all samples (consult the PI).

> **Note**
>
> The Olsen extractant ($NaHCO_3$ at pH 8.5) dissolves calcium phosphate precipitates and reduces Fe^{3+} and Al^{3+} activity, releasing adsorbed P. It works across a wide pH range but is the standard for calcareous and alkaline soils. The Bray-1 extractant dissolves Al-bound and Fe-bound P via fluoride complexation and acid dissolution; it underestimates P in calcareous soils because the acid is neutralized by free $CaCO_3$ (Kuo, 1996).

7.3. Olsen Extraction

1. Weigh 2.50 g of air-dried, sieved soil into a 125 mL Erlenmeyer flask
2. Add approximately 0.25 g of P-free activated charcoal (pre-washed with 0.5 M $NaHCO_3$)
3. Add 50 mL of 0.5 M $NaHCO_3$ extractant (pH 8.5)
4. Stopper and shake on the reciprocating shaker at 180 oscillations per minute for 30 minutes
5. Filter immediately through Whatman No. 42 filter paper into a clean flask or tube. The filtrate should be clear and colorless; if yellow-brown, the charcoal was insufficient or not pre-washed.
6. Proceed to colorimetric analysis (Section 6.5) within 24 hours. Store extracts at 4 C if analysis is delayed.

7.4. Bray-1 Extraction

1. Weigh 2.00 g of air-dried, sieved soil into a 125 mL Erlenmeyer flask
2. Add 20 mL of Bray-1 extractant (1:10 soil-to-solution ratio)
3. Shake on the reciprocating shaker at 180 oscillations per minute for 5 minutes (the short extraction time is critical; extended shaking can re-adsorb released P)
4. Filter immediately through Whatman No. 42 filter paper
5. Proceed to colorimetric analysis (Section 6.5) within 24 hours

> **Important**
>
> The Bray-1 extraction is exactly 5 minutes. Timing begins when the extractant contacts the soil. Over-extraction will underestimate available P due to re-sorption, while under-extraction will miss slowly dissolving forms.

7.5. Colorimetric Determination (Ascorbic Acid/Molybdate Blue)

This procedure follows Murphy & Riley (1962) as adapted by Kuo (1996).

1. Prepare the combined color reagent fresh (see Section 5, Reagents)
2. Pipette a 5 mL aliquot of the filtered extract into a clean test tube or cuvette
3. For Olsen extracts: acidify the aliquot with 1 drop (approximately 0.05 mL) of concentrated H_2SO_4 and swirl to expel CO_2 (the $NaHCO_3$ matrix effervesces; wait until bubbling stops before adding color reagent)
4. Add 0.8 mL of the combined color reagent to each tube
5. Mix by vortexing or inversion
6. Allow the color to develop for 15 minutes at room temperature. The solution turns blue in proportion to the phosphorus concentration.
7. Read the absorbance at 880 nm (or 882 nm) against a DI water blank within 30 minutes of color development
8. Determine the P concentration from the calibration curve (Section 6.6)

Alternative: Hach DR3900 PhosVer 3 method. If using the Hach spectrophotometer, follow the PhosVer 3 (ascorbic acid) method per SOP-L303, Program 490. Prepare standards in the same extractant matrix as the samples.

7.6. Calibration

1. Prepare a minimum of five working standards (e.g., 0, 0.1, 0.5, 1.0, 2.0, 5.0 mg/L P) by diluting the stock standard with the same extractant used for the samples
2. Treat each standard exactly as the samples (acidification for Olsen, color reagent addition, timing)
3. Read absorbance at 880 nm
4. Plot absorbance versus concentration and fit a linear regression. Acceptable r^2 is 0.995 or higher.

7.7. Calculations

Extractable P in soil (mg P / kg dry soil):

$$P_{\text{soil}} = \frac{C \times V}{M \times \left(\frac{100}{100+\theta_H}\right)}$$

where C = P concentration in the extract (mg/L), V = volume of extractant (mL), M = mass of air-dried soil (g), and θ_H = hygroscopic moisture (%, determined per SOP-L400). The term $\frac{100}{100+\theta_H}$ converts the air-dried mass to an oven-dry basis.

For the Olsen method with the standard parameters (2.50 g soil, 50 mL extractant):

$$P_{\text{soil}}\ (\text{mg/kg}) = C \times \frac{50}{2.50} \times \frac{100+\theta_H}{100} = C \times 20 \times \frac{100+\theta_H}{100}$$

For Bray-1 (2.00 g soil, 20 mL extractant):

$$P_{\text{soil}}\ (\text{mg/kg}) = C \times \frac{20}{2.00} \times \frac{100 + \theta_H}{100} = C \times 10 \times \frac{100 + \theta_H}{100}$$

8. Quality Control and Acceptance Criteria

8.1. Interpretation of Olsen P

Olsen P (mg/kg)	Classification	Agronomic Interpretation
Less than 5	Very low	P deficiency likely; fertilize
5 – 10	Low	P may limit yield; fertilize
10 – 20	Medium	Adequate for most crops
20 – 40	High	No response to added P expected
Greater than 40	Very high	Excess P; environmental risk of runoff losses

Thresholds are approximate and crop-dependent (Olsen et al., 1954; Sharpley et al., 2003). Consult local extension guidelines.

8.2. Interpretation of Bray-1 P

Bray-1 P (mg/kg)	Classification	Agronomic Interpretation
Less than 10	Very low	P deficiency likely
10 – 20	Low	Fertilize to build P levels
20 – 40	Medium	Adequate for most crops
40 – 80	High	No additional P needed
Greater than 80	Very high	Environmental risk

8.3. QC Requirements

- Prepare all standards in the same extractant matrix as the samples (matrix-matched calibration) to account for background color and ionic strength effects
- Run a reagent blank (extractant only, no soil) with each batch. The blank absorbance should be near zero at 880 nm.
- Run a laboratory reference soil with known extractable P in each batch. Recovery should be within +/- 15% of the established value.
- Run duplicate extractions on at least 10% of samples. Acceptable precision: relative percent difference (RPD) less than 15%.
- Calibration curve r^2 must be 0.995 or higher
- Check the mid-range standard after every 20 samples. Recalibrate if the check standard deviates by more than 10%.
- For Olsen extracts, verify the $NaHCO_3$ pH is 8.5 +/- 0.1 before each batch; pH drift changes extraction efficiency substantially.

9. Waste Disposal

Waste Type	Disposal Method
Olsen extracts ($NaHCO_3$)	Neutralize to pH 6–8 and drain, or collect for EH&S
Bray-1 extracts (NH_4F/HCl)	Collect in fluoride waste carboy; EH&S pickup. Do not drain.
Color reagent waste (molybdate/H_2SO_4)	Collect in inorganic waste; EH&S pickup
Used filter paper and soil	Standard trash (if non-hazardous soil)
Spent charcoal	Standard trash

Fluoride Waste

Bray-1 extracts contain ammonium fluoride. **Do not pour down the drain.** Collect all fluoride-containing waste in a labeled polyethylene carboy for EH&S hazardous waste pickup.

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11. Training Record

Before performing soil extractable P analyses independently, personnel must:

1. Read this SOP in full
2. Complete SOP-L002 Laboratory Safety and SOP-L003 Hazardous Materials Handling training
3. Review the SDS for ammonium fluoride (if using Bray-1)
4. Demonstrate preparation of the extractant, color reagent, and calibration standards under supervision
5. Run a practice batch (at least 5 samples + blank + reference soil + duplicate) with acceptable QC results
6. Be signed off by the PI or lab manager

12. Appendix A: Example Calculation in R

```r
library(tidyverse)

# --- Calibration curve ---
standards <- tibble(
  P_mg_L = c(0, 0.1, 0.5, 1.0, 2.0, 5.0),
  abs_880 = c(0.002, 0.018, 0.075, 0.148, 0.295, 0.740)
)

cal_model <- lm(abs_880 ~ P_mg_L, data = standards)
cat(sprintf("Calibration R-squared: %.5f\n", summary(cal_model)$r.squared))

# --- Sample calculation (Olsen method) ---
sample_abs <- 0.210            # Absorbance at 880 nm
soil_mass_g <- 2.50            # Air-dried soil (g)
extract_vol_mL <- 50           # Extractant volume (mL)
hygroscopic_moisture_pct <- 2.1 # Percent hygroscopic moisture

# Determine C from calibration
C_mg_L <- (sample_abs - coef(cal_model)[1]) / coef(cal_model)[2]

# Calculate soil extractable P (mg/kg dry soil)
P_soil_mg_kg <- C_mg_L * (extract_vol_mL / soil_mass_g) *
                ((100 + hygroscopic_moisture_pct) / 100)

cat(sprintf("Extract P concentration: %.3f mg/L\n", C_mg_L))
```

```r
cat(sprintf("Olsen P:                %.1f mg/kg dry soil\n", P_soil_mg_kg))

# Interpret
interpret <- case_when(
  P_soil_mg_kg < 5  ~ "Very low",
  P_soil_mg_kg < 10 ~ "Low",
  P_soil_mg_kg < 20 ~ "Medium",
  P_soil_mg_kg < 40 ~ "High",
  TRUE               ~ "Very high"
)
cat(sprintf("Classification:         %s\n", interpret))
```

13. Appendix B: Reagent Preparation Quick Reference

13.1. Olsen Extractant (0.5 M $NaHCO_3$, pH 8.5)

1. Dissolve 42.0 g $NaHCO_3$ in approximately 900 mL DI water
2. Adjust pH to 8.5 with 1 M NaOH (dropwise, with stirring)
3. Bring to 1 L with DI water
4. Check pH before each batch; re-adjust if needed
5. Prepare fresh weekly

13.2. Bray-1 Extractant (0.03 M NH_4F + 0.025 M HCl)

1. In a fume hood, dissolve 1.11 g NH_4F in approximately 900 mL DI water
2. Add 2.1 mL concentrated HCl (12 M)
3. Bring to 1 L with DI water
4. Store in polyethylene at room temperature. Stable for several weeks.

13.3. Combined Color Reagent (Murphy-Riley)

Mix in order, swirling between additions:

1. 50 mL of 5 N H_2SO_4
2. 15 mL of 4% ammonium molybdate
3. 5 mL of 0.274% antimony potassium tartrate
4. 30 mL of 1.76% ascorbic acid (freshly prepared)

Total volume: 100 mL. Prepare fresh for each analytical batch.

13.4. P Stock Standard (1000 mg/L P)

1. Dry KH_2PO_4 at 105 C for 2 hours; cool in a desiccator
2. Dissolve 4.394 g in DI water in a 1 L volumetric flask
3. Bring to volume. Store at 4 C. Stable for months.

14. Appendix C: BibTeX Entries

Append these to the project `references.bib`:

15. Appendix D: Troubleshooting

Symptom	Probable Cause	Corrective Action
Yellow-brown Olsen extract	Insufficient charcoal; charcoal not pre-washed	Increase charcoal; pre-wash with 0.5 M $NaHCO_3$
Calibration curve non-linear above 5 mg/L	Beer's Law limit exceeded	Dilute high samples; add lower standards
Bray-1 P unexpectedly low on calcareous soil	Acid neutralized by $CaCO_3$	Use Olsen method for soils with pH above 6.5
Blank reads above 0.01 absorbance	Contaminated glassware or reagent	Acid-wash glassware (SOP-L101); prepare fresh reagents
Color fades after 30 min	Ascorbic acid degradation	Read within 30 min; prepare ascorbic acid solution fresh daily
Duplicates disagree by more than 15% RPD	Soil heterogeneity; inconsistent extraction timing	Homogenize soil more thoroughly; use a timer for extraction
Reference soil out of range	Extractant pH drift (Olsen); reagent degradation	Verify $NaHCO_3$ pH; remake color reagent

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L406

Soil Organic Matter – Loss on Ignition

Standard Operating Procedure

SOP Number	SOP-L406
Revision	1.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes the estimation of soil organic matter (SOM) content using the loss-on-ignition (LOI) method. SOM is quantified by measuring the mass lost when oven-dried soil is combusted at 550 °C in a muffle furnace (Heiri et al., 2001; Nelson & Sommers, 1996).

2. Scope

- **Applies to:** Air-dried, sieved soil samples collected per SOP-F400 Soil Sampling and Processing.
- **Does not apply to:** Soils with high calcium carbonate content ($CaCO_3$ decomposes above ~450 °C and may inflate LOI values; consider an alternative method such as elemental analysis for calcareous soils (Heiri et al., 2001)).
- **Location:** EA Program laboratory, SGM 133.

3. Definitions

- **Ash-free dry mass (AFDM):** The mass remaining after removing water (drying at 105 °C) and organic matter (igniting at 550 °C); equivalent to the inorganic mineral mass of the sample.
- **Crucible:** A heat-resistant porcelain or ceramic container used to hold soil samples during furnace ignition; must be pre-ignited and weighed to constant mass before use.
- **Inorganic carbon (IC):** Carbon associated with carbonates ($CaCO_3$, $MgCO_3$); released as CO_2 during ignition above ~550 °C; can inflate LOI values in calcareous soils if ignition temperature is not controlled or if acid pre-treatment is omitted (Heiri et al., 2001).
- **Loss on ignition (LOI):** The percentage mass loss when an oven-dried soil is ignited at 550 °C for 4 hours: $LOI = (M_{105} - M_{550})/M_{105} \times 100$; used as a proxy for SOM content (Heiri et al., 2001).
- **Soil organic matter (SOM):** The organic fraction of soil, including plant and animal residues at various stages of decomposition, microbial biomass, and stabilized humus; estimated by LOI as approximately SOM $\approx 1.72 \times$ LOI (the Van Bemmelen factor) when organic carbon is the target (Nelson & Sommers, 1996).
- **Tare mass:** The pre-weighed mass of the empty, pre-ignited crucible; subtracted from all subsequent measurements to obtain sample mass.
- **Van Bemmelen factor:** The empirical conversion factor (1.724) relating organic carbon content to SOM by assuming that SOM contains 58% carbon by mass; use with caution for soils with unusual organic matter composition (Nelson & Sommers, 1996).

4. Responsibilities

Role	Responsibility
Lab PI	Approve protocol, ensure training compliance
Trained Personnel	Perform analysis, record and report results
New Students	Complete training before independent use

5. Safety and Hazards

Hazards

- **Thermal:** Muffle furnace operates at 550 °C; severe burn risk. Drying oven operates at 105 °C. Ceramic crucibles remain extremely hot for many minutes after removal from the furnace.
- **Physical:** Crucible tongs can slip; always use two hands when moving hot crucibles.

5.1. Required PPE

- ☐ Safety glasses
- ☐ Laboratory coat
- ☐ Heat-resistant gloves (mandatory when handling furnace-hot crucibles)

5.2. Emergency Procedures

Refer to SOP-L002 Laboratory Safety and SOP-L111 Muffle Furnace. For burns, cool with running water for at least 10 minutes and seek medical attention.

6. Equipment and Materials

6.1. Equipment

Item	Notes
Muffle furnace	Capable of 550 ±10 °C; programmable ramp preferred
Drying oven	Regulated at 105 ±2 °C
Analytical balance	Resolution 0.0001 g (0.1 mg)
Desiccator	With active color-indicating silica gel
Crucible tongs	Heat-resistant; long-handled

6.2. Consumables

Item	Notes
Porcelain or ceramic crucibles	Pre-ignited to constant mass
Weighing paper or spatulas	For soil transfer

7. Procedure

7.1. Crucible Preparation

1. Pre-ignite crucibles in the muffle furnace at 550 °C for at least 1 hour to remove any residual organic matter.
2. Transfer to a desiccator and cool to room temperature.
3. Weigh each crucible to the nearest 0.0001 g and record as the tare mass (W_{crucible}).

7.2. Oven Drying at 105 °C

1. Using a spatula, transfer 5–10 g of air-dried, sieved soil into a pre-weighed crucible.
2. Record the mass of crucible plus moist or air-dry soil.
3. Place uncovered crucibles in the drying oven at 105 °C for at least 24 hours.
4. Transfer crucibles to a desiccator and cool to room temperature (approximately 20–30 minutes).
5. Weigh to the nearest 0.0001 g and record as W_{105} (mass of oven-dried soil only, i.e., crucible + dry soil mass minus crucible tare).

7.3. Combustion at 550 °C

1. Transfer the crucibles containing oven-dried soil directly into the muffle furnace (Heiri et al., 2001).
2. Ramp the furnace to 550 °C (if programmable, use a ramp rate of approximately 10 °C per minute to prevent crucible cracking and reduce convective loss of fine particles).
3. Hold at 550 °C for 4 hours (Heiri et al., 2001).
4. Turn off the furnace and allow crucibles to cool inside the furnace until they can be safely transferred (below approximately 200 °C).
5. Transfer crucibles to a desiccator and cool to room temperature.
6. Weigh to the nearest 0.0001 g and record as W_{550} (mass of combusted soil, excluding crucible).

7.4. Calculation

Soil organic matter content is calculated as (Heiri et al., 2001):

$$\text{SOM (\%)} = \left(\frac{W_{105} - W_{550}}{W_{105}} \right) \times 100$$

where:

- W_{105} = mass of oven-dried soil (g)
- W_{550} = mass of soil after combustion at 550 °C (g)

To convert LOI (%) to approximate organic carbon (%OC), divide by 1.724 (the Van Bemmelen factor); note that this conversion assumes constant elemental composition and is less accurate for samples with unusual organic matter (Nelson & Sommers, 1996).

8. Quality Control

- Run analytical duplicates for at least 10% of samples; coefficient of variation between duplicates should be less than 5% (Heiri et al., 2001).
- Include an empty crucible blank in each furnace run to verify no mass gain from ambient contamination.
- Include a laboratory reference soil with known SOM content in each batch.
- Verify oven (105 °C) and furnace (550 °C) temperatures using a calibrated thermocouple at regular intervals.

9. Waste Disposal

Waste Type	Disposal Method
Combusted soil residue	Standard trash (inert mineral ash)
Crucibles	Clean and reuse

10. References

- Heiri et al. (2001)
- Nelson & Sommers (1996)
- SOP-L002 Laboratory Safety
- SOP-L111 Muffle Furnace

Heiri, O., Lotter, A. F., & Lemcke, G. (2001). Loss on ignition as a method for estimating organic and carbonate content in sediments: Reproducibility and comparability of results. *Journal of Paleolimnology*, *25*(1), 101–110. https://doi.org/10.1023/A:1008119611481

Nelson, D. W., & Sommers, L. E. (1996). Total carbon, organic carbon, and organic matter. In D. L. Sparks (Ed.), *Methods of soil analysis, part 3: Chemical methods* (pp. 961–1010). Soil Science Society of America. https://doi.org/10.2136/sssabookser5.3.c34

11. Related SOPs

SOP	Title	Relationship
L002	Laboratory Safety	General safety framework
L111	Muffle Furnace	Instrument operation
L400	Soil Moisture	Companion moisture determination
L407	Total Carbon	Direct combustion alternative

12. Training Record

Before performing this procedure independently, personnel must:

1. Read this SOP in full
2. Complete SOP-L111 Muffle Furnace training
3. Demonstrate safe operation of the muffle furnace and drying oven
4. Demonstrate proper use of the analytical balance and desiccator
5. Perform the procedure under supervision with acceptable duplicate precision
6. Be signed off by the PI or lab manager

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L407

Total Carbon – Shimadzu TOC-L with SSM-5000A

Standard Operating Procedure

SOP Number	SOP-L407
Revision	1.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes the operation of the Shimadzu TOC-L total organic carbon analyzer and the SSM-5000A Solid Sample Module for quantifying dissolved organic carbon (DOC) in soil extracts and total carbon (TC) or total organic carbon (TOC) in solid soil samples (Shimadzu Corporation, 2016, 2019).

2. Scope

- **Applies to:** K_2SO_4 extracts from the chloroform fumigation-extraction method (SOP-L409); water samples; and dried, ground solid soil samples.
- **Does not apply to:** Samples with extremely high salt concentrations that exceed the instrument's tolerance (consult the instrument manual); sea water or marine sediments with high salt content.
- **Location:** EA Program analytical laboratory, SGM 133.

3. Responsibilities

Role	Responsibility
Lab PI	Approve protocol, authorize instrument access
Instrument Manager	Maintain instrument, replace catalyst and consumables
Trained Personnel	Operate instrument, prepare standards and samples
New Students	Complete training before independent use

4. Definitions

- **Calibration verification standard (CVS):** A known-concentration standard analyzed at regular intervals during a run to confirm that instrument response has not drifted since initial calibration; acceptance criterion is within 10% of the certified value (Shimadzu Corporation, 2019).
- **Dissolved organic carbon (DOC):** Organic carbon in a liquid sample (water or soil extract) that passes through a 0.45 µm filter; quantified by the NPOC method on the Shimadzu TOC-L.
- **Inorganic carbon (IC):** Carbon present as carbonate (CO_3^{2-}), bicarbonate (HCO_3^-), and dissolved CO_2; removed from liquid samples by acidification and sparging; measured directly in soils at higher temperatures on the SSM-5000A.
- **NDIR detector:** Non-dispersive infrared detector; measures CO_2 evolved from sample combustion by absorption at 4.26 µm; the primary detection element of the TOC-L and SSM-5000A (Shimadzu Corporation, 2019).
- **NPOC (non-purgeable organic carbon):** The organic carbon fraction remaining after acidification and sparging to remove IC and volatile organic carbon; the operationally defined DOC fraction measured by the TOC-L for liquid samples.

- **SSM-5000A:** The Shimadzu Solid Sample Module that accepts dried, ground solid samples loaded in ceramic boats and combusts them at up to 900 °C to determine total carbon directly (Shimadzu Corporation, 2016).
- **TC (total carbon):** Sum of all carbon forms (organic and inorganic) in a sample; measured by direct high-temperature combustion of solid samples or by TC mode for liquid samples (Nelson & Sommers, 1996).
- **TOC (total organic carbon):** TC minus IC; in liquid samples, determined as NPOC; in solid soil samples, determined as TC minus IC or after acid treatment to remove carbonates before combustion (Nelson & Sommers, 1996).

5. Safety and Hazards

Hazards

- **Chemical:** Hydrochloric acid (HCl) is used to acidify liquid samples for IC removal; handle in a fume hood with appropriate PPE.
- **Thermal:** The combustion furnace operates at 680 °C (liquid mode) or up to 900 °C (SSM mode); do not contact the furnace chamber.
- **Pressurized gas:** Carrier gas (high-purity air or O_2) is supplied under pressure; inspect fittings for leaks.

5.1. Required PPE

- ☐ Safety glasses
- ☐ Laboratory coat
- ☐ Nitrile gloves

5.2. Emergency Procedures

Refer to SOP-L002 Laboratory Safety and the Shimadzu TOC-L instrument manual for instrument-specific emergency shutdowns.

6. Equipment and Materials

6.1. Equipment

Item	Notes
Shimadzu TOC-L	With ASI-L autosampler; SGM 133
SSM-5000A	Solid Sample Module; SGM 133
Analytical balance	0.01 mg resolution for solid sample weighing
0.45 µm syringe filters	For liquid sample pre-filtration
Autosampler vials	Acid-washed glass or certified TOC-clean

6.2. Reagents and Consumables

Item	Notes
Potassium hydrogen phthalate (KHP)	Primary standard for C calibration
Sodium bicarbonate / carbonate	IC calibration standards
2 M HCl	For carbonate removal from solid samples
High-purity air or O_2	Carrier gas for SSM-5000A combustion
Pre-combusted ceramic boats	900 °C prior to use; one per sample
0.5 M K_2SO_4	Method blank for extract analyses

7. Procedure

7.1. Startup

1. Verify carrier gas supply pressure (typically 200 kPa or per instrument manual (Shimadzu Corporation, 2019)).
2. Turn on the TOC-L main unit and allow the furnace to reach operating temperature (680 °C for liquid mode).
3. Launch LabSolutions TOC software on the connected computer.
4. Run a system blank check using deionized water to verify baseline stability; the blank value should be below 0.1 mg/L C.

7.2. Calibration

1. Prepare a multi-point calibration curve using KHP dissolved in deionized water.
2. Typical calibration range: 0, 1, 5, 10, 25, 50, 100 mg/L C (adjust to expected sample range).
3. For IC calibration (if measuring TC - IC = TOC), prepare sodium bicarbonate/carbonate standards.
4. Run calibration standards and verify that the correlation coefficient (r^2) is at least 0.999 (Shimadzu Corporation, 2019).
5. Save the calibration curve in the software.

7.3. Liquid Sample Analysis (DOC in Soil Extracts)

1. Filter all liquid samples through 0.45 µm syringe filters or pre-combusted glass fiber filters into clean autosampler vials.
2. For K_2SO_4 extracts, include 0.5 M K_2SO_4 blanks in the run.
3. Select the appropriate measurement mode in the software:
   - **NPOC mode** (recommended for most soil extracts): The instrument acidifies the sample with HCl, sparges with carrier gas to remove IC as CO_2, then combusts the remaining sample at 680 °C. The evolved CO_2 is measured by the NDIR detector.
   - **TC-IC mode** (if both total and inorganic carbon are needed): TC is measured by direct combustion; IC is measured by acidification only. TOC = TC - IC.

4. Load vials into the autosampler and start the run.
5. The instrument performs replicate injections (typically 3) and reports the mean and coefficient of variation.

> **High-Salt Extracts**
>
> If K_2SO_4 extracts have high salt content, use the instrument's automatic dilution function to extend catalyst and combustion tube life. Dilution factors are applied automatically to the reported concentrations (Shimadzu Corporation, 2019).

7.4. Solid Sample Analysis (SSM-5000A)

1. Switch the instrument to SSM mode in the LabSolutions software (Shimadzu Corporation, 2016).
2. Pre-combust ceramic boats at 900 °C to remove any residual carbon.
3. Weigh 10–50 mg of dried, finely ground soil into a clean ceramic boat using the analytical balance (record mass to 0.01 mg).
4. For TOC measurement (inorganic carbon removal): pre-treat the sample by adding a few drops of 2 M HCl to the boat, allow to react until effervescence stops, and dry at 105 °C before analysis (Nelson & Sommers, 1996).
5. For TC measurement (no acid pre-treatment): proceed directly.
6. Place the ceramic boat into the SSM sample port.
7. The SSM combusts the sample in an oxygen-rich environment at high temperature; the evolved CO_2 is carried to the NDIR detector.
8. The software reports total carbon content in mg C or as a percentage of sample mass.

7.5. Instrument Shutdown

1. After the final sample, run 3–5 deionized water blanks to flush the system.
2. Review all data in the software for outliers or failed injections.
3. Export data and save the run file.
4. If the instrument will not be used for several days, turn off the furnace and carrier gas per the instrument manual (Shimadzu Corporation, 2019).

8. Quality Control

- Run a calibration verification standard (mid-range) every 10–15 samples; acceptance criterion is within 10% of the known value.
- Include instrument blanks (deionized water) at the beginning, middle, and end of each run.
- Include method blanks (0.5 M K_2SO_4 for extract analyses) in each batch.
- Run sample duplicates at a rate of at least 1 per 10 samples.
- Monitor the coefficient of variation among replicate injections; re-run if CV exceeds 2% for liquid samples.
- Log catalyst replacement, combustion tube changes, and calibration dates in the instrument logbook (Shimadzu Corporation, 2019).

9. Maintenance

Task	Frequency
System blank check	Before each analytical run
Calibration verification	Each run (mid-range check standard)
Combustion tube inspection	Monthly or after approximately 500 injections
Catalyst replacement	As needed (when blank values increase or sensitivity decreases)
Drain trap and humidifier check	Weekly
Carrier gas cylinder replacement	As needed (monitor pressure gauge)

10. Waste Disposal

Waste Type	Disposal Method
Acidified liquid waste	Neutralize and dispose per EH&S guidelines
Spent catalyst	Collect for EH&S hazardous waste pickup
Used ceramic boats	Clean and reuse, or dispose in standard trash
K_2SO_4 extract waste	Aqueous waste; drain if approved, or collect

11. References

- Shimadzu Corporation (2019)
- Shimadzu Corporation (2016)
- Nelson & Sommers (1996)
- SOP-L002 Laboratory Safety
- SOP-L204 Shimadzu TOC-L (Instrument)
- SOP-L409 Microbial Biomass Carbon

Nelson, D. W., & Sommers, L. E. (1996). Total carbon, organic carbon, and organic matter. In D. L. Sparks (Ed.), *Methods of soil analysis, part 3: Chemical methods* (pp. 961–1010). Soil Science Society of America. https://doi.org/10.2136/sssabookser5.3.c34

Shimadzu Corporation. (2016). *SSM-5000A solid sample combustion unit: User manual.*

Shimadzu Corporation. (2019). *TOC-L series total organic carbon analyzer: User manual.*

12. Related SOPs

SOP	Title	Relationship
L002	Laboratory Safety	General safety framework
L204	Shimadzu TOC-L Instrument	Instrument startup/shutdown SOP

SOP	Title	Relationship
L406	Loss on Ignition	Complementary SOM method
L409	Microbial Biomass C	Source of K_2SO_4 extracts
L418	Water-Extractable DOC	Water extraction extracts

13. Training Record

Before operating this instrument independently, personnel must:

1. Read this SOP and the relevant sections of the Shimadzu TOC-L and SSM-5000A user manuals (Shimadzu Corporation, 2016, 2019)
2. Complete SOP-L003 Hazardous Materials Handling training
3. Observe a trained operator perform startup, calibration, sample analysis, and shutdown
4. Demonstrate competence with calibration standard preparation
5. Perform a supervised analytical run with acceptable QC results
6. Be signed off by the instrument manager or PI

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L408

Soil Respiration – Solvita CO_2 Burst Test

Standard Operating Procedure

SOP Number	SOP-L408
Revision	1.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes the measurement of soil microbial respiration using the Solvita CO_2 burst test. The method quantifies CO_2 evolution from rewetted, air-dried soil as an index of microbial activity and labile carbon availability (Haney et al., 2008a; Haney et al., 2008b).

The CO_2 burst exploits the "Birch effect," the well-documented pulse of microbial respiration that occurs when dry soil is rapidly rewetted (Birch, 1958; Fierer & Schimel, 2003). Drying and rewetting disrupts soil aggregates, lyses a fraction of the microbial biomass, and releases intracellular osmolytes, all of which provide readily mineralizable substrate that surviving microbes respire as CO_2 (Fierer & Schimel, 2002; Xiang et al., 2008). The magnitude of the burst integrates microbial biomass size, community physiological status, and the pool of labile organic carbon, making it a useful single-number indicator of biological soil fertility (Haney et al., 2008b).

2. Scope

- **Applies to:** Air-dried, sieved soil samples collected per SOP-F400 for microbial activity assessment
- **Does not apply to:** Compost maturity testing (see SOP-L413); field-moist basal respiration; long-term (7–28 day) incubation-based respirometry; or chamber-based field CO_2 flux measurements (see SOP-F406)
- **Location:** EA Program laboratory, SGM 133

3. Responsibilities

Role	Responsibility
Lab PI	Approve protocol, ensure training compliance
Lab Manager	Maintain Solvita kit inventory, verify probe expiration dates
Trained Personnel	Perform assay, record and report results
New Students	Complete training before independent use

4. Definitions

- **Basal respiration:** The rate of CO_2 production from soil under controlled, standardized conditions (specific moisture, temperature, incubation time) without additional substrate; reflects the size and activity of the soil microbial community.
- **CO_2 burst:** The pulse of CO_2 released from dry soil immediately upon rewetting; caused by osmotic stress on microorganisms, physical disruption of aggregates, and rapid oxidation of previously protected organic matter; the basis of the Solvita CO_2 Burst test.
- **Colorimetric gel pad:** The CO_2-sensitive indicator element in the Solvita kit; changes color in proportion to the CO_2 concentration in the jar headspace; read with a Solvita paddle reader or digitally.

- **Digital Color Reader (DCR):** The Solvita handheld device that reads the color of the gel pad and converts it to a numerical Solvita Index value.
- **Solvita Index:** A unitless scale (1–8) reported by the Solvita CO_2 Burst test; higher values indicate greater biological activity and organic matter availability; values correlate with potentially mineralizable nitrogen.
- **Soil microbial biomass:** The mass of living microbial cells (bacteria, fungi, protozoa) in soil; the primary driver of organic matter decomposition and nutrient cycling; estimated indirectly by CO_2 respiration or directly by fumigation-extraction (see SOP-L409).
- **Water-holding capacity (WHC):** The maximum amount of water a soil can hold after free drainage; expressed as a percentage of dry soil mass; the Solvita protocol adjusts sample moisture to a defined fraction of WHC for standardization.

5. Safety and Hazards

Hazards

- **Physical:** Minor risk of glass breakage from incubation jars. Handle jars carefully; clean up broken glass with a brush and dustpan, not bare hands.
- **Biological:** Soil samples may contain soil-borne pathogens. Wash hands thoroughly after handling soil. Do not touch your face during the procedure.

5.1. Required PPE

- ☐ Nitrile gloves
- ☐ Safety glasses
- ☐ Lab coat

6. Equipment and Materials

Item	Notes
Solvita CO_2 burst test kit	Should include enough probes for all samples, duplicates and controls.
Solvita Digital Color Reader (DCR)	For quantifying gel color change; calibrate before each batch
Incubation jars (475 mL)	Glass jars with screw-top lids
30 cc soil scoop	Provided with kit
Small beakers	Can be plastic or glass. Must fit inside incubation jar
Analytical balance	Capable of 0.1 g resolution
10ml pipette + pipette pump	For adding deionized water
Deionized water	From Milli-Q system or equivalent
Incubator or controlled room	Set to 25 +/- 1 C
Timer	24-hour countdown
Lab notebook or data sheet	For recording results

7. Procedure

7.1. Sample Preparation

1. Use soil that has been collected per SOP-F400.
2. If samples were stored at 4 C after sieving, allow them to equilibrate to room temperature (20–25 C) for at least 30 minutes before beginning the assay.
3. Sieve soils through a 2mm sieve. Use a mortar and pestle to break up soil clumps that did not pass through the sieve initially and resieve it. Discard any rocks or other materials that do not pass through the sieve.

> **Note**
>
> The CO_2 burst test is designed for air-dried soil. Drying standardizes the starting moisture across samples and maximizes the rewetting response. Results from field-moist soil are not comparable to published Solvita norms (Woods End Laboratories, 2019).

7.2. Incubation Setup

1. Measure 30ccs of the dried and sieved soils using a 30cc volume scoop and add it to a small beaker. Weight the soil (do not include weight of beaker) and record the exact mass to 0.1 g. Shake it gently to level the surface.
2. Gently sprinkle 9ccs of deionized water onto the surface of the soil using a pipette. The rewetting triggers the microbial CO_2 burst (Fierer & Schimel, 2003).
3. Remove a fresh CO_2-sensitive gel probe from its sealed foil packet. Inspect the gel: it should be attached to the probe, uniform in color with no cracks, discoloration, or signs of prior exposure. Discard compromised probes. Make sure not to touch the gel while handling the probe.
4. Insert the probe into the jar so that the gel face is exposed to the headspace above the soil surface. Do not bury the probe in soil.
5. Seal the jar immediately and securely.
6. Record the jar number, sample ID, soil mass, water volume added, and start time.

7.3. Incubation

1. Place sealed jars in the incubator set to 25 +/- 1 C
2. Incubate undisturbed for 24 hours
3. Do not open or move jars during incubation

7.4. Reading Results

1. After exactly 24 hours (+/- 15 minutes), remove the gel probe from the jar
2. Immediately insert the probe into the Solvita Digital Color Reader (DCR) and record the numerical reading

3. Record the color number and the corresponding CO_2-C concentration (mg CO_2-C kg^{-1} soil) from the DCR or the Solvita conversion table

> **Tip**
>
> Read probes promptly after removing them from the jar. Prolonged exposure to ambient air will alter the gel color and compromise the reading. The manufacturer recommends reading within 2 minutes of opening the jar.

7.5. Calculating Results

Express results on a dry-soil basis:

$$\text{CO}_2\text{-C (mg kg}^{-1}) = \frac{\text{DCR reading (mg CO}_2\text{-C)}}{\text{oven-dry soil mass (kg)}}$$

If the oven-dry mass is not known, calculate it from the air-dried mass and the gravimetric moisture content of a companion subsample determined by SOP-L400.

7.6. Cleanup

1. Dispose of used soil in the designated waste container (return to field site if uncontaminated, or standard trash)
2. Wash incubation jars thoroughly with soap and water; rinse with deionized water; dry completely before reuse
3. Dispose of used gel probes in standard trash

8. Quality Control

8.1. Routine QC

QC Element	Requirement
Duplicates	At least 1 per 10 samples; duplicates should agree within 15% relative percent difference (RPD)
Control	One empty, sealed jar with gel probe per batch; probe should read at or near baseline
Incubator temperature	Verify and record at start and end of each 24 h period; must be 25 +/- 1 C
DCR calibration	Calibrate per manufacturer instructions before each batch
Gel probe storage	Store sealed in foil packets at room temperature, away from light; check expiration dates before use

8.2. Corrective Actions

- If the blank reading exceeds the baseline by more than one color increment, check for CO_2 contamination sources (e.g., proximity to gas burners, poor jar seals) and repeat the batch
- If duplicate RPD exceeds 15%, re-run the sample pair. Persistent poor precision may indicate sample heterogeneity; increase the number of subsamples

9. Data Interpretation

The CO_2 burst is proportional to microbial biomass and labile carbon and is correlated with potential carbon, nitrogen, and phosphorus mineralization (Haney et al., 2008b). Typical ranges for the Solvita CO_2 burst test on agricultural and wildland soils are given below, adapted from Haney et al. (2008a) and the Solvita technical literature (Woods End Laboratories, 2019):

CO_2-C (mg kg^{-1})	Interpretation	Implication
< 20	Very low	Severely depleted microbial activity; very low labile C
20–50	Low	Limited biological activity; management intervention may be needed
50–100	Moderate	Adequate microbial activity for many soils
100–150	High	Active microbial community; good labile C supply
> 150	Very high	Highly active soil; common in recently amended or high-organic soils

! Important

These thresholds are general guidelines. Interpret results in the context of soil type, land use, climate, and management history. Comparisons are most meaningful when made across treatments within a single study using the same sampling and analytical protocol.

10. Waste Disposal

Waste Type	Disposal Method
Used soil	Return to field site or standard trash
Used gel probes	Standard trash
Deionized water rinse	Drain
Incubation jars	Wash, rinse, and reuse

11. References

Birch, H. F. (1958). The effect of soil drying on humus decomposition and nitrogen availability. *Plant and Soil*, *10*(1), 9–31. https://doi.org/10.1007/BF01343734

Fierer, N., & Schimel, J. P. (2002). Effects of drying-rewetting frequency on soil carbon and nitrogen transformations. *Soil Biology and Biochemistry*, *34*(6), 777–787. https://doi.org/10.1016/S0038-0717(02)00007-X

Fierer, N., & Schimel, J. P. (2003). A proposed mechanism for the pulse in carbon dioxide production commonly observed following the rapid rewetting of a dry soil. *Soil Science Society of America Journal*, *67*(3), 798–805. https://doi.org/10.2136/sssaj2003.7980

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12. Related SOPs

SOP	Title	Relationship
F400	Soil Sampling and Processing	Sample collection and preparation
F406	Soil Respiration Chambers	Field respiration complement
L002	Laboratory Safety	Required safety training
L400	Soil Moisture (Gravimetric)	Moisture correction for dry-mass basis
L406	Loss on Ignition (SOM)	Complementary organic matter index
L409	Microbial Biomass Carbon (CFE)	Direct biomass measurement
L413	Solvita Compost Maturity	Related Solvita protocol for compost

13. Training Record

Before performing this assay independently, personnel must:

1. Read this SOP in full
2. Complete SOP-L002 Laboratory Safety training

3. Complete SOP-F400 Soil Sampling training (for sample preparation context)
4. Review the Solvita CO_2 Burst protocol sheet included in the kit
5. Observe a trained operator perform the full procedure (setup through DCR reading)
6. Perform the assay under supervision with acceptable QC results (duplicate RPD within 15%; blank within acceptable range)
7. Be signed off by the PI or lab manager

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L409

Microbial Biomass Carbon – Chloroform Fumigation-Extraction

Standard Operating Procedure

SOP Number	SOP-L409
Revision	1.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes the determination of soil microbial biomass carbon (MBC) using the chloroform fumigation-extraction (CFE) method. Chloroform vapor lyses microbial cells, releasing intracellular carbon that is then extracted with 0.5 M K_2SO_4 and quantified as dissolved organic carbon (DOC) (Joergensen, 1996; Vance et al., 1987).

2. Scope

- **Applies to:** Field-moist soil samples collected per SOP-F400 Soil Sampling and Processing for microbial biomass assessment.
- **Does not apply to:** Air-dried or frozen soils (unless pre-validated for this method); microbial biomass nitrogen (requires additional analytical steps).
- **Location:** EA Program laboratory, SGM 133.

3. Responsibilities

Role	Responsibility
Lab PI	Approve protocol, ensure safety compliance
Trained Personnel	Perform fumigation and extraction, report results
New Students	Complete training and safety orientation before use

4. Definitions

- **Chloroform fumigation-extraction (CFE):** The standard method for measuring soil microbial biomass carbon; soil is fumigated with ethanol-free chloroform to lyse microbial cells, then extracted with K_2SO_4; MBC is calculated as the difference in extractable C between fumigated and unfumigated soils divided by the k_{EC} factor (Vance et al., 1987).
- **Extractable organic carbon (EOC):** The organic carbon recovered from soil in K_2SO_4 extract; includes a background pool of soluble organic carbon from non-microbial sources as well as carbon released from lysed microbial cells.
- k_{EC} **factor:** The extraction efficiency correction factor for microbial biomass carbon; commonly 0.45, meaning 45% of lysed microbial carbon is recovered in the extract; must be stated when reporting results (Joergensen, 1996; Vance et al., 1987).
- **Microbial biomass carbon (MBC):** The total carbon contained in the living soil microbial community (bacteria, fungi, protozoa, nematodes); expressed as mg C kg^{-1} oven-dry soil; a key indicator of soil biological health and nutrient cycling potential (Robertson et al., 1999).
- **Potentially mineralizable nitrogen (PMN):** The nitrogen that can be released from soil organic matter by microbial activity under aerobic conditions; correlated with microbial biomass and organic matter quality.
- **Unfumigated control:** Soil extracted with K_2SO_4 without prior chloroform fumigation; provides the background extractable carbon value subtracted from the fumigated extract to calculate MBC (Vance et al., 1987).

5. Safety and Hazards

5.1. Critical Safety Warning

Chloroform ($CHCl_3$) is a volatile, toxic solvent and a suspected carcinogen. All fumigation steps must be performed inside a fume hood. Do not inhale chloroform vapors. Use ethanol-free chloroform only (Food and Agriculture Organization of the United Nations, 2024).

5.2. Hazards

- **Chemical:** Chloroform (toxic, volatile, suspected carcinogen); 0.5 M K_2SO_4 (mild irritant).
- **Physical:** Vacuum desiccator under reduced pressure; risk of implosion if cracked.

5.3. Required PPE

☐ Nitrile gloves (double-gloving recommended when handling chloroform)
☐ Safety glasses or goggles
☐ Laboratory coat
☐ Work inside a fume hood at all times during fumigation

5.4. Emergency Procedures

In case of chloroform spill, refer to the SDS and SOP-L003 Hazardous Materials Handling. Evacuate the area if the spill is large; ventilate the space and notify the supervisor.

6. Equipment and Materials

6.1. Equipment

Item	Notes
Vacuum desiccator	Glass, with stopcock and vacuum line
Vacuum pump	Oil-free preferred
Fume hood	Required for all chloroform handling
Orbital or end-over-end shaker	For extraction step
Centrifuge	Capable of 4,000 × g (optional)
Filtration apparatus	With 0.45 µm glass fiber filters
Analytical balance	0.01 g resolution
Incubator or dark cabinet	For 24-hour fumigation at room temperature
TOC analyzer	Shimadzu TOC-L; see SOP-L407

6.2. Reagents and Consumables

Item	Notes
Ethanol-free chloroform	ACS grade; stored in fume hood
0.5 M K_2SO_4 solution	Prepare fresh or within 1 week
Glass fiber filters (0.45 µm)	Pre-combusted at 450 °C for 4 hours
Boiling chips or glass beads	For chloroform evaporation under vacuum
Extraction bottles (100 mL)	Screw-top, HDPE or glass
Filter funnels and flasks	Clean, acid-washed
Sample vials	Acid-washed, for filtered extracts

7. Procedure

7.1. Preparation

1. Prepare 0.5 M K_2SO_4 by dissolving 87.1 g K_2SO_4 per liter of deionized water. The 1:4 soil-to-extractant ratio is standard for CFE (Vance et al., 1987).
2. Pre-combust glass fiber filters at 450 °C for 4 hours and store in a clean container.
3. Determine the gravimetric moisture content of a separate subsample (dry at 105 °C for 24 hours per SOP-L400) so results can be expressed on an oven-dry-weight basis.

7.2. Fumigation

1. Weigh two paired subsamples of 15 g field-moist soil into labeled extraction bottles.
2. Place one set (fumigated) into the vacuum desiccator inside the fume hood.
3. Place a beaker containing approximately 30 mL of ethanol-free chloroform and a few boiling chips in the center of the desiccator.
4. Evacuate the desiccator using the vacuum pump until the chloroform begins to boil vigorously (approximately 2 minutes) (Vance et al., 1987).
5. Close the stopcock and incubate in the dark at room temperature (approximately 25 °C) for 24 hours (Food and Agriculture Organization of the United Nations, 2024; Vance et al., 1987).
6. The second set (unfumigated controls) is extracted immediately (proceed to §6.3).

7.3. Extraction

1. After 24 hours, release the vacuum inside the fume hood and remove the chloroform beaker.
2. Evacuate the desiccator repeatedly (3–5 times) to remove residual chloroform vapor from the soil.
3. Add 60 mL of 0.5 M K_2SO_4 to each bottle (both fumigated and unfumigated), achieving a 1:4 soil-to-extractant ratio (Vance et al., 1987).
4. Cap bottles and shake on an orbital or end-over-end shaker for 1 hour.
5. Allow the suspension to settle briefly, or centrifuge at 4,000 × g for 10 minutes.
6. Filter the supernatant through a pre-combusted 0.45 µm glass fiber filter into a clean sample vial.
7. Store filtered extracts at 4 °C if not analyzed immediately; analyze within 48 hours.

7.4. DOC Analysis

1. Analyze filtered extracts for dissolved organic carbon using the Shimadzu TOC-L per SOP-L407 Total Carbon.
2. Run 0.5 M K_2SO_4 blanks alongside samples.

7.5. Calculation

Microbial biomass carbon is calculated as (Joergensen, 1996; Vance et al., 1987):

$$\text{MBC} = \frac{C_{\text{fumigated}} - C_{\text{unfumigated}}}{k_{EC}}$$

where:

- $C_{\text{fumigated}}$ = DOC in the fumigated extract (mg C kg^{-1} oven-dry soil)
- $C_{\text{unfumigated}}$ = DOC in the unfumigated extract (mg C kg^{-1} oven-dry soil)
- $k_{EC} = 0.45$, the extraction efficiency coefficient (Joergensen, 1996; Vance et al., 1987)

> **Note**
>
> The unfumigated extract DOC value represents the background dissolved organic carbon pool and is often reported separately as an indicator of labile soil carbon (Robertson et al., 1999).

8. Quality Control

- Run analytical triplicates for at least 10% of samples; the coefficient of variation among replicates should be less than 15% (Food and Agriculture Organization of the United Nations, 2024).
- Include a reagent blank (0.5 M K_2SO_4 only, no soil) in each batch.
- Include a laboratory reference soil in each batch to monitor method consistency.
- Verify that the TOC calibration $r^2 \geq 0.999$ before reporting results.

9. Waste Disposal

Waste Type	Container	Disposal Method
Chloroform waste	Halogenated waste jug	EH&S hazardous waste pickup
K_2SO_4 extracts	Aqueous waste container	Drain with copious water (if approved) or collect for EH&S
Used soil	Solid waste bag	Standard trash
Used glass fiber filters	Standard trash	Standard trash

10. References

- Vance et al. (1987)
- Joergensen (1996)
- Food and Agriculture Organization of the United Nations (2024)
- Robertson et al. (1999)
- SOP-L003 Hazardous Materials Handling
- SOP-L400 Soil Moisture
- SOP-L407 Total Carbon

Food and Agriculture Organization of the United Nations. (2024). *Standard operating procedure for soil microbial biomass (Carbon): Chloroform fumigation-extraction method*. FAO. https://www.fao.org/soils-portal/soil-survey/soil-analysis/en/

Joergensen, R. G. (1996). The fumigation-extraction method to estimate soil microbial biomass: Calibration of the k_C value. *Soil Biology and Biochemistry*, *28*(1), 25–31. https://doi.org/10.1016/0038-0717(95)00102-6

Robertson, G. P., Coleman, D. C., Bledsoe, C. S., & Sollins, P. (1999). *Standard soil methods for long-term ecological research*. Oxford University Press.

Vance, E. D., Brookes, P. C., & Jenkinson, D. S. (1987). An extraction method for measuring soil microbial biomass C. *Soil Biology and Biochemistry*, *19*(6), 703–707. https://doi.org/10.1016/0038-0717(87)90052-6

11. Related SOPs

SOP	Title	Relationship
L003	Hazardous Materials	Chloroform handling
L400	Soil Moisture	Required companion analysis
L407	Total Carbon	DOC analysis of extracts
L408	Solvita CO_2 Burst	Complementary respiration index

12. Training Record

Before performing this procedure independently, personnel must:

1. Read this SOP and SOP-L003 Hazardous Materials Handling in full
2. Review the SDS for chloroform
3. Observe a trained operator perform the full fumigation-extraction procedure
4. Demonstrate competence with vacuum desiccator operation
5. Perform the procedure under supervision
6. Be signed off by the PI or lab manager

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L410

Hot-Plate Acid Digestion of Soils for Trace Metal Analysis

Standard Operating Procedure

SOP Number	SOP-L410
Revision	0.2
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the open-vessel, hot-plate acid digestion of soil, sediment, and sludge samples for near-total extraction of lead (Pb) and other trace metals, following US EPA Method 3050B (U.S. Environmental Protection Agency, 1996). The digested extract can be analyzed by ICP-OES, ICP-MS, or flame/ graphite furnace AAS. This is a near-total-dissolution method; silicates and some refractory oxides are not fully dissolved, but environmentally available metals are recovered.

For microwave-assisted digestion using the MARS 5 system, see SOP-L411. The microwave method is faster but processes fewer samples per batch; the hot-plate method described here is slower but requires only standard fume-hood equipment.

2. Scope

- **Applies to:** Soils, sediments, and sludge samples requiring trace metal analysis (Pb, Cd, Cu, Zn, Cr, As, Ni, Fe, Mn, and others), including urban soil lead surveys and photovoltaic panel site characterizations
- **Does not apply to:** Water samples (direct acidification or separate digestion); samples requiring total silicate dissolution (HF-based methods); microwave digestion (see SOP-L411)
- **Location:** EA Program laboratory, SGM 133

3. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, ensure training compliance
Lab Manager	Maintain equipment, stock reagents
Trained Personnel	Perform digestion per this SOP
New Students	Complete training before independent use; supervised for first digestion

4. Definitions

- **Aqua regia:** A 3:1 (v/v) mixture of HCl and HNO_3; dissolves noble metals (gold, platinum) and many sulfide minerals not attacked by HNO_3 alone; more aggressive than single-acid digestion but rarely necessary for soil and sediment.

- **Certified reference material (CRM):** A soil or sediment with certified elemental concentrations from an accredited producer (NIST, USGS); run alongside every digestion batch to verify accuracy.
- **Digestion blank (method blank):** A vessel containing only acid reagents and no soil, processed through the entire digestion procedure; detects contamination from acids, vessels, or the hot plate environment.
- **EPA Method 3050B:** The US EPA hot-plate acid digestion procedure for soils, sediments, and sludges; uses repeated refluxes with HNO_3 and H_2O_2 to dissolve environmentally available trace metals; the reference method for this SOP.
- **Matrix spike:** A sample to which a known concentration of analyte has been added before digestion; used to assess matrix-specific recovery and method accuracy.
- **Partial digestion:** Dissolution of the environmentally available or acid-soluble metal fraction of a sample (EPA 3050B); does not dissolve silicate minerals; appropriate for assessing bioavailability and regulatory compliance; distinguished from total digestion (which requires HF).
- **Reflux:** Heating an acid-sample mixture such that acid vapors condense and return to the vessel; increases digestion efficiency for resistant matrices.

5. Safety and Hazards

5.1. Critical Safety Warning

This procedure uses concentrated mineral acids (HNO_3, HCl) and hydrogen peroxide (H_2O_2) at elevated temperatures in open vessels. All acid handling and digestion must be performed in a certified chemical fume hood.

5.2. Hazards

- **Chemical:** Concentrated HNO_3 (15.8 M) is a severe oxidizer and corrosive. Concentrated HCl (12 M) is severely corrosive. 30% H_2O_2 is a reactive oxidizer that can cause exothermic reactions with organic matter.
- **Physical:** Hot plate operates at approximately 95 C; digestion vessels and watch glasses are hot. Burns from contact with heated glassware or splashing acid.
- **Inhalation:** HNO_3 digestion produces toxic NO_x fumes. HCl produces irritating HCl vapor. All heating must occur in the fume hood.

5.3. Required PPE

☐ Chemical splash goggles or face shield
☐ Lab coat
☐ Acid-resistant gloves (neoprene or heavy-duty nitrile, long-cuffed)
☐ Closed-toed shoes

5.4. Emergency Procedures

For acid splashes to skin or eyes, flush immediately at the eyewash station or safety shower for at least 15 minutes. For inhalation of fumes, move to fresh air. For acid spills, neutralize small spills with sodium bicarbonate; for large spills, evacuate and use the spill kit. Refer to SOP-L003 Hazardous Materials Handling.

> **!** Important
>
> Never add water to concentrated acid. Add acid slowly to samples. Never allow digestion vessels to go to dryness; this can volatilize analytes and damage vessels.

6. Equipment and Materials

6.1. Equipment

Item	Notes
Hot plate with temperature control	Capable of maintaining 95 C; placed inside fume hood
Digestion vessels	Teflon (PFA) beakers, glass beakers, or borosilicate digestion tubes
Watch glasses	Sized to cover the digestion vessels
Analytical balance (0.001 g)	See SOP-L102
Fume hood	Required for all steps involving acid and heating
Volumetric flasks (50 mL, Class A)	For final dilution
Graduated pipettes or dispensers	For acid delivery
Filter funnels and Whatman No. 41 filter paper	For clarifying digests
Tongs or heat-resistant clamps	For handling hot vessels

6.2. Reagents

Reagent	Grade / Notes
Nitric acid (HNO_3), concentrated (15.8 M)	Trace Metal grade
Hydrochloric acid (HCl), concentrated (12 M)	Trace Metal grade
Hydrogen peroxide (H_2O_2), approximately 30%	ACS reagent grade
Deionized water (Type I)	From *SOP-L11*

> **Note**
>
> All reagents must be Trace Metal grade or equivalent. Analyze a reagent blank before each batch; the blank must read below the method detection limit (MDL) for all target analytes.

7. Procedure

7.1. Sample Preparation

1. Air-dry the soil at room temperature or at 60 C until constant mass
2. Pass through a No. 10 sieve (2 mm) per SOP-F400 Soil Sampling
3. Homogenize thoroughly using a mortar and pestle
4. Determine gravimetric moisture on a companion subsample per SOP-L400

7.2. Glassware Cleaning

Acid-wash all vessels, volumetric flasks, and watch glasses per SOP-L101 Glassware Cleaning, Section 6.3 (10% HNO_3 acid bath). Rinse with reagent water and dry in a clean environment.

7.3. Weighing

1. Weigh 0.50–1.00 g of prepared soil into a clean digestion vessel
2. Record the exact mass to the nearest 0.001 g in the lab notebook

7.4. Initial Nitric Acid Digestion

1. Working in the fume hood, add approximately 10 mL of concentrated HNO_3 to the vessel
2. Cover with a watch glass
3. Place on the hot plate set to approximately 95 C. Do not allow the solution to boil.
4. Reflux gently until the initial vigorous reaction subsides and the volume begins to reduce. Do not allow the sample to go to dryness.
5. Continue heating for approximately 30–60 minutes, periodically lifting the watch glass to rinse the underside with DI water back into the vessel

7.5. Oxidation with H_2O_2

1. Remove the vessel from the hot plate and allow to cool slightly
2. Add 1–2 mL of 30% H_2O_2 dropwise. Wait for effervescence to subside between drops.
3. Return to the hot plate and resume heating at 95 C
4. Repeat the H_2O_2 additions until the reaction is minimal (typically 2–4 additions) and the digest appears light-colored

> ⚠ Warning
>
> Add H_2O_2 very slowly. The reaction with organic matter is exothermic and can cause violent spattering if added too quickly, especially to hot solutions. Always cool the vessel first.

7.6. HCl Treatment

1. Add approximately 5 mL of concentrated HCl to the digest
2. Cover and heat at approximately 95 C for 15–30 minutes
3. This step dissolves residual metal precipitates and improves recovery of metals such as Fe, Al, and Pb

7.7. Volume Reduction

1. Remove the watch glass and continue heating at approximately 95 C
2. Reduce the volume to approximately 5 mL
3. Monitor closely and do not allow the sample to go to dryness

> ❗ Important
>
> Evaporation to dryness will cause loss of volatile analytes (As, Se, Hg) and can bake residues onto the vessel walls, making quantitative recovery impossible.

7.8. Dilution and Filtration

1. Remove from the hot plate and allow to cool to room temperature
2. Rinse the vessel walls and watch glass with small portions of DI water
3. If undissolved material is present, filter through Whatman No. 41 filter paper into a clean 50 mL volumetric flask, rinsing the filter and vessel quantitatively
4. If the solution is clear, transfer directly to the volumetric flask
5. Bring to 50.0 mL with DI water
6. Mix thoroughly
7. Transfer to a clean, labeled polyethylene or polypropylene storage bottle. Store at 4 C until analysis.

8. Quality Control and Acceptance Criteria

8.1. QC Requirements

- **Method blank:** Include one vessel containing reagents only (no soil) in each digestion batch. The blank must read below the MDL for all target analytes.
- **Certified reference material (CRM):** Digest a CRM with known metal concentrations in each batch. Recovery: 80–120%.

- **Matrix spike:** Spike a duplicate sample with a known concentration of the target analyte(s). Recovery: 75–125%.
- **Duplicate digestions:** At least 10% of samples. Relative percent difference (RPD) less than 20%.
- **Calibration verification:** Verify with a mid-range check standard every 10 samples during instrumental analysis.

8.2. Calculations

Metal concentration in soil (mg/kg dry weight):

$$C_{\text{soil}} = \frac{C_{\text{digest}} \times V_{\text{final}}}{M_{\text{soil}}}$$

where C_{digest} = concentration in the digest (mg/L), V_{final} = final volume (mL, typically 50), and M_{soil} = mass of dried soil (g).

For soil dried at 60 C, apply a moisture correction to report on a 105 C oven-dry basis (see SOP-L400):

$$C_{\text{soil, OD}} = C_{\text{soil}} \times \frac{100 + \theta_{60}}{100 + \theta_{105}}$$

9. Waste Disposal

Waste Type	Disposal Method
Acid digest waste (all liquids from digestion)	Collect in labeled acid waste carboy; EH&S hazardous waste pickup
Used filter paper	Acid waste stream (contaminated with metals and acid)
Digested soil residue	Acid waste carboy
Spent H_2O_2	Small volumes may go into acid waste; large volumes, consult EH&S

Acid Waste

All waste from this procedure contains concentrated mineral acids and dissolved metals. **Do not pour down the drain.** Collect in a labeled polyethylene carboy for EH&S hazardous waste pickup.

10. References

- USEPA. (1996). Method 3050B: Acid digestion of sediments, sludges, and soils. Revision 2. In: *Test Methods for Evaluating Solid Waste, Physical/Chemical Methods* (SW-846). U.S. Environmental Protection Agency, Washington, DC.

- APHA, AWWA, WEF. (2012). *Standard Methods for Examination of Water and Wastewater*. 22nd ed. American Public Health Association (Eds.). Washington. 1360 pp.

U.S. Environmental Protection Agency. (1996). *Method 3050B: Acid digestion of sediments, sludges, and soils* (SW-846, Revision 2). U.S. Environmental Protection Agency. https://www.epa.gov/sites/production/files/2015-06/documents/epa-3050b.pdf

11. Training Record

Before performing hot-plate acid digestion independently, personnel must:

1. Read this SOP, SOP-L002 Laboratory Safety, and SOP-L003 Hazardous Materials Handling in full
2. Review the SDS for concentrated HNO_3, HCl, and 30% H_2O_2
3. Observe a trained operator perform a complete digestion cycle
4. Perform at least one complete digestion batch under direct supervision, including blank and CRM, with acceptable QC results
5. Be signed off by the PI or lab manager

12. Appendix A: Example Calculation

```
# Trace metal concentration from hot-plate digest
# EPA 3050B

C_digest_mg_L <- 1.24      # Pb concentration from ICP-MS (mg/L)
V_final_mL <- 50.0         # Final dilution volume (mL)
M_soil_g <- 0.752           # Mass of dried soil digested (g)

C_soil_mg_kg <- (C_digest_mg_L * V_final_mL) / M_soil_g

cat(sprintf("Digest concentration: %.3f mg/L Pb\n", C_digest_mg_L))
cat(sprintf("Soil mass:           %.3f g\n", M_soil_g))
cat(sprintf("Final volume:        %.1f mL\n", V_final_mL))
cat(sprintf("Soil Pb:             %.1f mg/kg\n", C_soil_mg_kg))

# Screening levels
cat(sprintf("\nCA DTSC residential screening level: 80 mg/kg\n"))
cat(sprintf("EPA RSL residential: 400 mg/kg\n"))
```

13. Appendix B: Method Comparison – Hot Plate vs. Microwave

Feature	Hot Plate (EPA 3050B, this SOP)	Microwave (EPA 3051A, SOP-L411)
Vessel type	Open (reflux under watch glass)	Sealed (pressurized)
Temperature	Approximately 95 C	175 C
Digestion time	2–4 hours (hands-on)	10 minutes (automated)
Sample throughput	Limited by hot plate space	Up to 24 vessels per run
Equipment required	Hot plate + fume hood	MARS 5 microwave system
Acid volume per sample	Approximately 15 mL total	10 mL
Organic matter handling	H_2O_2 oxidation step	Sealed vessel; pre-digest if vigorous
Metal recovery	Comparable for most elements	Comparable; slightly higher for volatile elements due to sealed system
Best for	Small batches; no access to microwave	Routine batches; time-sensitive work

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L411

Microwave-Assisted Acid Digestion of Soils for Trace Metal Analysis

Standard Operating Procedure

SOP Number	SOP-L411
Revision	0.2
Author(s)	Marc Los Huertos, Isaac Medina
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the microwave-assisted acid digestion of soil, sediment, and sludge samples for trace metal analysis, following US EPA Method 3051A (U.S. Environmental Protection Agency, 2007). The digested extract can be analyzed by flame atomic absorption spectrometry (FAAS), inductively coupled plasma mass spectrometry (ICP-MS), or other suitable methods. This is a near-total-dissolution method; silicates and some refractory oxides (SiO_2, TiO_2) may not fully dissolve but environmentally available metals are recovered.

For non-microwave hot-plate acid digestion, see *SOP-L65*.

2. Scope

- **Applies to:** Soils, sediments, and sludge samples requiring trace metal analysis (Pb, Cd, Cu, Zn, Cr, As, Ni, Fe, Mn, and others) at all EA Program sites, including urban soil lead surveys and photovoltaic panel site characterizations
- **Does not apply to:** Water samples (direct acidification or separate digestion per EPA 3005A); samples requiring complete silicate dissolution (use HF-based methods); organic tissue digestion
- **Location:** Advanced Laboratory, Seaver North (MARS 5 microwave); EA Program laboratory, SGM 133 (sample preparation and filtration)

3. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, ensure training compliance
Lab Manager	Maintain MARS 5 microwave, stock reagents
Trained Personnel	Perform digestion and filtration per this SOP
New Students	Complete training before independent use; must be supervised for first digestion run

4. Definitions

- **Closed-vessel digestion:** Digestion performed in sealed vessels under pressure; higher temperatures are achievable (up to 240 °C) compared to open-vessel hot-plate digestion; reduces loss of volatile elements and acid consumption.

- **EPA Method 3051A:** The US EPA microwave-assisted acid digestion method for soils, sediments, and sludges; uses HNO_3 (with optional HCl) in closed PTFE vessels at controlled temperature and pressure; the reference method for this SOP.
- **MARS system (Microwave Accelerated Reaction System):** The CEM Corporation microwave digestion system used in the EA Program lab; controls temperature and pressure in each vessel during digestion.
- **Maximum allowable working pressure (MAWP):** The pressure limit of the digestion vessel specified by the manufacturer; exceeding this limit risks vessel rupture; monitored by the MARS control software.
- **PTFE (polytetrafluoroethylene):** A chemically inert fluoropolymer used to fabricate digestion vessels; resistant to concentrated acids and temperatures up to ~260 °C; must be pre-cleaned with HNO_3 before use to remove trace metal contamination.
- **Ramp time:** The programmed time over which the microwave system increases temperature from ambient to the target; too-rapid ramping can generate excessive pressure and risk vessel failure.

5. Safety and Hazards

5.1. Critical Safety Warning

This procedure uses concentrated nitric acid (HNO_3) under high temperature and pressure in sealed vessels. Improper handling can cause severe acid burns, toxic fume exposure, or vessel rupture. Read the entire procedure and the MARS 5 operator's manual before beginning.

5.2. Hazards

- **Chemical:** Concentrated HNO_3 (15.8 M) is severely corrosive and produces toxic NO_x fumes. Optional HCl (12 M) is also severely corrosive. Both cause severe burns on skin and eye contact.
- **Physical:** Microwave vessels reach 175 C and develop significant internal pressure. Residual pressure and acid vapor remain in the headspace after digestion. Hot vessels can cause burns.
- **Inhalation:** NO_x and HCl fumes are toxic. All acid handling and vessel venting must occur in the fume hood.

5.3. Required PPE

- ☐ Chemical splash goggles (not safety glasses)
- ☐ Face shield (when opening hot vessels)
- ☐ Lab coat
- ☐ Neoprene or heavy-duty acid-resistant gloves (long-cuffed)
- ☐ Closed-toed shoes

5.4. Emergency Procedures

For acid splashes to skin or eyes, flush immediately at the eyewash station or safety shower for at least 15 minutes. For inhalation of fumes, move to fresh air and seek medical attention if

symptoms persist. For vessel rupture or spill, evacuate the area and notify the lab manager. Refer to SOP-L003 Hazardous Materials Handling.

6. Equipment and Materials

6.1. Equipment

Item	Notes
MARS 5 Microwave Accelerated Reaction System	CEM Corporation; with pre-programmed EPA 3051A method
Microwave digestion vessels with carbon fiber sleeves	Must be compatible with the MARS 5; pressure-relief mechanism required
Temperature and pressure sensors (if applicable)	Consult instrument supervisor
Fume hood	Required for all acid handling and vessel venting
Analytical balance (0.001 g)	See SOP-L102
Centrifuge	Capable of 2,000–3,000 rpm; see *SOP-L109* or *SOP-L108*
Mortar and pestle	For grinding dried soil
No. 10 sieve (2 mm mesh)	For sieving ground soil
Drying oven	Set to 60 C; see SOP-L105

6.2. Consumables

Item	Cat. No. / Notes
50 mL graduated centrifuge tubes	For dilution and centrifugation
15 mL centrifuge tubes	For filtered digest storage
Disposable syringes (10 mL)	Acid-resistant polypropylene
Syringe filters, 0.45 um, 25 mm	Fisherbrand Cat. No. 09-719D
100 mL beakers or aluminum crinkle dishes	For drying samples

6.3. Reagents

Reagent	Grade / Notes
Nitric acid (HNO_3), concentrated (15.8 M)	Trace Metal grade; Fisher A509P500 or equivalent
Hydrochloric acid (HCl), concentrated (12 M) (optional)	Trace Metal grade; for improved recovery of Al, Sb, Ba, Be, Cr, Fe, Mg, Ag, V (U.S. Environmental Protection Agency, 2007)

Reagent	Grade / Notes
Reagent water (Type I)	From *SOP-L11*; must be interference-free at target MDLs

> **Note**
>
> All reagents must be Trace Metal grade or equivalent. Analyze a reagent blank before use; the blank must read below the method detection limit (MDL) for all target analytes.

7. Procedure

7.1. Glassware and Vessel Cleaning

1. Acid-wash all vessels, centrifuge tubes, and volumetric ware per SOP-L101 Glassware Cleaning, Section 6.3 (10% HNO_3 acid bath)
2. Rinse with reagent water and dry in a clean environment
3. For microwave vessels, follow the manufacturer's cleaning protocol (CEM recommends a cleaning cycle with dilute HNO_3 between batches)

7.2. Sample Preparation

1. Weigh approximately 3–5 g of soil into a clean beaker or aluminum dish. Avoid obviously non-soil material (large rocks, twigs, leaves).
2. Dry at 60 C overnight in the drying oven. Do not exceed 60 C; higher temperatures can volatilize mercury and other semi-volatile metals.
3. Allow to cool. Using a clean mortar and pestle, grind the dried soil and pass through a No. 10 sieve (2 mm).

7.3. Microwave Digestion

1. Weigh 0.500 g (+/- 0.001 g) of the sieved, dried soil into a clean microwave vessel. For oil-contaminated or high-organic samples, use no more than 0.250 g. Record the exact mass.
2. Working in the fume hood, add 10.0 mL (+/- 0.1 mL) of concentrated HNO_3 (Trace Metal grade) to the vessel.

> **Vigorous Reaction**
>
> Adding acid to samples with high organic matter may cause immediate effervescence. If a vigorous reaction occurs, leave the vessel uncapped in the fume hood for 1–2 hours to allow pre-digestion before sealing.

3. *Optional:* For improved recovery of Al, Sb, Ba, Be, Cr, Fe, Mg, Ag, and V, substitute 9 mL HNO_3 + 3 mL concentrated HCl (aqua regia variant). Note: HCl can increase interferences with some ICP-MS analyses; consult the analyst.

4. Seal the vessels according to the manufacturer's instructions (CEM Corporation, 2015)
5. Place each sealed vessel into a slot on the vessel rack **with a carbon fiber sleeve in place**

7.4. Critical

Never place a vessel into a rack slot without its carbon fiber sleeve. The sleeve provides essential containment in the event of a vessel failure. Operating without the sleeve risks vessel explosion.

6. Secure the rack into the MARS 5 microwave
7. If applicable, connect the temperature and pressure sensors to the designated control vessel(s)
8. Select the pre-programmed EPA 3051A method. Verify the following parameters:

Parameter	Specification (EPA 3051A)
Ramp to 175 C	5.5 +/- 0.25 minutes
Hold at 175 +/- 5 C	4.5 minutes (remainder of 10 min total)
Maximum pressure	Per vessel rating; relief mechanism must activate before failure

9. With instructor verification that all vessels are sealed and the rack is properly installed, close the microwave door and start the program
10. After the program finishes, allow the vessels to cool inside the microwave for at least 5 minutes, then transfer to the fume hood for further cooling

7.5. Filtration and Extract Preparation

1. Working in the fume hood, carefully uncap each vessel and vent for 2–3 minutes to release residual acid vapor and pressure
2. Quantitatively transfer the digest into a clean 50 mL graduated centrifuge tube, rinsing the vessel walls with small portions of reagent water to ensure complete transfer
3. Dilute to the 25.0 mL mark with reagent water. Record the final volume. Keep all samples as close to the same volume as possible.
4. Cap and centrifuge at 2,000–3,000 rpm for 10 minutes or until the supernatant is clear

> **Note**
>
> Refractory materials (SiO_2, TiO_2, Al_2O_3) may remain undissolved and cause persistent turbidity. If the supernatant is not clear after centrifugation, allow the sample to stand overnight before filtering.

5. Using a clean 10 mL syringe, carefully withdraw 10 mL of supernatant without disturbing the pellet
6. Attach a fresh 0.45 um syringe filter (Fisherbrand 09-719D) and filter the supernatant into a clean 15 mL centrifuge tube
7. Use a new syringe and filter for each sample
8. Cap and label the filtered extract. Store at 4 C until analysis.

7.6. Disposal and Cleanup

1. Collect all acid digestion waste (vessel rinses, supernatant residues, centrifuge pellets in acid) in a labeled acid waste carboy in the fume hood. EH&S pickup.
2. Rinse and clean microwave vessels per the manufacturer's protocol
3. Dispose of used syringe filters and syringes in the acid waste stream (they are contaminated with concentrated HNO_3 digest)
4. Wipe down all bench surfaces with DI water
5. Remove and properly dispose of gloves; wash hands thoroughly

8. Quality Control and Acceptance Criteria

8.1. QC Requirements

- **Reagent blank:** Include one vessel containing only 10 mL HNO_3 (no soil) in each digestion batch. The blank must read below the MDL for all target analytes.
- **Laboratory control sample (LCS):** Digest a certified reference material (CRM) or laboratory reference soil with known metal concentrations in each batch. Recovery should be 75–125% of the certified value.
- **Matrix spike:** Spike a duplicate sample with a known concentration of the target analyte(s). Recovery should be 75–125%.
- **Duplicate digestions:** Process at least 10% of samples in duplicate. Relative percent difference (RPD) should be less than 20%.
- **Calibration verification:** After instrument analysis, verify the calibration with a mid-range check standard every 10 samples.

8.2. Calculations

Metal concentration in soil (mg/kg dry weight):

$$C_{\text{soil}} = \frac{C_{\text{digest}} \times V_{\text{final}}}{M_{\text{soil}}}$$

where C_{digest} = concentration in the digest (mg/L from ICP-MS or FAAS), V_{final} = final digest volume (mL, typically 25), and M_{soil} = mass of dried soil (g).

Since the soil was dried at 60 C (not 105 C), apply a moisture correction if reporting on a 105 C oven-dry basis:

$$C_{\text{soil, OD}} = C_{\text{soil}} \times \frac{100 + \theta_{60}}{100 + \theta_{105}}$$

where θ_{60} and θ_{105} are the moisture contents at 60 C and 105 C respectively (determined on a companion subsample per SOP-L400).

9. Waste Disposal

Waste Type	Disposal Method
Acid digest waste (vessels, rinses, pellets)	Collect in acid waste carboy; EH&S hazardous waste pickup
Used syringe filters and syringes	Acid waste stream (contaminated with HNO_3)
Centrifuge tube residues	Acid waste carboy
Digested soil pellets	Acid waste carboy (contain concentrated acid)

Acid Waste

All waste from this procedure contains concentrated HNO_3 and dissolved metals. **Do not pour down the drain.** Collect in a labeled polyethylene carboy for EH&S hazardous waste pickup.

10. References

CEM Corporation. (2015). *MARS 5 microwave accelerated reaction system: Operator's manual.* https://www.cem.com

U.S. Environmental Protection Agency. (2007). *Method 3051A: Microwave assisted acid digestion of sediments, sludges, soils, and oils* (SW-846, Revision 1). U.S. Environmental Protection Agency. https://www.epa.gov/sites/production/files/2015-12/documents/3051a.pdf

11. Training Record

Before performing microwave acid digestion independently, personnel must:

1. Read this SOP, SOP-L002 Laboratory Safety, and SOP-L003 Hazardous Materials Handling in full
2. Review the SDS for concentrated HNO_3 and HCl
3. Review the MARS 5 operator's manual (vessel loading, program selection, safety interlocks)(CEM Corporation, 2015)
4. Observe a trained operator perform a complete digestion cycle (vessel loading through filtration)
5. Perform at least one complete digestion batch under direct supervision, including a blank and a CRM, with acceptable QC results
6. Be signed off by the PI or lab manager

Important

First-time users must be directly supervised for their entire first digestion run. Independent operation is permitted only after sign-off.

12. Appendix A: Example Calculation

```
# Trace metal concentration from microwave digest
# EPA 3051A, analyzed by ICP-MS

C_digest_mg_L <- 0.452     # Pb concentration in filtered digest (mg/L)
V_final_mL <- 25.0         # Final volume after dilution (mL)
M_soil_g <- 0.503           # Mass of dried soil digested (g)

# Concentration in soil (mg/kg)
C_soil_mg_kg <- (C_digest_mg_L * V_final_mL) / M_soil_g

cat(sprintf("Digest concentration: %.3f mg/L Pb\n", C_digest_mg_L))
cat(sprintf("Soil mass:           %.3f g\n", M_soil_g))
cat(sprintf("Final volume:        %.1f mL\n", V_final_mL))
cat(sprintf("Soil Pb:             %.1f mg/kg\n", C_soil_mg_kg))

# Compare to screening levels
cat(sprintf("\nCalifornia DTSC residential screening level for Pb: 80 mg/kg\n"))
cat(sprintf("EPA RSL for Pb (residential): 400 mg/kg\n"))
if (C_soil_mg_kg > 400) {
  cat("WARNING: Exceeds EPA residential screening level\n")
} else if (C_soil_mg_kg > 80) {
  cat("NOTE: Exceeds CA DTSC residential screening level\n")
} else {
  cat("Below both screening levels\n")
}
```

13. Appendix B: Troubleshooting

Symptom	Probable Cause	Corrective Action
Vigorous reaction when acid added	High organic matter or carbonite content	Pre-digest uncapped in fume hood for 1–2 hours before sealing
Vessel pressure exceeds limit during run	Sample mass too large; excessive organic content	Reduce sample mass to 0.250 g; extend pre-digestion time
Supernatant not clear after centrifugation	Refractory oxides (SiO_2, TiO_2) remain undissolved	Allow to stand overnight; filter through 0.45 um; this is normal for EPA 3051A
Blank reads above MDL	Contaminated acid, vessels, or water	Use fresh Trace Metal grade acid; re-clean vessels; check DI water quality

Symptom	Probable Cause	Corrective Action
CRM recovery below 75%	Incomplete digestion; sample loss during transfer	Verify temperature reached 175 C; rinse vessels quantitatively; consider HNO_3/HCl mixture
CRM recovery above 125%	Contamination; calibration error	Check blank; recalibrate instrument; re-analyze
High RPD between duplicates	Soil heterogeneity; inconsistent weighing	Grind and sieve more thoroughly; weigh to 0.001 g precision

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L411

Nitrification Potential – Shaken Soil Slurry Method

Standard Operating Procedure

SOP Number	SOP-L411
Revision	0.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes a rapid method for measuring the potential rate of ammonium oxidation in soils using a shaken soil slurry. The method provides an estimate of the activity of the existing ammonium-oxidizing microbial population at the time of sampling. It is based on ISO 15685 (*Soil quality – Determination of potential nitrification and inhibition of nitrification – Rapid test by ammonium oxidation*) (International Organization for Standardization, 2012).

Ammonium oxidation is the first step in autotrophic nitrification. Soil is incubated in a buffered (pH 7.2) ammonium sulfate solution with sodium chlorate added to inhibit nitrite oxidation. The accumulation of nitrite (NO_2^-) over a 6-hour incubation period serves as the measure of potential nitrification activity. Because the generation time of ammonia-oxidizing bacteria exceeds 10 hours, the assay measures the activity of the population present at sampling, not population growth.

This method can be used to compare soils (e.g., polluted vs. reference), to screen the effects of management practices or chemical substances on nitrifying communities, and to evaluate the impact of biosolids or waste materials on soil nitrification.

2. Scope

- **Applies to:** Field-moist soils containing a population of nitrifying microorganisms; assessment of nitrification inhibition by chemical substances (except volatiles with Henry's constant $H > 1$), biosolid extracts, and polluted soil mixtures
- **Does not apply to:** Soils with no detectable nitrifying population; measurement of in situ nitrification rates (this is a potential activity assay under optimized conditions); volatile inhibitors
- **Location:** EA Program laboratory, SGM 133

3. Responsibilities

Role	Responsibility
Lab PI	Approve experimental design, ensure training compliance
Lab Manager	Prepare reagents, maintain shaking incubator
Trained Personnel	Perform assay, record and report results
New Students	Complete training before independent use

4. Definitions

- **Ammonia oxidation:** The first step of nitrification; conversion of NH_4^+ to NO_2^- by ammonia-oxidizing bacteria (AOB) and archaea (AOA); the rate-limiting step of nitrification in most soils.
- **Autotrophic nitrification:** Nitrification carried out by chemolithoautotrophic microorganisms (AOB, AOA, and nitrite oxidizers) that use the energy from ammonia oxidation to fix CO_2; distinguished from heterotrophic nitrification.
- **Chlorate inhibition:** Addition of sodium chlorate (ClO_3^-) to suppress nitrite oxidation, allowing NO_2^- to accumulate and be measured as the product of ammonia oxidation; used in some nitrification potential assays.
- **Nitrite oxidation:** The second step of nitrification; conversion of NO_2^- to NO_3^- by nitrite-oxidizing bacteria (NOB); not rate-limiting under normal conditions.
- **Nitrification potential (NP):** The short-term rate of NO_3^- (or NO_2^- + NO_3^-) production in a soil slurry amended with excess NH_4^+ and incubated aerobically; reflects the size and activity of the extant nitrifier community; expressed as µg NO_3^--N g^{-1} soil h^{-1}.
- **Slurry method:** The standard technique for measuring nitrification potential; soil is suspended in a buffered ammonium sulfate solution at a defined soil-to-solution ratio and shaken aerobically at 25 °C; sequential subsamples are taken and analyzed for NO_2^- + NO_3^-.

5. Safety and Hazards

Hazards

- **Chemical:** Sodium chlorate ($NaClO_3$) is an oxidizer; avoid contact with organic materials and combustible substances. Potassium chloride (4 M KCl) is a mild irritant. Ammonium sulfate is a mild irritant.
- **Physical:** The orbital shaking incubator has moving parts; keep hair and loose clothing clear. Centrifuge operation poses standard mechanical hazards.

5.1. Required PPE

☐ Safety glasses
☐ Lab coat
☐ Nitrile gloves

5.2. Emergency Procedures

For chemical spills, refer to SOP-L003 Hazardous Materials Handling. For sodium chlorate spills, do not use organic absorbents (sawdust, paper towels); use vermiculite or sand.

6. Equipment and Materials

6.1. Equipment

Item	Notes
Orbital shaking incubator	Thermostatically controlled at 25 +/- 2 C; capable of 175 rpm
Erlenmeyer flasks (250 mL)	One per replicate
Centrifuge	Capable of 3,000 x g; see *SOP-L108* or *SOP-L109*
Analytical balance	0.001 g resolution; see SOP-L102
Volumetric flasks (100 mL, 1 L)	Class A
Graduated cylinders	10, 100, 1000 mL
Pipettes	2 mL (for slurry sampling) and assorted
Test tubes with caps	For subsamples
Filter funnels and filter paper	High-filtration-speed paper; chemical purity can be below the highest grade
pH meter	Calibrated per SOP-L300

6.2. Reagents

Reagent	Concentration / Preparation
Distilled or deionized water	From *SOP-L11*
Potassium dihydrogen phosphate (KH_2PO_4)	0.2 mol/L solution
Dipotassium hydrogen phosphate (K_2HPO_4)	0.2 mol/L solution
Sodium chlorate ($NaClO_3$)	0.5 mol/L solution
Ammonium sulfate ($(NH_4)_2SO_4$)	Solid; 0.198 g per liter of test medium
Sodium bicarbonate ($NaHCO_3$)	5 mmol/L solution (optional; see Section 6.2)
Potassium chloride (KCl)	4 mol/L solution (extraction/stop solution)

7. Procedure

7.1. Stock Solution A (Phosphate Buffer)

Combine the following in a volumetric flask and bring to 200 mL with distilled water:

- 28 mL of 0.2 mol/L KH_2PO_4
- 72 mL of 0.2 mol/L K_2HPO_4

This yields a phosphate buffer at approximately pH 7.2.

7.2. Test Medium

Prepare 1 L of test medium as follows:

1. Transfer 10 mL of Stock Solution A to a 1 L volumetric flask
2. Add 10–30 mL of 0.5 mol/L $NaClO_3$ (see note below on concentration selection)
3. Add 0.198 g of $(NH_4)_2SO_4$
4. If a carbon source is needed (e.g., when testing biosolids with high nitrate), add 10 mL of 5 mmol/L $NaHCO_3$
5. Dilute to 1 L with distilled water
6. Verify that the pH is approximately 7.2; adjust if necessary

The final test medium contains approximately 1 mmol/L potassium phosphate buffer, 5–15 mmol/L sodium chlorate, and 1.5 mmol/L ammonium sulfate.

> **Note**
>
> The sodium chlorate concentration should be sufficient to inhibit biological nitrite oxidation without negatively affecting ammonium oxidation. If the appropriate concentration for your soil type is unknown, run a preliminary trial at 5, 10, and 15 mmol/L $NaClO_3$ to identify the optimal level.

7.3. Preparing the 4 M KCl Stop Solution

Dissolve 298.2 g KCl in distilled water and bring to 1 L in a volumetric flask. This solution is used to halt ammonium oxidation at each sampling time.

7.4. Soil Moisture Determination

Determine the gravimetric moisture content of the soil per SOP-L400 (dry at 105 C, 24 h). This value is needed to calculate the volume of water in the initial soil sample and to express results on a dry-mass basis.

7.5. Setting Up the Incubation

1. Weigh approximately 25 g of field-moist soil into each of at least three replicate 250 mL Erlenmeyer flasks
2. Calculate the volume of water already present in the soil:

$$V_{\text{water}} = M_{\text{soil}} \times \theta_g$$

where M_{soil} = mass of moist soil (g) and θ_g = gravimetric moisture content (g water / g dry soil).

3. Calculate the volume of test medium to add:

$$V_{\text{medium}} = V_{\text{total}} - V_{\text{water}}$$

where V_{total} = desired total liquid volume (typically 100 mL).

4. Add the calculated volume of test medium to each flask
5. Place the flasks upright on the orbital shaking incubator set to 25 +/- 2 C and 175 rpm (sufficient to keep solids suspended)

> **!** Important
>
> If testing chemical substances, dissolve them in the phosphate buffer and add to the test medium before diluting to 1 L. For substances with low water solubility, use mechanical dispersion or a low-toxicity vehicle (organic solvent, emulsifier). If a vehicle is used, include an additional control to assess its effect on ammonium oxidation.

7.6. Sampling the Slurry

1. At 2 h and 6 h after the start of incubation, shake each flask well to resuspend solids and withdraw a 2 mL aliquot of the slurry. Ensure the ratio of solution to soil is consistent across all samples.
2. Dispense the 2 mL aliquot into a test tube
3. Immediately add 2 mL of 4 M KCl to stop ammonium oxidation
4. Centrifuge at 3,000 x g for 2 minutes, or filter through high-speed filter paper
5. Transfer the clear supernatant or filtrate to a clean, labeled tube

7.7. Nitrite Analysis

Analyze the extracts for NO_2^--N using a suitable colorimetric method (e.g., ISO 14256-2; diazotization with sulfanilamide and N-(1-naphthyl)ethylenediamine). Extracts may be stored at 4–8 C but must be analyzed within 24 hours.

8. Quality Control and Acceptance Criteria

8.1. Calculations

Potential ammonium oxidation rate:

$$\text{Rate} = \frac{[\text{NO}_2^-\text{-N}]_{6h} - [\text{NO}_2^-\text{-N}]_{2h}}{\Delta t \times M_{\text{dry}}} \times V_{\text{total}}$$

where the NO_2^--N concentrations are in ug/mL (= mg/L), Δt = time interval (4 h), M_{dry} = dry mass of soil (g), and V_{total} = total liquid volume in the flask (mL). Report results as ng NO_2^--N per g dry soil per hour.

Inhibition (%) by a test chemical:

$$\text{Inhibition (\%)} = \frac{R_{\text{control}} - R_{\text{treatment}}}{R_{\text{control}}} \times 100$$

where R = ammonium oxidation rate.

Toxicity assessment for polluted soil mixtures:

When mixing polluted and unpolluted (reference) soils, the polluted soil is considered toxic if:

$$A_m + s_m < 0.9 \times \frac{A_c + A_p}{2}$$

where A_m = mean activity in the mixture, s_m = standard deviation in the mixture, A_c = mean activity in the control soil, and A_p = mean activity in the polluted soil (each tested separately). This threshold applies with a minimum of four replicates.

8.2. QC Requirements

- Use a minimum of three replicates per treatment
- Four replicates are required when applying the toxicity assessment equation above
- Include a negative control (soil with no ammonium addition) and a positive control (reference soil with known nitrification activity) in each batch
- Verify that ammonium oxidation is linear between the 2 h and 6 h sampling points. If non-linearity is suspected, add sampling at 0 h and 4 h to characterize the time course.
- Blank controls (test medium without soil) should show no significant nitrite accumulation
- Report the coefficient of variation among replicates. A CV greater than 15% suggests sampling inconsistency or soil heterogeneity; consider additional replicates.

9. Waste Disposal

Waste Type	Disposal Method
Soil slurries (non-hazardous)	Drain liquid; dispose of soil in standard trash
KCl extracts	Drain with copious water
$NaClO_3$-containing solutions	Collect in oxidizer waste; EH&S pickup. Do not mix with organic waste.
Used filter paper and tubes	Standard trash (if non-hazardous soil)

10. References

International Organization for Standardization. (2012). *Soil quality – determination of potential nitrification and inhibition of nitrification – rapid test by ammonium oxidation* (ISO 15685:2012). International Organization for Standardization. https://www.iso.org/standard/55080.html

11. Training Record

Before performing the nitrification potential assay independently, personnel must:

1. Read this SOP in full
2. Complete SOP-L002 Laboratory Safety and SOP-L003 Hazardous Materials Handling training
3. Review the SDS for sodium chlorate
4. Complete SOP-L400 Soil Moisture training
5. Observe a trained operator perform the full assay from slurry setup through nitrite analysis
6. Perform the assay under supervision with acceptable replicate precision (CV less than 15%)
7. Be signed off by the PI or lab manager

12. Appendix A: Example Calculation

```
# Nitrification potential calculation per ISO 15685

# Input data
no2_2h_mg_L <- 0.15       # NO2-N at 2 h (mg/L in the extract)
no2_6h_mg_L <- 0.95       # NO2-N at 6 h (mg/L in the extract)
delta_t_h <- 4             # Time interval (h)
soil_moist_g <- 25.0       # Mass of field-moist soil (g)
theta_g <- 0.22            # Gravimetric moisture (g water / g dry soil)
V_total_mL <- 100          # Total liquid volume in the flask (mL)

# Dry mass of soil
M_dry_g <- soil_moist_g / (1 + theta_g)

# Potential ammonium oxidation rate (ng NO2-N / g dry soil / h)
# Convert mg/L to ug/mL (same units), then multiply by V_total to get
# total ug in the flask, divide by dry mass and time, convert to ng.
rate_ng_per_g_per_h <- ((no2_6h_mg_L - no2_2h_mg_L) *
                         V_total_mL / (delta_t_h * M_dry_g)) * 1000

cat(sprintf("Dry soil mass:            %.2f g\n", M_dry_g))
cat(sprintf("Delta NO2-N:              %.2f mg/L\n",
            no2_6h_mg_L - no2_2h_mg_L))
cat(sprintf("Nitrification potential:  %.1f ng NO2-N / g dry soil / h\n",
            rate_ng_per_g_per_h))
```

13. Appendix B: Reagent Preparation Quick Reference

Reagent	Preparation
0.2 mol/L KH_2PO_4	Dissolve 27.22 g in 1 L distilled water
0.2 mol/L K_2HPO_4	Dissolve 34.84 g in 1 L distilled water
Stock Solution A	28 mL KH_2PO_4 + 72 mL K_2HPO_4 + 100 mL water
0.5 mol/L $NaClO_3$	Dissolve 53.23 g in 1 L distilled water
4 mol/L KCl	Dissolve 298.2 g in 1 L distilled water
5 mmol/L $NaHCO_3$	Dissolve 0.420 g in 1 L distilled water
Test medium (1 L)	10 mL Stock A + 10–30 mL $NaClO_3$ + 0.198 g $(NH_4)_2SO_4$ + water to 1 L

14. Appendix C: Troubleshooting

Symptom	Probable Cause	Corrective Action
No nitrite accumulation	Soil has no active nitrifying population; $NaClO_3$ concentration too high	Test a reference soil in parallel; reduce $NaClO_3$ to 5 mmol/L
Non-linear NO_2^- accumulation	Nitrifier population growing during incubation; substrate depletion	Add intermediate time points (0 h, 4 h); use only the linear portion of the curve
High variability among replicates	Heterogeneous soil; inconsistent slurry sampling	Sieve and mix soil thoroughly; ensure slurry is well-shaken before each aliquot
Nitrite detected in controls (no NH_4^+)	Background nitrification from soil organic N	Note in results; subtract control values from treatment values
$NaClO_3$ appears to inhibit ammonium oxidation	Concentration too high for this soil	Run a $NaClO_3$ dose-response trial (5, 10, 15 mmol/L)

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L413

Compost Maturity – Solvita CO_2/NH_3 Test

Standard Operating Procedure

SOP Number	SOP-L413
Revision	0.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the determination of compost maturity and stability using the Solvita Compost Maturity Test (Woods End Laboratories). The test simultaneously measures carbon dioxide (CO_2) and ammonia (NH_3) emissions from a compost sample during a 4-hour incubation, providing a dual indicator of biological activity and nitrogen stabilization. The intersection of the two readings yields a Compost Maturity Index (CMI) that indicates whether compost is immature, active, curing, or finished (Vargas et al., 2005).

CO_2 evolution reflects ongoing microbial decomposition of organic carbon. NH_3 volatilization indicates incomplete nitrogen stabilization and is associated with odor, phytotoxicity, and high pH. True compost maturity is reached when both gases have declined to low levels. Because the CO_2 and NH_3 cycles do not always track together, the dual-measurement approach provides a more reliable maturity assessment than either gas alone.

This SOP is distinct from SOP-L408 Solvita CO_2 Burst, which measures rewetted *soil* respiration over 24 hours using only a CO_2 paddle.

2. Scope

- **Applies to:** Assessment of maturity and stability of composted organic materials, including green waste, food waste, biosolids compost, manure compost, and vermicompost
- **Does not apply to:** Soil respiration measurements (see SOP-L408); raw feedstock characterization; long-term (7–28 day) laboratory respirometry; finished potting mixes where compost is a minor component
- **Location:** EA Program laboratory, SGM 133

3. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, ensure training compliance
Lab Manager	Maintain Solvita kit inventory, stock gel paddles
Trained Personnel	Perform assay, record and report results
New Students	Complete training before independent use

4. Definitions

- **Compost maturity:** A measure of how fully decomposed and stabilized a compost product is; mature compost no longer undergoes rapid decomposition, does not cause phytotoxicity, and can be safely applied to crops or soils.
- **CO_2 index (Solvita):** The Solvita scale value (1–8) corresponding to the CO_2 concentration in the headspace above moist compost; lower values indicate more active decomposition (less mature); higher values indicate stabilization.
- **Maturity index:** A combined score calculated from the CO_2 and NH_3 Solvita indices; the primary output of the Solvita compost maturity test; used by USCC, US Composting Council, and others to certify compost quality.
- **NH_3 index (Solvita):** The Solvita scale value (1–5) corresponding to the NH_3 concentration in the headspace above moist compost; high NH_3 (low index) indicates immature compost with excess ammoniacal nitrogen that can harm plants.
- **Phytotoxicity:** Inhibition of plant germination or growth caused by immature compost; typically results from high NH_3 concentrations, volatile fatty acids, or other metabolites produced during active decomposition.
- **Solvita paddle:** A plastic stick holding two gel-pad sensors (CO_2 and NH_3) inserted into the compost jar headspace during the test incubation; color changes are read with the Solvita DCR reader.

5. Safety and Hazards

Hazards

- **Biological:** Compost may contain elevated microbial loads, fungal spores, and trace pathogens. Handle as a potential biohazard. Avoid inhaling dust when opening bags or scooping dry compost.
- **Chemical:** NH_3 released from immature compost is an irritant at high concentrations. Work in a well-ventilated area when handling fresh or actively decomposing compost.
- **Physical:** Minor risk of glass breakage from incubation jars.

5.1. Required PPE

☐ Nitrile gloves
☐ Safety glasses
☐ Lab coat
☐ Dust mask (recommended when handling dry or dusty compost)

5.2. Emergency Procedures

If compost dust is inhaled and respiratory irritation occurs, move to fresh air. For skin contact with compost, wash thoroughly with soap and water. Refer to SOP-L002 Laboratory Safety.

6. Equipment and Materials

6.1. Equipment

Item	Notes
Solvita Compost Maturity Test kit	Includes CO_2 and NH_3 gel paddles, incubation jars, scoop, and color charts
Solvita Digital Color Reader (DCR)	Recommended for quantitative readings; provides numerical color values
Incubation jars (approx. 475 mL)	Glass or polycarbonate; provided with kit
Timer	4-hour countdown
Thermometer	For recording ambient and sample temperature
Lab notebook or data sheet	For recording results

6.2. Consumables

Item	Notes
Solvita CO_2 gel paddles	One per test; store sealed at room temperature in the dark
Solvita NH_3 gel paddles	One per test; store sealed at room temperature in the dark
Solvita CMI Calculator spreadsheet	Available from Woods End / solvita.com for precise index calculation

7. Procedure

7.1. Sample Collection and Preparation

1. Collect a representative compost sample of at least 500 g from the pile or windrow. Sample from the interior of the pile (at least 30 cm below the surface) to avoid surface drying artifacts.
2. If the compost is very heterogeneous, collect from multiple locations and composite the subsamples
3. The sample should be at "natural" moisture, not artificially wetted or dried. Compost that has been stored at 4 C should be brought to room temperature (20–25 C) before testing. Allow at least 30 minutes.
4. Gently break up large clumps by hand but do not sieve or grind the compost. The test is designed for material in its as-produced condition.

! Important

Do not test compost that is above 40 C (i.e., still in the thermophilic phase). Elevated temperatures accelerate gas production and will give artificially high CO_2 and NH_3 readings

that do not reflect the maturity of the material once cooled.

7.2. Setting Up the Test

1. Fill a clean Solvita incubation jar loosely to the fill line with compost using the supplied scoop. Do not pack or compress the material. The volume should be approximately 150–200 mL.
2. Remove one CO_2 gel paddle and one NH_3 gel paddle from their sealed foil packets
3. Inspect each paddle: the gel should be uniform in color with no cracks, discoloration, or signs of prior exposure. Discard any compromised paddles.
4. Insert both paddles into the jar so that the gel faces are exposed to the headspace above the compost surface. The paddles should stand upright and not be buried in the compost.
5. Seal the jar immediately and securely
6. Record the jar number, sample ID, compost source, ambient temperature, and start time
7. Start the 4-hour timer

7.3. Incubation

1. Place sealed jars at room temperature (20–25 C) in a location shielded from direct sunlight and drafts
2. Do not open, move, or disturb jars during the 4-hour incubation

Note

The standard incubation time is 4 hours. This is shorter than the 24-hour incubation used for the Solvita soil CO_2 burst test (SOP-L408) because compost produces gas at much higher rates than soil.

7.4. Reading Results

1. After exactly 4 hours, open the jar and immediately remove both paddles
2. Read each paddle promptly. Prolonged exposure to ambient air will alter the gel color.

Using the Solvita Color Charts (visual method):

3. Compare the CO_2 paddle to the Solvita CO_2 color chart. Record the color number (1–8 scale, where 1 = highest CO_2 / least stable, 8 = lowest CO_2 / most stable).
4. Compare the NH_3 paddle to the Solvita NH_3 color chart. Record the color number (1–5 scale, where 1 = highest NH_3 / least mature, 5 = lowest NH_3 / most mature).

Using the Solvita Digital Color Reader (DCR, recommended):

3. Insert the CO_2 paddle into the DCR and record the numerical reading
4. Insert the NH_3 paddle into the DCR and record the numerical reading
5. The DCR values can be entered into the Solvita CMI Calculator spreadsheet for a more precise Maturity Index

7.5. Determining the Compost Maturity Index (CMI)

Use the CO_2 and NH_3 color numbers to determine the CMI from the Solvita lookup table (printed in the kit manual) or the CMI Calculator spreadsheet:

CMI	Interpretation	Recommended Action
1	Raw, very immature	Continue active composting
2	Immature, highly active	Continue active composting
3	Immature, active	Continue composting/curing
4	Moderately immature	Begin curing phase
5	Mid-range maturity	Continue curing
6	Moderately mature	Approaching readiness
7	Mature	Ready for most applications
8	Very mature, highly stable	Fully finished compost

Tip

Most regulatory agencies and compost quality programs consider a CMI of 6 or above as "mature" and suitable for unrestricted use. A CMI of 7–8 indicates a fully finished product with minimal phytotoxicity risk. For sensitive applications (seed germination, potting mixes), target CMI 7 or 8.

7.6. Cleanup

1. Dispose of used compost in the designated waste container or return it to the compost pile
2. Wash incubation jars thoroughly with soap and water; rinse with deionized water and allow to air-dry
3. Dispose of used gel paddles in standard trash

8. Quality Control and Acceptance Criteria

8.1. QC Requirements

- Run duplicate tests on at least 10% of samples (two jars from the same homogenized compost). Duplicate CO_2 readings should agree within 1 color number; NH_3 readings within 1 color number.
- Include a blank jar (empty, sealed with both paddles) in each batch to check for ambient CO_2 and NH_3 interference. Both paddles should read at or near their maximum (most stable) values.
- Verify that the compost sample temperature is between 20 and 40 C before testing. Record the temperature.
- Calibrate the DCR per the manufacturer's instructions before each batch if using the digital reader.
- Store gel paddles sealed in their foil packets, at room temperature, away from light. Check expiration dates; do not use expired paddles.

8.2. Complementary Tests

For a comprehensive compost quality assessment, consider pairing the Solvita test with:

Parameter	SOP / Method
pH and electrical conductivity	SOP-L402 (1:5 compost-water extract)
Moisture content	SOP-L400 (105 C, 24 h)
Organic matter (LOI)	SOP-L406
Total C and N (C:N ratio)	SOP-L407 and elemental analysis
Seed germination bioassay	*TBD*

9. Waste Disposal

Waste Type	Disposal Method
Used compost	Return to compost pile or standard trash
Used gel paddles	Standard trash
Jar wash water	Drain

10. References

Vargas, D. C. nas., Monedero, M. S., Urpilainen, S., Kamilaki, A., & Stentiford, E. (2005). Assessing the stability and maturity of compost at large-scale plants. *Ingenier' ia*, *9*(2), 25–30.

11. Training Record

Before performing the Solvita compost maturity test independently, personnel must:

1. Read this SOP in full
2. Complete SOP-L002 Laboratory Safety training
3. Review the Solvita Compost Maturity Test User's Guide (included in the kit)
4. Observe a trained operator perform the full procedure from sample collection through CMI determination
5. Perform at least one test under supervision with acceptable duplicate precision (within 1 color number)
6. Be signed off by the PI or lab manager

12. Appendix A: Solvita Compost Maturity Index Lookup Table

The following table reproduces the general structure of the Solvita CMI lookup. Consult the kit manual or CMI Calculator spreadsheet for the official version.

	$NH_3 = 1$	$NH_3 = 2$	$NH_3 = 3$	$NH_3 = 4$	$NH_3 = 5$
$CO_2 = 1$	1	1	1	2	2
$CO_2 = 2$	1	2	2	3	3
$CO_2 = 3$	2	2	3	4	4
$CO_2 = 4$	2	3	4	5	5
$CO_2 = 5$	3	4	5	6	6
$CO_2 = 6$	3	4	5	6	7
$CO_2 = 7$	4	5	6	7	7
$CO_2 = 8$	5	5	6	7	8

Note: This table is approximate. Use the official Solvita color charts and CMI Calculator for definitive results.

13. Appendix B: Interpreting Results in Context

13.1. CO_2 Color Number Interpretation

Color No.	CO_2 Level	Stability Interpretation
1–2	Very high	Actively decomposing; unstable
3–4	Moderate to high	Active composting phase
5–6	Low to moderate	Curing; approaching stability
7–8	Very low	Stable; decomposition essentially complete

13.2. NH_3 Color Number Interpretation

Color No.	NH_3 Level	Maturity Interpretation
1	Very high	Strong odor; phytotoxic; immature
2	High	Significant ammonia; not ready
3	Moderate	Some ammonia; continue curing
4	Low	Approaching maturity
5	Very low / none	Nitrogen stabilized; mature

13.3. Common Patterns

CO_2	NH_3	Interpretation
High	High	Actively decomposing with unstable N; classic immature compost
High	Low	Carbon-rich material still decomposing but N is stabilized; may need more time
Low	High	Unusual; possible anaerobic conditions or recent N-rich amendment; investigate

CO_2	NH_3	Interpretation
Low	Low	Both carbon and nitrogen cycles complete; finished compost

14. Appendix C: Troubleshooting

Symptom	Probable Cause	Corrective Action
CO_2 paddle reads 1 (max activity) after 4 h	Compost is very fresh or sample was too warm	Verify sample temperature was below 40 C; confirm composting has progressed past thermophilic phase
NH_3 paddle reads 1 (max ammonia)	High free ammonia; fresh manure or urea input	Continue composting; ensure adequate C:N ratio (target 25:1–30:1)
Both paddles show no color change (max stability)	Compost is fully mature, or paddles expired	Verify paddle expiration date; confirm with a known immature sample
Duplicates disagree by more than 1 color number	Heterogeneous compost; inconsistent fill level	Homogenize sample more thoroughly; fill jars to consistent level
CO_2 high but NH_3 low	High-carbon, low-N material (e.g., wood chip compost)	Normal for C-rich substrates; continue composting until CO_2 declines
CO_2 low but NH_3 high	Possible anaerobic pocket; recent N amendment	Turn the pile to aerate; reduce N inputs; retest in 1–2 weeks

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L414

Soil Carbon and Nitrogen Analysis by Flash EA-IRMS

Standard Operating Procedure

SOP Number	SOP-L414
Revision	1.1
Author(s)	Marc Los Huertos, Kyle McCarty
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes the complete workflow for determining δ^{13}C, δ^{15}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 δ^{13}C, δ^{15}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 (δ^{2}H, δ^{18}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).
- **δ^{13}C:** Stable carbon isotope ratio expressed in per mil (‰) relative to Vienna Pee Dee Belemnite (VPDB): $\delta^{13}C = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1\right) \times 1000$ where $R = {}^{13}C/{}^{12}C$ (Coplen, 2011).
- **δ^{15}N:** Stable nitrogen isotope ratio expressed in per mil (‰) relative to atmospheric N_2 (AIR): $\delta^{15}N = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1\right) \times 1000$ where $R = {}^{15}N/{}^{14}N$ (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 O_2 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 O_2 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 µm)	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 N_2 and CO_2 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 δ^{13}C; IAEA-N1 + IAEA-N2 for δ^{15}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 δ^{13}C and δ^{15}N must recover within ±0.2‰ 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 >= 3) must show SD <= 0.15‰.
- 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

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L415

Carbonate Isotope Analysis by GasBench II-IRMS

Standard Operating Procedure

SOP Number	SOP-L415
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Author(s)	Marc Los Huertos, Kyle McCarty
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes the determination of δ^{13}C and δ^{18}O in carbonate minerals and sediments using phosphoric acid digestion and headspace analysis on the GasBench II coupled to the Delta V IRMS (Coplen, 2011; Kim et al., 2007; Swart et al., 1991). It covers sample preparation, vial flushing and acidification, the analytical run, and post-analysis data correction.

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

2. Scope

- **Applies to:** Carbonate-bearing samples (calcite, dolomite, aragonite, shell, foraminifera, soil carbonates, cement, and similar materials) analyzed for δ^{13}C (VPDB) and δ^{18}O (VPDB or VSMOW) by phosphoric acid digestion and continuous-flow IRMS.
- **Does not apply to:** DIC in water, breath gas, CO_2 at atmospheric concentrations, or water equilibration methods (these use the GasBench but with different sample preparation and Isodat methods).
- **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 GasBench and PAL
Certified User	All steps from sample prep through data reduction
Student Researcher	May perform sample prep and data reduction under supervision

4. Definitions

- **Acid fractionation factor (α^*):** The equilibrium isotope fractionation between carbonate mineral and CO_2 produced by phosphoric acid digestion; temperature-dependent and mineral-specific (Kim et al., 2007).
- δ^{13}C (VPDB): Stable carbon isotope ratio in per mil (‰) referenced to Vienna Pee Dee Belemnite (VPDB); the conventional scale for carbonate and organic carbon (Coplen, 2011).
- δ^{18}O (VPDB or VSMOW): Stable oxygen isotope ratio in per mil (‰); carbonates are commonly reported on VPDB, which relates to VSMOW by $\delta^{18}O_{\text{VPDB}} \approx 0.97001 \times \delta^{18}O_{\text{VSMOW}} - 29.99$ (Coplen, 2011).
- **Exetainer vial:** 12 mL borosilicate glass vial with a septum-capped lid used for sample reaction and headspace analysis.
- **GasBench II:** Continuous-flow peripheral that samples headspace CO_2 from sealed exetainer vials through a flushed needle system and delivers it to the IRMS via the ConFlo IV.

- **PAL autosampler:** CTC Analytics autosampler integrated with the GasBench that positions vials under the injection needle for automated headspace sampling.
- **Phosphoric acid digestion:** Reaction of carbonate with 100% H_3PO_4 at 50 °C to release CO_2 quantitatively from calcite and aragonite (Swart et al., 1991).
- **VPDB (Vienna Pee Dee Belemnite):** The primary international reference scale for $\delta^{13}C$ and $\delta^{18}O$ in carbonates.
- **VSMOW (Vienna Standard Mean Ocean Water):** The primary international reference scale for $\delta^{18}O$ and $\delta^{2}H$ in water and atmospheric gases.

5. Safety and Hazards

See SOP-L208, Section 4 for the full hazard list. Hazards specific to this application:

⚠ Application-Specific Hazards

- **100% phosphoric acid (H_3PO_4):** Corrosive. Handle in the fume hood. Wear nitrile gloves and safety glasses at all times.
- **Pressurized vials:** Flushed vials contain He under slight pressure; use the PAL needle carefully to avoid splashing acid.

5.1. Required PPE

☐ Safety glasses
☐ Lab coat
☐ Nitrile gloves (acid handling and vial preparation)

5.2. Required Training

- SOP-L002 Laboratory Safety
- SOP-L003 Hazardous Materials Handling (acid handling)
- SOP-L208 IRMS General Instrument Use (must be a certified IRMS User)

6. Equipment and Materials

Item	Location	Notes
Exetainer vials (12 mL)	SGM Rm 133	Check septum integrity before use
100% phosphoric acid	Fume hood, SGM Rm 133	Corrosive; store in acid cabinet
He gas (ultra-high purity)	SGM Rm 135	For vial flushing
Flushing manifold / needle	SGM Rm 133	10-port manifold preferred
Micro-spatula	SGM Rm 133	For weighing carbonate powder
Micro-balance	SGM Rm 133	Tare before each vial
GasBench II + PAL autosampler	SGM Rm 135	Confirm needle and trap condition

Item	Location	Notes
Certified reference materials	SGM Rm 133	e.g., NBS-18, NBS-19, IAEA-603
Heating block (50 °C)	SGM Rm 133	For overnight reaction

7. Estimated Time

Task	Time
Vial preparation and flushing	1–2 h (for a 40-vial batch)
Acid addition and reaction	1 h (calcite); overnight (dolomite)
Analytical run (per injection)	~8 min x 11 injections/vial
Data reduction (R script)	30–60 min

8. Procedure

8.1. Vial Preparation

1. Label exetainer vials with sample ID and position number.
2. Weigh 0.1–0.5 mg carbonate powder into each vial (adjust for expected calcite content).
3. Cap vials tightly with septum caps.
4. Flush each vial with He (UHP) for 5–8 min using the flushing manifold to remove atmospheric CO_2.

8.2. Acid Addition

1. In the fume hood, add 3–5 drops (~100 µL) of 100% H_3PO_4 to each vial through the septum using a syringe.
2. Incubate at 50 °C for 1 h (calcite and aragonite) or overnight (dolomite) in the heating block.
3. Allow vials to equilibrate to room temperature before loading into the PAL tray.

8.3. Sequence Design

1. Open Isodat Sequence Editor and load the GasBench carbonate method.
2. Build the sequence: 3 blank vials (flushed, no sample), reference materials, unknowns (1 RM per 8 unknowns), check standards.
3. Set the PAL to collect 11 injections per vial; the first 2–3 are discarded in post-processing.
4. Save the sequence file as `YYYYMMDD_Project_SeqID_User.seq`.

8.4. Analytical Run

1. Complete the pre-analysis checklist in SOP-L208.
2. Load the PAL tray with vials in correct order.
3. Press **Start** in Isodat and monitor the first 5 injections.

8.5. Post-Analysis Data Correction

Apply corrections using the R script in the project repository:

```
library(tidyverse)

raw <- read_csv("data/raw/YYYYMMDD_Project_SeqID_User_raw.csv")

# Each vial has 11 injections; discard injections 1-3
clean <- raw |>
  filter(injection >= 4) |>
  group_by(vial_id) |>
  summarise(
    d13C_mean = mean(d13C_raw),
    d18O_mean = mean(d18O_raw),
    d13C_sd   = sd(d13C_raw),
    d18O_sd   = sd(d18O_raw),
    n_inj     = n()
  )

# Scale normalization (two-point) using certified RMs
# Drift correction across run order
# QC: flag vials where within-vial SD > 0.15 permil
```

9. Quality Control and Acceptance Criteria

- Reference materials must recover within ±0.2‰ (δ^{13}C) and ±0.3‰ (δ^{18}O) of certified values after corrections.
- Within-vial SD (injections 4–11) must be <0.15‰ for both isotopes.
- Blank vials must produce no CO_2 peak above noise.

10. Data Management

1. Export raw Isodat data immediately after the run as `.csv`.
2. Save as `data/raw/YYYYMMDD_Project_SeqID_User_raw.csv` in the project repository.
3. Commit raw data and the correction script per SOP-L801.

11. Waste Disposal

Waste Type	Disposal Method
Used exetainer vials and acid	Acid waste carboy per SOP-L006
Gloves, Kimwipes	Standard trash unless acid-contaminated

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

Kim, S.-T., Mucci, A., & Taylor, B. E. (2007). Phosphoric acid fractionation factors for calcite and aragonite between 25 and 75 °C: revisited. *Chemical Geology*, *246*(3–4), 135–146. https://doi.org/10.1016/j.chemgeo.2007.08.005

Swart, P. K., Burns, S. J., & Leder, J. J. (1991). Fractionation of the stable isotopes of oxygen and carbon in carbon dioxide during the reaction of calcite with phosphoric acid as a function of temperature and technique. *Chemical Geology: Isotope Geoscience Section*, *86*(2), 89–96. https://doi.org/10.1016/0168-9622(91)90055-2

13. Related SOPs

SOP	Title	Relationship
L002	Laboratory Safety	Required prerequisite
L003	Hazardous Materials Handling	Acid handling
L208	IRMS General Instrument Use	Instrument-level procedures
L414	Soil CN by Flash EA-IRMS	Companion application SOP
L802	Quality Control and Quality Assurance	General QC framework

14. Training Record

Before performing carbonate isotope analysis independently, personnel must:

1. Read this SOP and SOP-L208 in full
2. Be a certified IRMS User (SOP-L208, Training Record)
3. Complete SOP-L003 Hazardous Materials Handling (acid handling)
4. Observe a GasBench carbonate run from vial preparation through data export
5. Perform a supervised run with acceptable QC results
6. Complete a supervised data reduction session
7. Be signed off by the lab manager or PI

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L416

Cation Exchange Capacity (CEC)

Standard Operating Procedure

SOP Number	SOP-L416
Revision	0.1
Author(s)	
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes the determination of cation exchange capacity (CEC) and exchangeable base cations (Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}) in soils using the ammonium acetate (NH_4OAc) saturation and displacement method at pH 7.0 (Chapman, 1965; Sumner & Miller, 1996). CEC quantifies the total capacity of a soil to hold exchangeable cations and is a fundamental indicator of soil fertility, buffering capacity, and nutrient retention (Sparks, 2003).

2. Scope

- **Applies to:** Air-dried, sieved (< 2 mm) mineral soils from all EA Program sites. The method is also applicable to compost and organic amendments, although CEC values for highly organic materials should be interpreted with caution due to pH-dependent charge (Sumner & Miller, 1996).
- **Does not apply to:** Soils dominated by variable-charge minerals (e.g., highly weathered Oxisols, Andisols) where CEC measured at pH 7.0 may overestimate field-effective CEC. For such soils, a compulsive exchange method at field pH may be more appropriate (Gillman & Sumpter, 1979). Does not apply to water samples.
- **Location:** EA Program laboratory, SGM 133

3. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, ensure training compliance
Trained Personnel	Prepare extracts, record and report results
New Students	Complete training before independent use

3.1. Required Training

Students using this SOP should be trained on:

- SOP-L002 Laboratory Safety
- SOP-L400 Soil Moisture

4. Definitions

- **Buffered salt solution:** A reagent at a defined pH used to saturate exchange sites with a known index cation (e.g., NH_4^+ at pH 7.0 in the ammonium acetate method); the buffer pH controls which exchange sites are measured.
- **Base saturation:** The percentage of CEC occupied by base cations (Ca^{2+}, Mg^{2+}, K^+, Na^+); calculated as the sum of base cations divided by CEC, expressed as %; important for liming and fertility management.
- **Cation exchange capacity (CEC):** The total capacity of a soil to hold exchangeable cations, expressed in $cmol_c$ kg^{-1} (or meq 100 g^{-1}); governed by clay mineralogy and organic matter content; the higher the CEC, the greater the soil's buffering capacity against acidification and nutrient leaching.
- **Effective CEC (ECEC):** CEC measured at the soil's natural pH (using an unbuffered salt such as 1 M KCl or $BaCl_2$); represents the actual exchange capacity at field conditions; lower than pH 7-buffered CEC in acid soils.
- **Exchange sites:** Negatively charged surfaces on clay minerals and organic matter that hold cations electrostatically; released by displacing the sorbed cations with a high-concentration competing ion.
- **Index cation:** The cation used to saturate exchange sites before measurement (e.g., NH_4^+ in the ammonium acetate method, Ba^{2+} in the compulsive exchange method); subsequently displaced and quantified to calculate CEC.

5. Safety and Hazards

⚠ Hazards

- **Chemical:** 1 M ammonium acetate (NH_4OAc) is a mild irritant. Concentrated acetic acid used in reagent preparation is corrosive and produces irritating vapors. Concentrated ammonium hydroxide (NH_4OH) used for pH adjustment is caustic and produces strong vapors. Ethanol (95%) is flammable.
- **Physical:** Reciprocating shaker can pinch fingers if containers are not secured properly.

❗ Engineering Controls

Prepare 1 M NH_4OAc from concentrated reagents inside a chemical fume hood. Once diluted to working concentration, the solution can be used on the open bench.

5.1. Required PPE

- ☐ Safety glasses or chemical splash goggles (goggles when preparing reagents)
- ☐ Laboratory coat
- ☐ Nitrile gloves
- ☐ Closed-toed shoes

5.2. Emergency Procedures

For chemical spill or splash, refer to SOP-L002 Laboratory Safety or call campus safety. For acetic acid or ammonium hydroxide contact with eyes, flush immediately with water for at least 15 minutes.

6. Equipment and Materials

6.1. Equipment

Item	Notes
Reciprocating or end-over-end shaker	For batch extraction
Centrifuge	Capable of 2,000–3,000 x g
Vacuum filtration apparatus	Buchner funnel, flask, and vacuum source
Analytical balance	Readable to 0.01 g
Drying oven	105 °C for moisture subsamples
pH meter	For adjusting NH_4OAc to pH 7.0
Volumetric flasks	1 L and 250 mL

6.2. Reagents and Consumables

Item	Notes
Ammonium acetate (NH_4OAc)	ACS reagent grade; or prepare from glacial acetic acid + NH_4OH
Ethanol (95%)	Reagent grade, to wash excess $NH_4{}^+$
Potassium chloride (KCl), 2 M	Or 10% NaCl; for displacing adsorbed $NH_4{}^+$
Whatman #42 filter paper	Or equivalent, for filtration
Centrifuge tubes (50 mL)	Polypropylene
Volumetric flasks (250 mL)	For collecting leachates
DI water	

7. Procedure

7.1. Reagent Preparation

7.1.1. M Ammonium Acetate (pH 7.0)

1. In a fume hood, dissolve 77.08 g of ammonium acetate ($NH_4C_2H_3O_2$, MW 77.08) in approximately 800 mL of DI water in a 1 L volumetric flask.
2. Adjust to pH 7.0 using dilute acetic acid (to lower pH) or dilute ammonium hydroxide (to raise pH).
3. Bring to 1 L with DI water and mix thoroughly.

> **Tip**
>
> Prepare this solution the day before extraction. It is stable indefinitely at room temperature in a capped container. Verify pH before each use, as CO_2 absorption can lower the pH over time.

7.1.2. M Potassium Chloride

Prepare per SOP-L403, Section 7.1.

7.2. Moisture Subsample

A companion gravimetric moisture determination is required to express results on an oven-dry soil basis.

1. Set aside approximately 10 g of soil from each sample for moisture determination
2. Dry at 105 °C for 24 hours and record the dry weight
3. Calculate gravimetric moisture content per SOP-L400 Soil Moisture

7.3. Ammonium Acetate Saturation (Exchangeable Cations)

1. Weigh 5.00 g of air-dried, sieved (< 2 mm) soil into a labeled 50 mL centrifuge tube. Record the exact mass to 0.01 g.
2. Add 30 mL of 1 M NH_4OAc (pH 7.0) to the tube. Cap securely.
3. Place on the reciprocating shaker for 30 minutes.
4. Centrifuge at 2,000–3,000 x g for 5 minutes. Decant the clear supernatant into a 250 mL volumetric flask. Retain the soil pellet.
5. Repeat steps 2–4 two additional times (three extractions total), combining all supernatants in the same 250 mL volumetric flask.
6. Bring the combined extract to 250 mL with 1 M NH_4OAc and mix. This extract is used for exchangeable cation analysis (Ca^{2+}, Mg^{2+}, K^+, Na^+) by ICP-OES, AAS, or flame photometry (Helmke & Sparks, 1996).

7.4. Ethanol Wash

The ethanol wash removes excess (non-exchanged) $NH_4{}^+$ from the soil without displacing adsorbed $NH_4{}^+$ (Sumner & Miller, 1996).

1. Add 30 mL of 95% ethanol to the soil pellet remaining in the centrifuge tube. Cap and shake for 5 minutes.
2. Centrifuge at 2,000–3,000 x g for 5 minutes. Discard the supernatant ethanol wash.

3. Repeat the ethanol wash two additional times (three washes total).

> **!** Critical Step
>
> Incomplete removal of excess NH_4OAc will cause CEC to be overestimated. Ensure three full ethanol washes with clear supernatant before proceeding.

7.5. Displacement of Adsorbed NH_4^+ (CEC Determination)

1. Add 30 mL of 2 M KCl to the washed soil pellet. Cap and shake for 30 minutes.
2. Centrifuge at 2,000–3,000 x g for 5 minutes. Decant the supernatant into a clean 250 mL volumetric flask.
3. Repeat steps 1–2 two additional times (three displacements total), combining all supernatants in the same flask.
4. Bring the combined KCl extract to 250 mL with 2 M KCl and mix.
5. Determine the NH_4^+-N concentration in this extract by colorimetric analysis (indophenol blue method), flow injection, or steam distillation (Mulvaney, 1996). This value represents the CEC.

7.6. Cleanup

1. Discard spent soil in standard solid waste.
2. Rinse all glassware and centrifuge tubes with DI water.
3. Return reagents to labeled storage areas.

8. Calculations

8.1. Cation Exchange Capacity

$$\text{CEC (cmol}_c\text{/kg)} = \frac{C_{\text{NH}_4} \times V_{\text{KCl}} \times 10^{-3}}{m_{\text{dry}} \times 14.01} \times 100$$

where:

- C_{NH_4} = NH_4^+-N concentration in the KCl displacement extract (mg N/L)
- V_{KCl} = total volume of combined KCl extract (mL; typically 250 mL)
- m_{dry} = oven-dry equivalent mass of soil (g), calculated as $m_{\text{air-dry}}/(1 + \theta_g)$
- 14.01 = atomic mass of nitrogen (g/mol)
- The factor of 100 converts from mol_c/g to cmol_c/kg

8.2. Exchangeable Cations

Report exchangeable Ca^{2+}, Mg^{2+}, K^+, and Na^+ from the NH_4OAc extract (§6.3) in $cmol_c/kg$:

$$\text{Exchangeable cation (cmol}_c\text{/kg)} = \frac{C_{\text{cation}} \times V_{\text{extract}} \times \text{valence}}{M_{\text{cation}} \times m_{\text{dry}}} \times \frac{1}{10}$$

where C_{cation} is in mg/L, V_{extract} in mL, M_{cation} is the atomic mass (Ca = 40.08, Mg = 24.31, K = 39.10, Na = 22.99), and valence is 2 for Ca and Mg and 1 for K and Na.

8.3. Base Saturation

$$\text{Base Saturation (\%)} = \frac{Ca^{2+} + Mg^{2+} + K^+ + Na^+}{\text{CEC}} \times 100$$

where all values are in $cmol_c/kg$.

8.4. Interpretation

CEC ($cmol_c/kg$)	Classification	Typical Soil Textures
< 5	Very low	Sands, degraded tropicals
5–15	Low	Sandy loams, kaolinitic clays
15–25	Medium	Loams, silt loams
25–40	High	Clay loams, smectitic soils
> 40	Very high	Heavy clays, organic soils

Source: adapted from Sparks (2003) and Brady & Weil (2008).

9. Quality Control and Acceptance Criteria

- Include a reagent blank (NH_4OAc and KCl through the full procedure without soil) in each batch. NH_4^+-N in the blank must be below the method detection limit.
- Run extraction duplicates on at least 10% of samples. Duplicate results should agree within 10% RPD.
- Include a laboratory reference soil with known CEC when available.
- Sum of exchangeable base cations should not exceed CEC. If the sum substantially exceeds CEC, suspect incomplete ethanol washing or analytical error.
- Verify NH_4OAc pH is 7.0 ± 0.05 before each extraction batch.

10. Waste Disposal

Waste Type	Container	Disposal Method
NH_4OAc extracts	Drain with water	Low hazard; drain permitted
Ethanol washes	Flammable waste carboy	EH&S pickup
KCl extracts	Drain with water	Low hazard; drain permitted
Spent soil	Standard solid waste	Trash

11. References

- Sumner & Miller (1996)
- Chapman (1965)
- Sparks (2003)
- Helmke & Sparks (1996)
- Mulvaney (1996)
- Gillman & Sumpter (1979)
- Brady & Weil (2008)
- Ross & Ketterings (1995)
- SOP-L002 Laboratory Safety
- SOP-L400 Soil Moisture
- SOP-L403 Nitrate and Ammonium – KCl Extraction

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Gillman, G. P., & Sumpter, E. A. (1979). A proposed method for the measurement of exchange properties of highly weathered soils. *Australian Journal of Soil Research*, *17*(1), 129–139. https://doi.org/10.1071/SR9790129

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Mulvaney, R. L. (1996). Nitrogen: Inorganic forms. In D. L. Sparks, A. L. Page, P. A. Helmke, & R. H. Loeppert (Eds.), *Methods of soil analysis, part 3: Chemical methods* (pp. 1123–1184). Soil Science Society of America. https://doi.org/10.2136/sssabookser5.3.c38

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Sparks, D. L. (2003). *Environmental soil chemistry* (2nd ed.). Academic Press. https://doi.org/10.1016/B978-012656446-4/50001-3

Sumner, M. E., & Miller, W. P. (1996). Cation exchange capacity and exchange coefficients. In D. L. Sparks, A. L. Page, P. A. Helmke, & R. H. Loeppert (Eds.), *Methods of soil analysis, part 3: Chemical methods* (pp. 1201–1229). Soil Science Society of America. https://doi.org/10.2136/sssabookser5.3.c40

12. Training Record

Before performing CEC analysis independently, personnel must:

1. Read this SOP in full
2. Complete SOP-L002 Laboratory Safety training
3. Review the SDS for ammonium acetate, acetic acid, ammonium hydroxide, and ethanol
4. Complete SOP-L400 Soil Moisture training
5. Observe a trained operator perform the full extraction sequence
6. Perform the procedure under supervision with acceptable duplicate precision (RPD $< 10\%$)
7. Be signed off by the PI or lab manager

13. Appendix A: Example Calculation

```r
# --- CEC Calculation Example ---

# Input values
nh4_mg_L    <- 42.5    # NH4-N in KCl displacement extract (mg/L)
vol_KCl_mL  <- 250     # Total volume of combined KCl extract (mL)
mass_air_g  <- 5.00    # Air-dried soil mass (g)
moisture    <- 0.032   # Gravimetric moisture content (decimal)

# Oven-dry mass
mass_dry_g <- mass_air_g / (1 + moisture)

# CEC in cmol_c/kg
cec <- (nh4_mg_L * vol_KCl_mL * 1e-3) / (mass_dry_g * 14.01) * 100

cat(sprintf("Oven-dry soil mass: %.3f g\n", mass_dry_g))
cat(sprintf("CEC: %.1f cmol_c/kg\n", cec))

# --- Exchangeable Cation Example (Ca from NH4OAc extract) ---
ca_mg_L      <- 85.2   # Ca concentration in NH4OAc extract (mg/L)
vol_ext_mL   <- 250    # Total volume of NH4OAc extract (mL)
ca_mw        <- 40.08  # Atomic mass of Ca
ca_valence   <- 2

exch_ca <- (ca_mg_L * vol_ext_mL * ca_valence) / (ca_mw * mass_dry_g) / 10

cat(sprintf("Exchangeable Ca: %.1f cmol_c/kg\n", exch_ca))
```

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L417

Soil Aggregate Stability – Wet Sieving Method

Standard Operating Procedure

SOP Number	SOP-L417
Revision	0.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the determination of soil aggregate stability using the wet sieving method. Aggregate stability quantifies the resistance of soil structural units to disintegration when subjected to disruptive forces such as wetting, raindrop impact, and mechanical disturbance. It is a key

indicator of soil physical quality, erosion susceptibility, and the effects of management practices (tillage, cover cropping, organic amendments) on soil structure (Kemper & Rosenau, 1986; Nimmo & Perkins, 2004).

2. Scope

- **Applies to:** Air-dried mineral soil samples sieved to a defined aggregate size class (typically 1–2 mm or 2–4 mm). Samples must have been collected and processed per SOP-F400 Soil Sampling.
- **Does not apply to:** Sandy soils with negligible aggregation, organic soils (histosols), or aggregate characterization by dry sieving alone
- **Location:** EA Program laboratory, SGM 133

3. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, ensure training compliance
Lab Manager	Maintain wet sieving apparatus
Trained Personnel	Follow this SOP, report results
New Students	Complete training before independent use

4. Definitions

- **Aggregate:** A cluster of soil particles (sand, silt, clay, organic matter) bound together by organic compounds, fungal hyphae, microbial exudates, and electrostatic forces; the basic structural unit of soil.
- **Aggregate stability (wet-sieve method):** A measure of soil aggregate resistance to disruption by the mechanical action of water immersion and oscillation; expressed as the percentage of aggregates retained on a defined sieve size (e.g., 0.25 mm or 2 mm) after wet sieving.
- **Dispersant (sodium hexametaphosphate):** A chemical added to the control (dispersed) sample to break down all aggregates; used to measure the sand correction in aggregate stability calculations.
- **Mean weight diameter (MWD):** A summary statistic for aggregate size distribution calculated as the weighted mean of aggregate size fractions after wet or dry sieving; higher MWD indicates better structural stability.
- **Slaking:** The rapid collapse of aggregates upon sudden immersion in water, caused by compression of entrapped air; fast wetting promotes slaking and reduces aggregate stability measurements.
- **Water-stable aggregate (WSA):** An aggregate that remains intact after defined wet-sieving; the fraction used to calculate aggregate stability.

5. Safety and Hazards

Hazards

- **Chemical:** Sodium hexametaphosphate (dispersing agent) is a mild irritant. Hydrogen peroxide (if used for organic matter correction) is a strong oxidizer.
- **Physical:** Drying oven operates at 105 C; burn risk from hot containers.

5.1. Required PPE

- ☐ Safety glasses
- ☐ Lab coat
- ☐ Nitrile gloves (when handling dispersing solution or H_2O_2)
- ☐ Heat-resistant gloves (when handling containers from the oven)

5.2. Emergency Procedures

For chemical splashes, flush with water for at least 15 minutes. Refer to SOP-L002 Laboratory Safety and SOP-L003 Hazardous Materials Handling.

6. Equipment and Materials

6.1. Equipment

Item	Location	Notes
Wet sieving apparatus (Yoder-type or Eijkelkamp)	SGM 133	Oscillating sieve holder
Nested sieves (e.g., 2 mm, 1 mm, 0.5 mm, 0.25 mm, 0.053 mm)	SGM 133	Stainless steel, 8 cm diameter
Drying oven (105 C)	SGM 133	
Analytical balance (0.001 g)	SGM 133	See SOP-L200
Desiccator with active desiccant	SGM 133	
Pre-weighed aluminum drying tins	SGM 133	

6.2. Reagents and Consumables

Item	Notes
Deionized water	For wetting and sieving
Sodium hexametaphosphate solution (5 g/L)	For dispersing residual aggregates (sand correction)

Item	Notes
Hydrogen peroxide (30%), optional	For removing organic matter if sand correction requires it

7. Procedure

7.1. Sample Preparation

1. Air-dry soil samples at room temperature until friable (typically 48–72 hours)
2. Gently break large clods along natural planes of weakness by hand; do not grind or crush
3. Dry-sieve the sample through a nest of sieves to isolate the desired aggregate size class (e.g., 1–2 mm or 2–4 mm)
4. Discard material outside the target size range for this analysis
5. Weigh out replicate subsamples (typically 4.0 g each, minimum 3 replicates per sample) into labeled containers

7.2. Pre-Wetting (Capillary Wetting)

Rapid wetting causes slaking, which may overestimate instability for some research questions. If slow pre-wetting is desired:

1. Place each subsample on a moistened filter paper in a closed container
2. Allow capillary wetting for 10 minutes (or a duration standardized for the study)
3. Proceed to wet sieving

If the goal is to assess slaking resistance under rapid wetting (the more common approach), omit the pre-wetting step and place air-dried aggregates directly onto the sieve.

7.3. Wet Sieving

1. Place the pre-weighed aggregates on the top sieve of the wet sieving apparatus (e.g., 0.25 mm for a single-sieve method, or the top of a nested stack)
2. Raise the water level in the sieving cans until the sieves are submerged to the appropriate depth (typically 1–2 cm above the sieve mesh)
3. Allow the aggregates to soak for 5 minutes (standardize this time for the study) before starting oscillation
4. Start the wet sieving apparatus. The standard cycle is 3 cm stroke at 34 cycles per minute for 3 minutes (Kemper & Rosenau, 1986). Record the exact settings used.
5. After sieving, carefully raise the sieves from the water

7.4. Collection and Drying

1. Backwash the material retained on each sieve into a pre-weighed aluminum tin using a wash bottle with deionized water

2. Transfer any material that passed through the finest sieve (the material in the sieving can) into a separate tin if size fractionation is desired
3. Dry all tins at 105 C for 24 hours (or until constant mass)
4. Cool in a desiccator and weigh to the nearest 0.001 g

7.5. Sand Correction

The material retained on the sieve after wet sieving includes both water-stable aggregates and primary sand particles that are inherently larger than the sieve openings. To correct for sand:

1. Add 50 mL of 5 g/L sodium hexametaphosphate to the dried residue in each tin
2. Allow to soak for at least 2 hours (or overnight) to fully disperse remaining aggregates
3. Pour the dispersed slurry back through the same sieve used in the wet sieving step
4. Rinse thoroughly with deionized water
5. Backwash the retained sand into a clean tin, dry at 105 C, cool, and weigh

7.6. Calculations

Water-stable aggregates (WSA) as a fraction of the total sample, corrected for sand:

$$\mathrm{WSA}(\%) = \frac{M_{\text{stable}} - M_{\text{sand}}}{M_{\text{total}} - M_{\text{sand}}} \times 100$$

where:

- M_{stable} = oven-dry mass of material retained on the sieve after wet sieving (g)
- M_{sand} = oven-dry mass of sand retained after dispersal (g)
- M_{total} = initial oven-dry equivalent mass of the subsample (g; corrected for moisture using gravimetric moisture from SOP-L400)

If a nested sieve set is used, calculate the mean weight diameter (MWD):

$$\mathrm{MWD} = \sum_{i=1}^{n} \bar{x}_i \, w_i$$

where $\bar{x}_i$ is the mean diameter of the ith size fraction and w_i is the proportion of the total sample weight in that fraction (after sand correction) (Van Bavel, 1950).

8. Quality Control and Acceptance Criteria

- Run a minimum of 3 replicates per sample; report the mean and standard deviation
- The coefficient of variation among replicates should be less than 15%
- Include a laboratory reference soil in each batch to monitor method consistency over time
- Record all apparatus settings (stroke length, frequency, sieving duration) so results are reproducible
- *TBD – identify or prepare a reference soil for long-term QC tracking*

9. Waste Disposal

Waste Type	Disposal Method
Sieved soil and sand	Standard trash or return to sample stock
Sodium hexametaphosphate solution	Drain with copious water (non-hazardous at this concentration)
H_2O_2 waste (if used)	Dilute and drain, or collect for EH&S
Used aluminum tins	Clean and reuse, or recycle

10. References

Kemper, W. D., & Rosenau, R. C. (1986). Aggregate stability and size distribution. In A. Klute (Ed.), *Methods of soil analysis, part 1: Physical and mineralogical methods* (2nd ed., pp. 425–442). Soil Science Society of America. https://doi.org/10.2136/sssabookser5.1.2ed.c17

Nimmo, J. R., & Perkins, K. S. (2004). Aggregate stability and size distribution. In J. H. Dane & G. C. Topp (Eds.), *Methods of soil analysis, part 4: Physical methods* (pp. 317–328). Soil Science Society of America. https://doi.org/10.2136/sssabookser5.4.c14

Van Bavel, C. H. M. (1950). Mean weight-diameter of soil aggregates as a statistical index of aggregation. *Soil Science Society of America Journal*, *14*, 20–23. https://doi.org/10.2136/sssaj1950.036159950014000C0005x

11. Training Record

Before performing this procedure independently, personnel must:

1. Read this SOP in full
2. Complete SOP-L002 Laboratory Safety training
3. Complete SOP-L400 Soil Moisture training (required for moisture correction)
4. Observe a trained operator perform the full procedure including sand correction
5. Perform the analysis under supervision with acceptable replicate precision (CV less than 15%)
6. Be signed off by the PI or lab manager

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L418

Water-Extractable Dissolved Organic Carbon (Soil)

Standard Operating Procedure

SOP Number	SOP-L418
Revision	0.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the extraction and quantification of water-extractable dissolved organic carbon (WEOC) from soil. WEOC represents the fraction of soil organic matter that is readily

soluble in water and includes simple sugars, organic acids, amino acids, and other low-molecular-weight compounds. It is a sensitive indicator of labile carbon availability, microbial substrate supply, and changes in soil management or land use (Ghani et al., 2003; Jones et al., 2006).

WEOC is distinct from the K_2SO_4-extractable DOC measured in the chloroform fumigation-extraction method (SOP-L409) and from total soil organic carbon (SOP-L407). The water extraction uses no salt, making it a closer analogue to the dissolved organic carbon that would leach from soil under natural rainfall or irrigation.

2. Scope

- **Applies to:** Mineral and organic soil samples, compost, and organic amendments. Samples should be collected per SOP-F400 Soil Sampling and stored at 4 C (for field-moist analysis) or air-dried.
- **Does not apply to:** Water samples (analyze directly on the TOC-L), K_2SO_4 extracts for microbial biomass carbon (see SOP-L409), or total soil carbon by combustion (see SOP-L407)
- **Location:** EA Program laboratory, SGM 133

3. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, ensure training compliance
Trained Personnel	Perform extraction and submit samples for TOC analysis
New Students	Complete training before independent use

4. Definitions

- **Dissolved organic carbon (DOC):** Organic carbon that passes through a 0.45 µm filter; in soil extracts, includes low-molecular-weight organic acids, fulvic acids, amino acids, and other water-soluble organic compounds.
- **Hot-water extractable carbon (HWEC):** A more aggressive extraction using hot water (80 °C); releases more labile organic carbon than cold-water extraction; a closer proxy for microbially available carbon and total labile carbon.
- **KHP (potassium hydrogen phthalate):** The primary standard for TOC calibration; a pure, stable organic acid weighed accurately to prepare standards of known carbon concentration.
- **Labile organic carbon:** Readily decomposable organic carbon fractions that turn over on timescales of days to months; includes microbial biomass, water-extractable organic carbon, and dissolved organic carbon; sensitive to management and land use.
- **NPOC (non-purgeable organic carbon):** The organic carbon remaining in a sample after acidification and sparging to remove inorganic carbon; the quantity measured by the Shimadzu TOC-L and the operational definition of DOC.

- **Soil-to-water ratio:** The ratio of soil mass to extraction water volume (typically 1:5 or 1:10 w/v) in the water extraction step; must be reported alongside results and held constant within a study.

5. Safety and Hazards

Hazards

- **Physical:** Drying oven (105 C) and orbital shaker pose minor physical hazards.
- **Biological:** Soil samples may harbor pathogens. Wash hands after handling soil.
- **Chemical:** Concentrated HCl (if used for acidification before TOC analysis) is corrosive.

5.1. Required PPE

- ☐ Safety glasses
- ☐ Lab coat
- ☐ Nitrile gloves

5.2. Emergency Procedures

For acid splashes, flush with water for at least 15 minutes. Refer to SOP-L002 Laboratory Safety.

6. Equipment and Materials

6.1. Equipment

Item	Location	Notes
Orbital or end-over-end shaker	SGM 133	
Centrifuge (capable of 4,000 x g)	SGM 133	
Vacuum filtration apparatus or syringe filters	SGM 133	
Shimadzu TOC-L analyzer	SGM 133	See SOP-L204
Analytical balance (0.01 g)	SGM 133	See SOP-L200
Drying oven (105 C)	SGM 133	For gravimetric moisture

6.2. Reagents and Consumables

Item	Notes
Deionized water (Type I or II, 18.2 MOhm)	Carbon-free; verify with TOC blank

Item	Notes
50 mL centrifuge tubes (polypropylene)	Pre-rinsed with DI water
0.45 um membrane or syringe filters	Pre-combusted glass fiber (GF/F) or PVDF; do not use cellulose acetate (leaches DOC)
Pre-combusted 40 mL glass vials with PTFE-lined caps	For TOC-L analysis
2 M HCl	For acidifying extracts if NPOC mode is used

7. Procedure

7.1. Sample Preparation

1. If using field-moist soil, determine gravimetric moisture content on a separate subsample per SOP-L400
2. If using air-dried soil, pass through a 2 mm sieve and determine residual moisture content

7.2. Extraction

1. Weigh 5.0 g (oven-dry equivalent) of soil into a 50 mL centrifuge tube. Record the exact mass.
2. Add 25 mL of deionized water (1:5 soil-to-water ratio) (Ghani et al., 2003; Jones et al., 2006)
3. Cap the tube and shake on an end-over-end or orbital shaker at approximately 150 rpm for 1 hour at room temperature
4. Centrifuge at 4,000 x g for 10 minutes to separate the supernatant

> **Tip**
>
> Some protocols use a hot water extraction (80 C for 16 hours) to capture a larger fraction of labile carbon (Ghani et al., 2003). If hot water extraction is desired, specify the temperature and duration explicitly in the lab notebook and report, as the two methods are not directly comparable.

7.3. Filtration

1. Filter the supernatant through a 0.45 um filter into a pre-combusted glass vial
2. If using syringe filters, discard the first 2–3 mL of filtrate to flush any carbon leached from the filter
3. Collect at least 15 mL of filtrate for TOC analysis (enough for multiple injections plus rinse volume)

7.4. Storage

1. If extracts cannot be analyzed within 24 hours, acidify to pH less than 2 with 2 M HCl (approximately 1 drop per 10 mL) and store at 4 C
2. Analyze within 7 days of extraction; prolonged storage may alter DOC concentrations even when acidified

7.5. DOC Analysis

1. Analyze filtered extracts on the Shimadzu TOC-L in NPOC mode per SOP-L204
2. Prepare a deionized water blank and extraction blanks (DI water processed through the full extraction procedure) alongside samples
3. Run calibration standards (potassium hydrogen phthalate, KHP) as described in SOP-L204

7.6. Calculations

$$\text{WEOC (mg C kg}^{-1}\text{ dry soil)} = \frac{(C_{\text{sample}} - C_{\text{blank}}) \times V_{\text{extract}}}{m_{\text{soil}}}$$

where:

- C_{sample} = DOC concentration in the filtered extract (mg C/L)
- C_{blank} = DOC concentration in the extraction blank (mg C/L)
- V_{extract} = total volume of extractant added (L; e.g., 0.025 L)
- m_{soil} = oven-dry mass of soil extracted (kg)

8. Quality Control and Acceptance Criteria

- Include extraction blanks (DI water only, no soil) in each batch to quantify background carbon from filters, tubes, and water
- Run sample duplicates for at least 10% of samples; CV between duplicates should be less than 10%
- Include a laboratory reference soil in each batch
- Verify that extraction blank DOC is less than 1 mg/L; if higher, troubleshoot water quality and filter blanks
- Instrument QC follows SOP-L204 requirements (calibration verification, instrument blanks, replicate injection CV less than 2%)

9. Waste Disposal

Waste Type	Disposal Method
Soil residue after extraction	Standard trash or return to sample stock
Filtered water extracts	Drain with copious water (non-hazardous)

Waste Type	Disposal Method
Acidified extracts	Neutralize and drain, or collect for EH&S
Used filters	Standard trash

10. References

Ghani, A., Dexter, M., & Perrott, K. W. (2003). Hot-water extractable carbon in soils: A sensitive measurement for determining impacts of fertilisation, grazing and cultivation. *Soil Biology and Biochemistry*, *35*(9), 1231–1243. https://doi.org/10.1016/S0038-0717(03)00186-X

Jones, D. L., Willett, V. B., Stockdale, E. A., Sherlock, R., & Murphy, D. V. (2006). Dissolved organic carbon dynamics and fluxes in agricultural soils. *Geoderma*, *135*, 200–211.

11. Training Record

Before performing this procedure independently, personnel must:

1. Read this SOP in full
2. Complete SOP-L002 Laboratory Safety training
3. Complete SOP-L400 Soil Moisture training (required for moisture correction)
4. Be trained on SOP-L204 Shimadzu TOC-L or have extracts analyzed by a trained operator
5. Perform the extraction under supervision with acceptable duplicate precision (CV less than 10%)
6. Be signed off by the PI or lab manager

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L419

Soil Color – Munsell Lab Confirmation

Standard Operating Procedure

SOP Number	SOP-L419
Revision	0.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the determination of soil color under controlled laboratory lighting using the Munsell Soil Color Charts. Soil color is a rapid, non-destructive indicator of organic matter content, mineralogy, drainage condition, and degree of weathering. It is a required observation in soil profile descriptions and is used in soil taxonomy (e.g., distinguishing Mollisols by dark surface colors) (Lynn & Pearson, 1993; Soil Survey Division Staff, 2017).

Field color determinations (see SOP-F400) are affected by variable natural lighting, sun angle, and observer fatigue. This laboratory procedure standardizes the observation under a controlled illuminant to improve reproducibility and provide a reference-quality color record.

2. Scope

- **Applies to:** All soil and sediment samples for which a reference-quality Munsell color determination is needed, including both moist and air-dried conditions
- **Does not apply to:** Rock or mineral color determinations using the Munsell Rock Color Chart, instrumental colorimetry or spectrophotometry, or quick field assessments (which remain valuable but are less reproducible)
- **Location:** EA Program laboratory, SGM 133

3. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, ensure training compliance
Trained Personnel	Perform color determinations, record results
New Students	Complete training before independent use

4. Definitions

- **Chroma:** The colorimetric purity or saturation of a color; the third component of Munsell notation (e.g., the 4 in 10YR 4/4); higher chroma values indicate more saturated, vivid colors; low chroma (less than or equal to 2) in subsurface horizons indicates gleying and periodic saturation.
- **Hue:** The dominant spectral color in Munsell notation (e.g., 10YR, 7.5YR, 2.5Y, Gley 1); corresponds to the wavelength of the dominant reflected light; the first element of a Munsell color designation.
- **Munsell color system:** A standardized color notation developed by A.H. Munsell expressing color as three independent components: Hue, Value, and Chroma; used universally in soil science for objective color description.
- **Redoximorphic features:** Color patterns in soil caused by reduction and oxidation of iron and manganese under fluctuating saturation conditions; include masses (concentrations), depletions (zones of low chroma), and concretions; diagnostic for hydric soils.
- **Standard illuminant (D65):** A standardized daylight spectrum at 6,500 K color temperature; the reference lighting condition for accurate Munsell color matching; overcast north-sky light or a D65 light box approximates this condition.
- **Value:** The lightness or darkness of a color in Munsell notation; ranges from 0 (black) to 10 (white); the second component in Munsell notation (e.g., the 4 in 10YR 4/4).

5. Safety and Hazards

Hazards

- **Biological:** Soil samples may contain pathogens. Wash hands after handling soil.
- **Physical:** No significant hazards. If using a spray bottle to moisten samples, avoid wetting electronic equipment or Munsell charts.

5.1. Required PPE

☐ Nitrile gloves
☐ Lab coat

6. Equipment and Materials

Item	Notes
Munsell Soil Color Charts (current edition)	Replace when pages are faded, stained, or damaged
Daylight-balanced lamp (D65 or equivalent, 5,000–6,500 K)	Position to illuminate the sample and chart evenly without glare
Spray bottle with deionized water	For moistening air-dried samples
White ceramic tile or neutral gray card	For calibrating eye to neutral
Spatula or palette knife	For preparing a smooth sample surface
Lab notebook or standardized data sheet	For recording results

7. Procedure

7.1. Lighting Setup

1. Position the daylight-balanced lamp so it illuminates the sample and the Munsell chart at approximately the same angle and distance
2. Avoid mixing light sources (turn off overhead fluorescent or LED room lights if their color temperature differs significantly from the reference lamp)
3. Allow the lamp to warm up for at least 5 minutes before beginning observations

Note

If a daylight-balanced lamp is not available, work near a north-facing window under overcast sky conditions. Avoid direct sunlight, which changes color temperature with sun angle and clouds.

7.2. Sample Preparation

1. For **moist** color: if the sample is already field-moist, proceed directly. If it has been air-dried, moisten a subsample by adding deionized water drop by drop with a spray bottle or pipette until the sample glistens but no free water pools on the surface (Soil Survey Division Staff, 2017)
2. For **dry** color: use the air-dried sample without adding water
3. Use a spatula to press the soil into a smooth, flat surface (approximately 2–3 cm diameter) on a white or neutral background. A rough surface creates shadows that bias the reading toward darker values.
4. If the sample is heterogeneous (e.g., redoximorphic features), determine the color of the matrix and the color of each distinct mottle or concentration separately

7.3. Color Determination

1. Hold the Munsell chart adjacent to the prepared sample so both are under the same illumination
2. Look through the apertures on the chart page, placing the sample behind each hole, to find the closest match
3. First identify the correct **hue** page (e.g., 10YR, 7.5YR, 2.5Y)
4. Then match the **value** (lightness, vertical axis) and **chroma** (saturation, horizontal axis) within that page
5. Record the Munsell notation in the format: hue value/chroma (e.g., 10YR 4/3)
6. If the sample falls between two chips, record the interpolated notation or the nearest chip with a note (e.g., "10YR 4/3 to 4/4")
7. Repeat the determination for both moist and dry conditions

> **Tip**
>
> Color vision varies between individuals and can shift with fatigue. For critical determinations, have two observers independently determine the color and compare results. If readings differ by more than one chip in any dimension, re-examine jointly.

7.4. Recording Results

Record the following for each sample:

- Sample ID and depth
- Munsell notation (moist)
- Munsell notation (dry)
- Descriptive color name from the chart (e.g., "dark yellowish brown")
- Any redoximorphic features (mottle color, abundance, size, contrast)
- Observer name(s)
- Date and lighting conditions

7.5. Special Cases

Redoximorphic features: When mottles or concentrations are present, record the matrix color and the color, abundance (few, common, many), size (fine, medium, coarse), and contrast (faint, distinct, prominent) of each type of feature per the standards in the Soil Survey Manual (Soil Survey Division Staff, 2017).

Very dark samples (value 2 or less): These may be difficult to distinguish on the chart. Prepare a thinner smear and confirm under slightly brighter illumination.

Effervescent or calcareous samples: Note the presence of carbonates (tested with dilute HCl per SOP-L402), as carbonate coatings affect apparent color.

8. Quality Control and Acceptance Criteria

- Perform duplicate readings (by the same or a second observer) on at least 10% of samples
- Acceptable agreement: readings should match within one chip in value and one chip in chroma on the same hue page
- Inspect the Munsell chart for fading or staining at the start of each session; replace if the neutral chip (N/) no longer appears true gray
- Record the lamp model and approximate color temperature in the lab notebook

9. Waste Disposal

Waste Type	Disposal Method
Used soil subsamples	Return to sample container or standard trash
No chemical waste generated	–

10. References

Lynn, W., & Pearson, M. J. (1993). Soil color, mineralogy, and classification. In J. M. Bigham & E. J. Ciolkosz (Eds.), *Soil color* (pp. 71–91). Soil Science Society of America. https://doi.org/10.2136/sssaspecpub31.c5

Soil Survey Division Staff. (2017). *Soil survey manual*. Government Printing Office. https://www.nrcs.usda.gov/resources/guides-and-instructions/soil-survey-manual

11. Training Record

Before performing Munsell color determinations independently, personnel must:

1. Read this SOP in full
2. Review the introductory pages of the Munsell Soil Color Charts

3. Practice matching at least 10 samples under supervision and compare readings with a trained observer
4. Demonstrate agreement within one chip in value and chroma on duplicate determinations
5. Be signed off by the PI or lab manager

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L420

Soil Labile Amino-Nitrogen – Solvita SLAN Test

Standard Operating Procedure

SOP Number	SOP-L420
Revision	0.2
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the determination of soil labile amino-nitrogen (amino-N) using the Solvita SLAN test (Woods End Laboratories) (Brinton, 2016b). The SLAN test measures a chemically labile fraction of organic nitrogen present in soil humus as amino-sugars and related amino compounds. These forms represent a "potentially available" nitrogen pool that lies between the immediately available mineral nitrogen (NH_4^+ and NO_3^-) measured by conventional extraction and the total nitrogen pool measured by combustion or Kjeldahl digestion.

The test uses alkali hydrolysis with 2 mol L^{-1} NaOH to release NH_3 from amino-N compounds in soil (Mulvaney & Khan, 2001). The liberated NH_3 diffuses into a Solvita gel probe during a 24-hour room-temperature incubation. The color change of the gel is proportional to the quantity of labile amino-N and is quantified using the Solvita Digital Color Reader (DCR) in NH_3 mode, yielding results in mg kg^{-1} amino-N.

The SLAN procedure is chemically analogous to the Illinois Soil Nitrogen Test (ISNT), which uses the same 2 mol L^{-1} NaOH reagent but requires heating. The SLAN test offers two practical advantages over the ISNT: heating is not needed, and fewer reagents are required (Moore et al., 2019a). In combination with the Solvita CO_2 Burst test (SOP-L408), SLAN provides a complementary assessment of biological soil fertility by pairing a measure of microbial activity (CO_2 respiration) with a measure of organic nitrogen reserves (Brinton, 2016a).

This SOP is distinct from SOP-L408, which measures microbial CO_2 respiration from rewetted soil, and from SOP-L403, which measures immediately available mineral nitrogen by KCl extraction.

2. Scope

- **Applies to:** Air-dried, sieved mineral soils from all EA Program sites; assessment of organic nitrogen reserves and humus quality; screening for management effects on labile nitrogen pools
- **Does not apply to:** Measurement of immediately available mineral nitrogen (NH_4^+ or NO_3^-; see SOP-L403 or SOP-L404); total soil nitrogen determination (see SOP-L407 and elemental analysis); compost maturity testing (see SOP-L413); field-moist soils (samples must be air-dried before testing)
- **Location:** EA Program laboratory, SGM 133

3. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, ensure training compliance

Role	Responsibility
Lab Manager	Maintain SLAN kit inventory, prepare and store NaOH solution, verify probe expiration dates
Trained Personnel	Perform assay, record and report results
New Students	Complete training before independent use

4. Definitions

- **Alkali-hydrolyzable nitrogen (AHN):** The fraction of organic nitrogen released by hydrolysis in sodium hydroxide solution; operationally defined by the extraction conditions; highly correlated with amino acid nitrogen and microbially available N (Mulvaney & Khan, 2001).
- **Amino acid nitrogen:** Organic nitrogen in the form of free amino acids and simple peptides; the most labile fraction of soil organic nitrogen; the primary substrate measured by the Solvita SLAN test.
- **Diffusion cell:** The sealed incubation jar used in the Solvita SLAN test in which NaOH releases NH_3 from hydrolyzed soil nitrogen into the headspace, where it is captured and measured by a gel probe.
- **Digital Color Reader (DCR):** The Solvita handheld device that reads gel probe color and converts it to a numerical amino-N concentration (mg kg^{-1}); set to NH_3 mode for SLAN measurements.
- **Gel probe (SLAN):** The colorimetric sensor in the Solvita SLAN test that changes color in proportion to accumulated NH_3; read with the DCR after exactly 24 hours of incubation.
- **Labile amino nitrogen (LAN):** The nitrogen fraction estimated by the Solvita SLAN test; a proxy for readily mineralizable organic nitrogen; expressed as mg N kg^{-1} dry soil (Moore et al., 2019b).
- **Nitrogen mineralization:** The biological conversion of organic nitrogen to inorganic forms (NH_4^+ and eventually NO_3^-) by soil microorganisms; the primary process by which plant-available N is replenished from organic matter; positively correlated with LAN (Moore et al., 2019a).

5. Safety and Hazards

Hazards

- **Chemical:** Sodium hydroxide (NaOH) at 2 mol L^{-1} is strongly corrosive and can cause severe burns to skin, eyes, and mucous membranes. Handle with care; refer to the NaOH Safety Data Sheet (SDS) posted in the lab. The SLAN test requires use of a strong reagent and is not intended for casual or unsupervised use.
- **Biological:** Soil samples may contain soil-borne pathogens. Wash hands thoroughly after handling soil.
- **Physical:** Minor risk of glass breakage from incubation jars. Handle jars carefully.

5.1. Required PPE

- ☐ Nitrile gloves (chemical-resistant)
- ☐ Safety glasses or splash goggles
- ☐ Lab coat

5.2. Emergency Procedures

- For NaOH contact with skin, immediately flush the affected area with copious water for at least 15 minutes. Remove contaminated clothing.
- For NaOH contact with eyes, flush with water at the eyewash station for at least 15 minutes and seek medical attention.
- For spills, neutralize with dilute acid (e.g., 0.1 M HCl or vinegar) or contain with absorbent material, then clean up and dispose per institutional guidelines.
- Refer to SOP-L002 Laboratory Safety for general emergency procedures.

6. Equipment and Materials

6.1. Equipment

Item	Notes
Solvita SLAN test kit	Includes NH_3 gel probes, incubation jars with silicone-gasketed lids, 50 mL plastic beakers, color chart, and manual
Solvita Digital Color Reader (DCR)	Set to NH_3 mode; calibrate per manufacturer instructions
Incubation jars (~250 mL)	Glass with silicone-gasketed screw-top lids; provided with kit
Analytical balance (0.01 g)	See SOP-L102
Graduated cylinder (25 or 50 mL)	For dispensing NaOH solution
Timer	24-hour countdown
Lab notebook or data sheet	For recording results

6.2. Consumables and Reagents

Item	Notes
Solvita NH_3 gel probes	One per test; store sealed at room temperature in the dark; check expiration dates
50 mL plastic beakers (cups)	Provided with kit; one per test
Sodium hydroxide (NaOH), 2 mol L^{-1}	Prepare from ACS-grade NaOH pellets; see Section 5.1

6.3. Reagent Preparation

2 mol L^{-1} NaOH solution:

1. Weigh 80.0 g of ACS-grade NaOH pellets.
2. Dissolve in approximately 800 mL of deionized water in a 1 L volumetric flask or heat-resistant beaker. The dissolution is exothermic; add pellets slowly and stir.

> ⚠ Warning
>
> Always add NaOH to water, never water to NaOH. Use a heat-resistant container and allow the solution to cool to room temperature before bringing to final volume.

3. Bring to a final volume of 1000 mL with deionized water.
4. Transfer to a labeled, tightly sealed polyethylene bottle. NaOH solutions absorb CO_2 from the atmosphere; keep sealed when not in use.
5. Label with: reagent name, concentration, date prepared, preparer's initials, and expiration date (6 months from preparation).

7. Procedure

7.1. Sample Preparation

1. Use soil that has been air-dried and sieved through a 2 mm sieve per SOP-F400. Do not grind the soil.
2. If samples were stored at 4 °C after sieving, allow them to equilibrate to room temperature (20–25 °C) for at least 30 minutes before beginning the assay.
3. Gently mix each sample to ensure homogeneity before weighing.

> ℹ Note
>
> The SLAN test is designed for air-dried soil. Results from field-moist soil are not comparable to published SLAN norms or the manufacturer's interpretation guidelines (Brinton, 2016b).

7.2. Incubation Setup

1. Weigh 4.0 g of air-dried, sieved soil into a clean 50 mL plastic beaker. Record the exact mass to 0.01 g.
2. Place the beaker containing soil inside a clean, dry 250 mL incubation jar.
3. Using the graduated cylinder, add 20 mL of 2 mol L^{-1} NaOH directly to the soil in the beaker.
4. Remove a fresh NH_3-sensitive gel probe from its sealed foil packet. Inspect the gel: it should be uniform in color with no cracks, discoloration, or signs of prior exposure. Discard any compromised probes.

5. Place the gel probe in the air space surrounding the plastic beaker. The gel face must be exposed to the headspace and must not contact the NaOH solution or the soil.
6. Seal the jar immediately and securely with the silicone-gasketed lid.
7. Record the jar number, sample ID, soil mass, and start time.

> **Important**
>
> Work efficiently once the NaOH is added. NH_3 begins volatilizing immediately upon contact with the soil; seal the jar as quickly as possible to capture the full emission.

7.3. Incubation

1. Place sealed jars at room temperature (20–25 °C), shielded from direct sunlight and drafts.
2. Incubate undisturbed for 24 hours.
3. Do not open, move, or disturb jars during incubation.

7.4. Reading Results

1. After exactly 24 hours (±30 minutes), open the jar and immediately remove the gel probe.
2. Read the probe promptly; prolonged exposure to ambient air will alter the gel color.

Using the Solvita Digital Color Reader (DCR – recommended):

3. Set the DCR to NH_3 mode.
4. Insert the gel probe into the DCR and record the numerical reading.
5. The DCR reading corresponds to amino-N concentration in mg kg^{-1} per the manufacturer's calibration (Brinton, 2016b).

Using the Solvita Color Chart (visual – semi-quantitative only):

3. Compare the gel probe to the Solvita NH_3 color chart.
4. Record the color number.

> **Tip**
>
> Read probes within 2 minutes of opening the jar. The DCR is strongly recommended for all quantitative work; the color chart provides semi-quantitative screening only.

7.5. Recording Results

Record the following for each test:

- Date and time (start and end of incubation)

- Sample ID, site, and depth
- Soil mass (g)
- NaOH volume added (mL)
- DCR reading (NH_3 mode) or color chart number
- Amino-N concentration (mg kg^{-1})
- Any anomalies (unusual soil color or odor, probe defects)

7.6. Cleanup

1. Carefully pour the NaOH-soil slurry into the designated waste container. Do not pour corrosive solutions down the drain without neutralization (see Section 8).
2. Rinse plastic beakers and incubation jars with deionized water. Dry completely before reuse.
3. Dispose of used gel probes in standard trash.

8. Quality Control and Acceptance Criteria

8.1. QC Requirements

QC Element	Requirement
Duplicates	At least 1 per 10 samples; RPD within 15%
Blank	One jar with 20 mL NaOH, no soil, and a gel probe per batch; probe should show no or minimal color change
DCR calibration	Calibrate per manufacturer instructions before each batch; set to NH_3 mode
Gel probe storage	Store sealed in foil at room temperature, away from light; check expiration dates; never use expired probes
NaOH solution	Verify 2 mol L^{-1}; check expiration; keep tightly sealed

8.2. Corrective Actions

- If the blank reading exceeds baseline by more than one color increment, check for NH_3 contamination sources (nearby ammonium reagents, compost, inadequate ventilation) and repeat the batch.
- If duplicate RPD exceeds 15%, re-run the sample pair. Persistent poor precision may indicate sample heterogeneity; increase homogenization effort before weighing.
- If NaOH solution appears cloudy or shows precipitate, prepare a fresh solution.

8.3. Limitations

The SLAN test does not detect nitrate or urea, and normal ammonium fertilizer rates do not interfere with the measurement. Known limitations include:

- The test may not be sensitive in very low-organic-matter soils (e.g., sandy soils with less than 1% SOM), where results may fall below the detection limit.

- Color matching by visual chart is subjective; the DCR is required for quantitative reporting.
- SLAN measures a chemically defined labile N pool, not the biologically available fraction directly; interpret alongside other soil health indicators.

8.4. Interpretation Guide

SLAN Amino-N (mg kg^{-1})	Interpretation	Implication
Less than 30	Very low	Severely depleted organic N reserves; management intervention warranted
30–60	Low	Limited labile N pool; consider cover crops or organic amendments
60–120	Moderate	Adequate organic N reserves for many soils
120–200	High	Good labile N pool; indicative of healthy humus
Greater than 200	Very high	Abundant organic N reserves; common in high-SOM, no-till, or prairie soils

Thresholds are approximate guidelines adapted from Woods End Laboratories and published field studies (Moore et al., 2019c, 2019b). Interpret SLAN values in the context of soil organic matter content, soil texture, and management history.

8.5. Complementary Tests

Parameter	SOP / Method
Microbial respiration (CO_2 Burst)	SOP-L408
Mineral nitrogen (NO_3^-, NH_4^+)	SOP-L403
Nitrate quick test	SOP-L404
Soil organic matter (LOI)	SOP-L406
Total C and N	SOP-L407 and elemental analysis
pH and electrical conductivity	SOP-L402

9. Waste Disposal

Waste Type	Disposal Method
NaOH-soil slurry	Neutralize with dilute HCl to ~pH 7 before draining, or collect in labeled corrosive waste container per SOP-L006
Used gel probes	Standard trash
Deionized water rinses	Drain
Incubation jars and beakers	Wash, rinse with DI water, and reuse
Unused NaOH solution (expired)	Neutralize before disposal per SOP-L006

10. References

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11. Related SOPs

SOP	Title	Relationship
F400	Soil Sampling and Processing	Sample collection and preparation
L002	Laboratory Safety	Required safety training
L400	Soil Moisture (Gravimetric)	Moisture correction for dry-mass basis
L403	Nitrate and Ammonium (KCl)	Complementary mineral N test
L404	Nitrate Quick Test	Complementary field N screening
L406	Loss on Ignition (SOM)	Complementary organic matter index
L407	Total Carbon	Complementary total C and N
L408	Solvita CO_2 Burst	Paired biological activity test

12. Training Record

Before performing the Solvita SLAN test independently, personnel must:

1. Read this SOP in full
2. Complete SOP-L002 Laboratory Safety training
3. Review the Solvita SLAN Official Instructions manual (included in the kit or available from solvita.com)
4. Demonstrate safe handling and preparation of 2 mol L^{-1} NaOH solution
5. Observe a trained operator perform the full procedure from sample weighing through DCR reading

6. Perform at least one test under supervision with acceptable QC results (duplicate RPD within 15%; blank within acceptable range)
7. Be signed off by the PI or lab manager

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L475

Flash EA Soil Combustion for IRMS Analysis

Standard Operating Procedure

SOP Number	SOP-L475
Revision	1.1
Author(s)	Marc Los Huertos, Kyle McCarty
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes the complete workflow for analyzing soil and sediment samples for δ^{15}N, δ^{13}C, and elemental C and N content using the Thermo Scientific Flash IRMS Elemental Analyzer (EA) coupled to the Delta V Isotope Ratio Mass Spectrometer (IRMS) through the ConFlo IV continuous-flow interface (Thermo Fisher Scientific, 2008b, 2010a). It covers sample preparation, instrument warm-up, sequence creation, running the analysis, shutdown, and data reduction.

For general instrument operation, gas checks, and pre-analysis tests, see SOP-L208. For the full data correction workflow (blank, linearity, scale normalization, and drift correction), see SOP-L414.

2. Scope

- **Applies to:** Soil, sediment, and plant tissue samples analyzed for δ^{13}C, δ^{15}N, and elemental concentrations (%C, %N) by combustion EA-IRMS in NC (dual reactor) mode
- **Does not apply to:** Sulfur isotope analysis (NCS mode), GasBench carbonate analysis (see SOP-L415), TC/EA hydrogen and oxygen isotope analysis, or liquid samples
- **Location:** Sample preparation in SGM Rm 133; analysis in the Oxtoby Environmental Isotope Lab, SGM Rm 135

3. Responsibilities

Role	Responsibility
Lab PI	Approve methods, ensure training compliance
Lab Manager	Prepare and verify instrument methods; maintain reactors
Super-User	Supervise trainees; perform minor maintenance
Certified User	Prepare samples, build sequences, run analyses
Student Researcher	Sample weighing and data reduction (no unsupervised runs)

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; Thermo Fisher Scientific, 2010a).
- **Certified user:** A student, staff, or faculty member qualified to prepare samples and run the IRMS without supervision, as designated by the lab PI.
- **ConFlo IV:** Interface hub connecting the Flash EA and other peripherals to the Delta V IRMS; handles reference-gas delivery and peak switching (Thermo Fisher Scientific, 2008a).
- **δ^{13}C:** Stable carbon isotope ratio expressed in per mil (‰) relative to Vienna Pee Dee Belemnite (VPDB): $\delta^{13}C = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000$ where $R = {}^{13}C/{}^{12}C$ (Coplen, 2011).
- **δ^{15}N:** Stable nitrogen isotope ratio expressed in per mil (‰) relative to atmospheric N_2 (AIR): $\delta^{15}N = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000$ where $R = {}^{15}N/{}^{14}N$ (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 (Thermo Fisher Scientific, 2010a).
- **Isodat:** Thermo Fisher software for analytical sequence design, instrument control, and raw isotope data export (Thermo Fisher Scientific, 2010b).
- **Linearity correction:** Adjustment applied to account for the dependence of measured δ values on peak amplitude at the detector (Paul et al., 2007).
- **Reference material (RM):** A substance with certified or consensus δ values used to anchor measured values to the VPDB or AIR scale; at least two RMs spanning the expected sample range are required for two-point scale normalization (Coplen, 2011; Werner & Brand, 2001).
- **Scale normalization:** Two-point (or multi-point) correction that maps measured δ values to the international isotope-ratio scale using certified reference materials (Coplen, 2011; Paul et al., 2007).
- **Super-user:** A staff or faculty member qualified to run the IRMS and perform minor maintenance (gas replacement, reactor exchange, etc.).
- **Tin capsule:** 5×9 mm pressed-tin container used to encapsulate solid samples; tin ignites in the O_2 pulse, raising combustion temperature briefly to ensure complete combustion.
- **Zero-enrichment test:** Reference gas on/off pulses used to verify that the IRMS measures consistent δ values before analysis; performed during instrument warm-up.

5. Safety and Hazards

Hazards

- **Pressurized gases:** He, O_2, N_2, CO_2. Verify cylinder pressures before each session. He tank pressure must be above 500 psi, regulated to 50 psi.
- **Hot surfaces:** The Flash EA combustion reactor operates above 1000 °C. Use caution when handling ash fingers or reactor tubes.
- **Sharps:** Syringes, tweezers, and capsule-crimping tools can cause puncture or cut injuries.
- **Chemical:** Chromium oxide, silvered cobaltous/cobaltic oxide, and reduced copper used in the reactor packing are irritants; follow standard laboratory handling precautions.

User Responsibilities

The lab manager verifies that methods are working properly, but **Certified Users** are responsible for preparing their own samples, creating run sequences, overseeing runs, and conducting data reduction.

5.1. Required PPE

☐ Safety glasses

- ☐ Laboratory coat
- ☐ Nitrile gloves (especially when handling reactor packing materials)

5.2. Emergency Procedures

Refer to SOP-L208 for instrument-specific emergency procedures and SOP-L002 for general lab safety.

6. Required Materials

6.1. Consumables and Reagents (NC Dual Reactor)

Item	ThermoFisher P/N	EA Consumables P/N
Quartz reactor (set of 2)	468 20070	C1069
Ash finger (quartz insert)	N/A	C1098
Silvered cobaltous/cobaltic oxide	338 24500	B1120
Chromium oxide	338 22900	B1099
Reduced copper wires	338 35300	B1016
Quartz wool	338 22200	—
Magnesium perchlorate (Anhydrone)	281 13102	—

6.2. Capsules and Weighing Supplies

- Tin capsules (appropriate size for your sample mass)
- Tungsten oxide (WO_3) – approximately 2 parts per sample for combustion aid (if required by method)
- Aluminum crucibles (for oven-drying soils)
- Weighing paper, cell wells, tweezers, spatulas
- Kimwipes, gloves

6.3. Standard Reference Materials

Prepare calibrated internal standards for every batch. Confirm which standards you need **before** approaching the lab. Standards on hand (certified values):

Reference Standard	δ^{13}C (VPDB)	δ^{15}N (AIR)
LOCSS	–26.66	7.30
HOCSS	–26.27	4.42
Urea	–43.26	–0.56
USGS24	–16.05	—
USGS40	–26.39	–4.52
USGS41a	36.55	47.6

(Coplen, 2011; Werner & Brand, 2001)

Important

Lab standards are shared resources. Do not use them without approval from a super-user, the lab technician, or the PI. You are expected to replace what you use.

7. Sample Preparation

7.1. Drying and Grinding

1. Weigh soil or sediment samples into aluminum crucibles.
2. Oven-dry at 105 °C until constant mass.
3. Grind dried samples to a fine, homogeneous powder.

7.2. Elemental Pre-screening

Submit a subset of samples to elemental analysis (EA) to determine %C and %N before committing the full batch. This determines the correct sample mass to target the optimal signal range.

7.3. Sample Weighing

The target sample mass depends on %C and %N concentrations (Thermo Fisher Scientific, 2010a):

Typical %C	Target Sample Mass	Notes
> 5% C	3–5 mg	Dilute if signal is too high
1–5% C	7–15 mg	Standard range
< 1% C	15–25 mg	May require longer run times

1. Fold a tin capsule using tweezers (clean hands; no skin oils).
2. Weigh the target sample mass into the capsule.
3. Add WO_3 if required for combustion aids.
4. Crimp the capsule tightly so no sample can escape during handling.
5. Place crimped capsules in sequence order in a cell-well plate.
6. Record the exact mass of each sample in the data reduction spreadsheet.

Tip: Clean Tweezers and Capsules

Oils and residue from fingers contaminate tin capsules, introducing C and N blanks. Handle capsules only with clean, dry tweezers (Thermo Fisher Scientific, 2010a).

8. Instrument Warm-Up and Pre-Analysis Checks

See SOP-L208 for the complete warm-up procedure, gas pressure verification, zero-enrichment test, and linearity check. The Flash EA should be at operating temperature and passing all pre-analysis tests before loading samples.

9. Sequence Design

1. Open Isodat on the acquisition computer (Thermo Fisher Scientific, 2010b).
2. Create a new sequence with the following elements in order:
   - **Empty capsule blanks** (4–6): establish blank baseline
   - **Reference materials** (2–3 each of two or more certified RMs): for scale normalization (Coplen, 2011)
   - **Conditioning samples** (2–3): first samples in the run; typically sacrificial or low-priority
   - **Unknown samples** (bracketed by RMs every 10–12 unknowns)
   - **Closing reference materials** (2–3 of each RM): for drift correction (Paul et al., 2007)
3. Enter sample names, masses, and expected %C/%N for each row.
4. Assign the correct Isodat method to the sequence.
5. Save the sequence file before starting.

10. Running the Analysis

1. Verify carrier gas pressure (He > 500 psi, regulated to 50 psi).
2. Load capsules into the autosampler carousel in sequence order.
3. Press **Start Sequence** in Isodat.
4. Monitor the first 3–4 samples to confirm:
   - N_2 and CO_2 peaks are within the expected mass range
   - Peak shapes are symmetric and well-separated
   - Reference material δ values are within $\pm 0.3‰$ of expected values (Werner & Brand, 2001)
5. If values are drifting, stop the run, identify the cause, and restart after correction.

11. Shutdown

1. After the final sample, allow the sequence to complete fully.
2. Follow the shutdown procedure in SOP-L208.
3. Export the raw data file from Isodat and save to the project directory.

12. Data Reduction

Data reduction follows the protocol in SOP-L414, which covers:

1. Blank correction (empty tin capsule contributions) (Paul et al., 2007)
2. Linearity correction (δ vs. peak area regression)
3. Two-point scale normalization to the VPDB / AIR scale using certified RMs (Coplen, 2011)
4. Drift correction (linear regression through bracketing RMs)
5. Uncertainty estimation and quality flags

13. Definitions of Certification Levels

Level	Capabilities
Lab PI	Unrestricted access; certify super-users
Super-User	Independent operation; certify certified users; replace He, O_2 cylinders; change reactors
Certified User	All steps from sample prep through data reduction; no unsupervised maintenance
Student Researcher	Sample prep and data reduction only, under supervision

14. References

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- Thermo Fisher Scientific (2008b)
- Thermo Fisher Scientific (2008a)
- Thermo Fisher Scientific (2010b)
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15. Related SOPs

SOP	Title	Relationship
L002	Laboratory Safety	General safety framework
L208	Delta V IRMS General Instrument	Instrument startup and QC
L414	Soil CN by Flash EA-IRMS	Full data correction workflow
L415	Carbonate GasBench IRMS	Complementary IRMS application

16. Training Record

Before running analyses independently, personnel must:

1. Read this SOP and SOP-L208 in full
2. Be assigned a certification level by the Lab PI
3. Observe a Super-User or Certified User run a complete sequence from sample loading through data reduction
4. Run a supervised batch of at least 10 unknown samples with RM checks, achieving results within ±0.3‰ of expected values for both C and N
5. Be signed off by the Lab PI or designated Super-User