



Environmental Analysis Program

Pomona College
Biogeochemistry Laboratory

SOP-L116

Sample Grinding and Homogenization – Ball Mill, Mortar and Pestle

Standard Operating Procedure

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1. Purpose

This procedure describes the grinding and homogenization of dried soil, sediment, plant tissue, and other solid environmental samples to produce a fine, uniform powder suitable for chemical, elemental, and isotopic analysis. Many analytical methods require a homogeneous subsample of 0.5–20 mg (e.g., EA-IRMS capsules, [SOP-L414](#)) or 0.5–1.0 g (e.g., acid digestion, [SOP-L410](#), [SOP-L411](#)). Without thorough grinding and homogenization, small subsamples drawn from an incompletely mixed bulk sample may not represent the true composition of that sample, introducing analytical error unrelated to the instrument or method (Bremner, 1996; Nelson & Sommers, 1996). Particle-size reduction to $\leq 150\ \mu\text{m}$ also improves combustion completeness in high-temperature elemental analyzers and reduces between-capsule mass variance in EA-IRMS work (Sollins et al., 1996; Thermo Fisher Scientific, 2010).

2. Scope

- **Applies to:** Dry grinding and homogenization of air-dried or oven-dried (60–105 °C) solid samples, including mineral soils, organic soils, sediments, dried plant tissue, compost, and biochar, using agate or steel mortar and pestle, ball mill, or mechanical grinder.
- **Does not apply to:** Wet grinding or homogenization of fresh tissue (cryogenic grinding for molecular biology applications); sieving-only sample preparation (see [SOP-F400](#) for 2 mm sieving); bead beating for DNA extraction (integral to [SOP-L700](#)); ball milling specifically for TC and EA-IRMS (see [SOP-L117](#)).
- **Location:** EA Program laboratory, SGM 134, Edmunds 017 - Rock Grinding

3. Responsibilities

Role	Responsibility
Lab PI	Approve SOP
Lab Manager	Maintain grinding equipment, replace worn components
Trained Personnel	Grind and homogenize samples per this SOP
New Students	Complete training before independent use

4. Definitions

Term	Definition
Grinding	Mechanical size reduction of solid particles by impact, compression, or abrasion (Bernhardt, 1994).

Term	Definition
Homogenization	Mixing and size reduction to produce a sample in which any subsample of a given mass has the same composition as any other subsample of that mass (Nelson & Sommers, 1996).
Particle size (d_{50})	Median particle diameter by mass; the most common target for soil analysis is 150 μm (U.S. No. 100 mesh sieve) (Nelson & Sommers, 1996).
Cross-contamination	Transfer of material from a previously processed sample to a subsequent one via residue retained on grinding equipment surfaces (Boutton, 1991).
Sacrificial subsample	A small amount of the upcoming sample ground and discarded before collecting the analytical aliquot, to purge residual material from equipment surfaces (Boutton, 1991).
Respirable crystalline silica (RCS)	Quartz and cristobalite particles $\leq 10 \mu\text{m}$ aerodynamic diameter released during grinding of mineral soils; an IARC Group 1 carcinogen (International Agency for Research on Cancer, 1997).
Air-dried	Dried at ambient temperature ($\leq 40^\circ\text{C}$) until mass is constant; the standard pre-treatment for 2 mm sieving (Nelson & Sommers, 1996).

5. Safety and Hazards

Hazards

- **Respirable dust and silica:** Grinding dry soil and plant tissue generates fine respirable dust including crystalline silica (quartz), which is an IARC Group 1 carcinogen at particle sizes $\leq 10 \mu\text{m}$ (International Agency for Research on Cancer, 1997). Always perform grinding inside a fume hood or wear a NIOSH-approved N95 respirator.
- **Contaminated soils:** Soils from legacy-contaminated sites (Pb, As, Hg, pesticides, PAHs) pose additional inhalation and dermal hazards during grinding. Consult the Lab PI before processing (U.S. Environmental Protection Agency, 2007).
- **Noise:** Ball mills and mechanical grinders can exceed 85 dB. Wear hearing protection for runs > 1 min (National Institute for Occupational Safety and Health, 1998).
- **Pinch/crush hazards:** Milling cups and clamps are heavy. Secure milling cups before starting the mill and keep hands clear during operation.
- **Heat:** Milling cups may get hot if samples are run for extended periods of time. Use protective gloves to handle hot items, or wait until they have cooled down enough to avoid burns.
- **Cross-contamination:** Residual sample in grinding equipment is the primary quality

risk in sequential grinding. Thorough cleaning between samples is essential (Section 6.5).

5.1. Required PPE

- ☐ Safety glasses
- ☐ Lab coat
- ☐ Nitrile gloves
- ☐ N95 dust mask or work in fume hood
- ☐ Hearing protection (ball mill or mechanical grinder)

5.2. Emergency Procedures

For dust inhalation, move to fresh air immediately. For cuts from broken equipment, apply first aid per [SOP-L002](#). Report all incidents to the Lab Manager.

6. Equipment and Materials

6.1. Grinding Equipment

Item	Location	Best For
Agate mortar and pestle (various sizes)	SGM 134	Small batches (< 50 g); trace metal work (low contamination)
Ceramic (porcelain) mortar and pestle	SGM 134	General purpose; not for trace metals (contributes Al, Si)
Ball mill (planetary or mixer mill)	Edmunds 017 - Rock Grinding	Small batches (< 5g); estimated particle size
Mechanical grinder (Wiley mill, Thomas mill)	TBD	Plant tissue; coarse initial grinding

6.2. Accessories

Item	Notes
Stainless steel spatula	Transferring sample
Brush (soft bristle, nylon)	Cleaning mortar and milling cups
Lint-free wipes (Kimtech)	Wiping surfaces
70% ethanol spray bottle	Decontamination
Compressed air (can or line)	Blowing dust from crevices
Test sieves (No. 60 = 250 μm ; No. 100 = 150 μm ; No. 200 = 75 μm)	Verifying particle size

Item	Notes
Labeled sample vials or coin envelopes	Storing ground samples
Analytical balance	Per SOP-L200
Weighing boat/paper	Used for weighing samples

7. Procedure

7.1. Sample Selection and Drying

Samples must be thoroughly dry before grinding. Residual moisture causes particles to clump, impedes size reduction, and can promote microbial activity that alters sample chemistry during storage (Nelson & Sommers, 1996).

1. Use air-dried samples sieved to 2 mm ([SOP-F400](#)), or oven-dry per the drying requirements in the table below:

Analytical Pathway	Drying Temperature	Rationale
TC, LOI, metals, or general chemistry	105 °C to constant mass	Standard gravimetric drying; isotope fractionation not a concern (Nelson & Sommers, 1996)
$\delta^{15}\text{N}$, $\delta^{13}\text{C}$ (EA-IRMS)	40–60 °C to constant mass	Temperatures > 60 °C volatilize ^{14}N -enriched NH_4^+ , artificially elevating $\delta^{15}\text{N}$ (Kool et al., 2011; Gutiñas2012?)
Plant tissue (any analysis)	60 °C to constant mass	Higher temperatures degrade labile N compounds and alter tissue $\delta^{15}\text{N}$ (Kalra, 1998)

2. Cool samples to room temperature in a desiccator before grinding.
3. Record drying temperature, start and end times, and initial and final mass in the lab notebook.

7.2. Mortar and Pestle (Manual Grinding)

Manual grinding with mortar and pestle is appropriate for small batches (< 50 g), hard materials requiring careful size control, and laboratories where a ball mill is unavailable (Nelson & Sommers, 1996).

1. Place a clean, dry mortar on a stable bench surface. If working with contaminated soil or generating visible dust, work inside a fume hood.

2. Add a small amount of dried sample (5–20 g) to the mortar.
3. Grind with a circular and pressing motion, periodically scraping material from the sides back to the center.
4. Continue grinding until the sample passes through the target sieve (No. 60 [250 μm] for general chemistry; No. 100 [150 μm] for EA-IRMS (Thermo Fisher Scientific, 2010)). For plant tissue, grind to a uniform fine powder.
5. Transfer the ground sample to a labeled vial or coin envelope with a clean spatula.
6. Record the sample ID and particle size achieved.

Tip

For hard or fibrous samples (e.g., woody roots, calcium carbonate nodules), pre-crush coarsely with a hammer inside a thick plastic bag before transferring to the mortar. This reduces grinding time and wear on the mortar.

7.3. Ball Mill (Mechanical Grinding)

Ball milling provides more reproducible particle size reduction and higher throughput than manual grinding, and is preferred for batches > 20 samples or where particle size uniformity is critical (Bernhardt, 1994).

1. Select the appropriate milling cup and ball material (*confirm which materials we have for balls/jars*):

Material	Best Use Case	Notes
Stainless steel	General chemistry (non-metal)	Faster; may contribute Fe, Cr, Ni, Mn
Ceramic	TBD	TBD

2. Load the sample and milling balls into the milling cup. The milling cup's volume should be occupied roughly by 1/3 sample, 1/3 grinding ball(s) and 1/3 air.

Safe Running

- **Filling:** Sample quantity should not be below 25% of the milling cup's holding capacity.
- **Material** Milling ball and milling cup material must always be the same.
- **Single Running** Do not run machine with only one milling cup loaded. If you only need to use one milling cup, still load the second one completely empty (no sample nor milling balls)

3. Tightly seal milling cup and load it into the milling cup holder. Make sure to insert cups in the correct orientation, as shown below:



Figure 1: *Orientation of milling cup for left hand side holder. Cup should be mirrored for the right hand side holder.*

4. Turn locking pin into the locked position as shown in the image below and turn the handwheel until maximum clamping range is reached. Close hood.



Figure 2: *Locked and unlocked pin positions*

5. Set speed and duration per the table below or per the analytical SOP:

Sample Type	Frequency (Hz)	Duration	Target Size
Organic soil	30	2 min	$\leq 75 \mu\text{m}$
Quartz Sand	30	1 min	N/A

- Run the mill. If the milling cup becomes warm to the touch, alternate 2-minute grinding intervals with 1-minute rest periods to prevent thermal alteration of labile organic matter and N compounds (Kool et al., 2011).
- After run ends, open the hood and unlock the locking pin before removing milling cups. Wait 30 seconds after stopping before opening the milling cup (dust settles). Open inside the fume hood or with N95 protection.
- Transfer the ground sample to a labeled container.
- If required, verify particle size by passing a subsample through the target sieve.
- Perform cleaning run with quartz sand in between sample runs. Add the same volume of quartz sand as the samples you are running, and the milling balls you are using. The quartz run will help clean the cups and remove any sample that might have gotten stuck to the cup. After the quartz run, clean cup thoroughly according to the “Cleaning Between Samples” section.

7.4. Mechanical Grinder (Plant Tissue)

A Wiley-type or Thomas-type cutting mill provides rapid coarse grinding of dried plant tissue and is generally faster than mortar and pestle for batch processing (Kalra, 1998).

- Set the screen mesh to the target particle size (1 mm [No. 18] or 0.5 mm [No. 35]).
- Feed dried tissue into the hopper slowly; do not overload.
- Collect ground material from the output chamber.
- For analyses requiring finer particle size (e.g., EA-IRMS), follow with mortar and pestle or ball mill to reduce further (Thermo Fisher Scientific, 2010).

7.5. Cleaning Between Samples

Cross-contamination is the primary quality risk in sequential grinding. For isotope work, residues as small as 0.05 mg C from a high-carbon sample can produce measurable $\delta^{13}\text{C}$ bias in a subsequent low-carbon sample (Boutton, 1991). Clean thoroughly between every sample:

- Disassemble the mortar/pestle or milling cup and balls.
- Brush out all visible residue with a soft-bristle brush.
- Wipe all surfaces with a lint-free tissue dampened with 70% ethanol.
- Trace metal work:** rinse with 10% HNO_3 (trace-metal grade), then triple-rinse with Type I deionized water (18.2 M Ω), and air-dry (U.S. Environmental Protection Agency, 2007).
- Isotope work:** rinse with Type I deionized water, wipe with ethanol, and blow dry with clean compressed air.

6. **Wiley mill:** run a small amount of clean quartz sand through the mill between samples to abrade residues from the cutting chamber, then brush and wipe.
7. Allow all equipment to dry completely before grinding the next sample.

! Important

When transitioning between samples with very different compositions (e.g., high-carbon compost followed by low-carbon mineral soil), grind a sacrificial subsample of the next sample and discard it before collecting the analytical aliquot. This purges micro-residues that ethanol-wiping alone may not remove (Boutton, 1991). This step is mandatory for EA-IRMS work.

7.6. Storage

1. Transfer ground powder to a labeled vial (glass or polypropylene with a polyethylene-lined cap). Avoid foam-gasketed caps, which can off-gas organic vapors.
2. Label with: sample ID, date ground, equipment and milling cup material, and target particle size.
3. Store in a desiccator at room temperature. Ground samples have large surface area and are hygroscopic; even brief exposure to humid air can add 0.5–2% mass as adsorbed water (Nelson & Sommers, 1996).
4. For long-term storage (> 2 weeks), seal with Parafilm and store at -20°C .
5. Record grinding date, equipment, milling cup material, speed, and duration in the lab notebook and sample tracking spreadsheet.

8. Quality Control

QC Check	Frequency	Acceptance Criterion
Particle size verification (sieve)	$\geq 10\%$ of samples per batch	$\geq 90\%$ by mass passes target sieve; re-grind failures
Equipment blank (clean milling cup 2 min)	Once per batch and after new milling cup	No visible powder; TC blank < 0.05 mg C if applicable
Certified reference material (CRM) – trace metals	1 per batch (trace metal work)	Recovered elemental concentration within 10% of certified value (U.S. Environmental Protection Agency, 2007)
Reference standard – isotopes (USGS40, USGS41a)	1 per batch (isotope work)	$\delta^{13}\text{C}$ within ± 0.5 ‰; $\delta^{15}\text{N}$ within ± 0.5 ‰ of certified value (Brand et al., 2014)
Sample replicate (duplicate grind)	1 per 10 samples	%C or %N: $\text{CV} \leq 5\%$; isotopes: $\text{SD} \leq 0.3$ ‰
Sacrificial subsample log	Every inter-sample cleaning (isotope work)	Recorded in lab notebook

Document grinding equipment and milling cup material for every batch; different materials introduce different trace elements and the record is essential for troubleshooting contamination (Bernhardt, 1994).

9. Waste Disposal

Waste Type	Disposal Method
Grinding residue (non-hazardous soil/plant)	Standard solid waste or return to sample stock
Grinding residue (contaminated soil)	Hazardous solid waste per SOP-L006
Quartz sand (cleaning aid)	Standard solid waste
Used ethanol wipes	Standard solid waste (non-halogenated)
Worn grinding balls or milling cups (agate, ceramic)	Standard solid waste
Worn grinding components (stainless steel)	Metal recycling
HNO ₃ rinse waste	Acid waste per SOP-L006

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11. Related SOPs

SOP	Title	Relationship
F400	Soil Sampling and Processing	2 mm sieving before grinding
F608	Plant Tissue Sampling	Dried tissue requiring grinding
L105	Drying Ovens	Pre-grinding drying
L117	Ball Mill Prep for TC and EA-IRMS	Instrument-specific ball mill protocol
L200	Balance Calibration	Weighing ground subsamples
L406	Loss on Ignition	Downstream analysis
L410	Hot-Plate Acid Digestion	Requires ground sample
L411	MARS Microwave Digestion	Requires ground sample
L414	EA-IRMS Combustion	Requires finely ground sample
L415	Carbonate GasBench-IRMS	Requires ground carbonate sample

12. Training Record

Before performing carbonate isotope analysis independently, personnel must:

1. Read this SOP in full and sign the SOP acknowledgment form.
2. Complete [SOP-L002 Laboratory Safety](#) training.
3. Demonstrate proper mortar-and-pestle technique including grinding motion and inter-sample cleaning.
4. If using the ball mill, demonstrate proper milling cup loading, speed/time selection, and milling cup cleaning per Section 7.5.
5. Grind at least 5 samples under supervision with acceptable particle size verification (all material passes the target sieve).
6. Be signed off by the PI or Lab Manager.

Trainee	Trainer	Date	Initials