



Environmental Analysis Program

Pomona College
Biogeochemistry Laboratory

SOP-L603

Plant Tissue Drying, Grinding, and Preparation for Analysis

Standard Operating Procedure

SOP Number	SOP-L603
Revision	0.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

FOR INTERNAL USE ONLY

This document is the property of the Environmental Analysis Program at Pomona College.
Unauthorized distribution is not permitted.

Table of contents

1 Purpose	1
2 Scope	2
3 Responsibilities	2
4 Definitions	2
5 Safety and Hazards	3
5.1 Required PPE	3
5.2 Emergency Procedures	3
6 Equipment and Materials	3
6.1 Equipment	3
6.2 Consumables	3
7 Procedure	4
7.1 Sample Receiving and Cleaning	4
7.2 Drying	4
7.3 Coarse Grinding (Wiley Mill)	4
7.4 Fine Grinding (for IRMS or Homogeneity-Critical Analyses)	5
7.5 Weighing into Tin Capsules (for EA-IRMS)	5
8 Quality Control	5
9 Waste Disposal	6
10 References	6
11 Related SOPs	6
12 Training Record	6

1. Purpose

This SOP describes the laboratory procedures for drying, grinding, and preparing plant tissue samples for subsequent elemental, isotopic, or nutrient analysis. Proper sample preparation is essential for obtaining representative, reproducible analytical results. The procedures follow standard methods for plant analysis (Jones, 2001; Kalra, 1998; Mills & Jones, 2004).

2. Scope

- **Applies to:** Leaf, stem, root, litter, and other plant tissue samples collected from EA Program field sites, prepared for total carbon and nitrogen (EA-IRMS), total phosphorus, metal analysis (ICP-MS after digestion), or other elemental determinations
- **Does not apply to:** Fresh tissue assays that require unfixed material (e.g., chlorophyll extraction from fresh leaves, enzyme activity assays); seed or fruit tissue with high oil content (requires freeze-drying); soil organic matter (see [SOP-L406](#))
- **Location:** EA Program laboratory, SGM 133

3. Responsibilities

Role	Responsibility
Lab PI	Approve drying protocol and grinding method
Trained Personnel	Process samples per this SOP
New Students	Complete training before independent use

4. Definitions

- **Acid washing (plant tissue):** Cleaning tissue with dilute HCl to remove surface contamination (dust, soil particles, fertilizer residues) before analysis for trace metals or nutrients; may be inappropriate for samples requiring analysis of acid-labile compounds.
- **Air drying:** Drying plant tissue at ambient temperature (typically 20–40 °C) to a stable moisture content; used as a preliminary step before oven drying; preserves enzyme activity and RNA better than direct oven drying.
- **Ball milling:** Size reduction by grinding samples between hardened steel or ceramic balls inside a rotating vessel; the preferred method for producing a homogeneous powder from fibrous plant tissue for EA-IRMS and elemental analysis.
- **Critical tissue concentration:** The minimum concentration of a nutrient required for maximum growth; below this level the plant is deficient; above it, growth is normal or the nutrient may be toxic at very high levels.
- **Lyophilization (freeze drying):** Removal of water from tissue under vacuum after rapid freezing; preserves cell structure, volatile compounds, and biological activity better than oven drying; required for enzyme, RNA, and some metabolite analyses.
- **Subsampling:** Drawing a representative small portion from a bulk dried, ground sample for analysis; requires thorough mixing and homogenization of the bulk powder before subsampling to ensure the subsample is representative.

5. Safety and Hazards

Hazards

- **Physical:** Drying ovens operate at 60–105 °C; burn risk from hot trays and glassware. The Wiley mill and ball mill contain rapidly moving parts; keep hands, hair, and loose clothing away from the grinding chamber.
- **Noise:** The Wiley mill generates noise above 85 dBA. Wear hearing protection during operation.
- **Dust:** Grinding produces fine particulate matter. Work in a well-ventilated area or use a dust mask, especially when processing large batches.
- **Biological:** Field-collected plant tissue may harbor ticks, spiders, or allergenic compounds (e.g., poison oak resin). Inspect samples before handling; wear gloves.

5.1. Required PPE

- ☐ Safety glasses or goggles
- ☐ Lab coat
- ☐ Nitrile gloves
- ☐ Hearing protection (Wiley mill operation)
- ☐ Dust mask or N95 respirator (grinding large batches)

5.2. Emergency Procedures

For burns from the oven, cool affected area under running water. Refer to [SOP-L002 Laboratory Safety](#).

6. Equipment and Materials

6.1. Equipment

Item	Notes
Drying oven	Forced-air convection; set to 60 °C (standard) or 105 °C (rapid dry)
Wiley mill (Thomas Scientific)	With 1 mm (20-mesh) screen
Ball mill or mortar and pestle	For fine grinding (< 250 μ m) for IRMS
Analytical balance	0.0001 g readability (for weighing into tin capsules)
Desiccator with desiccant	For cooling dried samples

6.2. Consumables

Item	Notes
Paper bags or aluminum foil trays	For oven drying; labeled with pencil
Labeled sample bags (polyethylene or paper)	For storing ground tissue
Tin capsules (5 x 9 mm)	For EA-IRMS analysis
Kimwipes	For cleaning mill between samples
70% ethanol (spray bottle)	For decontaminating equipment

7. Procedure

7.1. Sample Receiving and Cleaning

1. Log incoming samples in the laboratory tracking spreadsheet. Verify site ID, date, species or tissue type, and collector.
2. If tissue was not rinsed in the field, briefly rinse leaf or stem material with deionized water to remove surface soil, dust, and epiphytes. Do not soak, as leaching of soluble compounds will occur (Kalra, 1998).
3. Blot excess water with paper towels.
4. For root samples, remove adhering soil by gentle agitation in DI water; multiple rinses may be required.

7.2. Drying

1. Place cleaned tissue in labeled paper bags or foil trays. Do not overload bags; tissue should be loosely arranged to allow air circulation.
2. Dry at 60 °C in the forced-air oven for a minimum of 48 hours, or until constant mass is achieved (two consecutive weighings 4 hours apart differ by less than 0.1% of sample mass) (Jones, 2001).
3. For samples intended only for total C and N by combustion (not volatile compound analysis), drying at 70 °C is acceptable and reduces drying time.

! Important

Do not exceed 70 °C for samples intended for nitrogen analysis, as volatile nitrogen compounds may be lost at higher temperatures. Do not exceed 60 °C for samples intended for analysis of volatile organic compounds, essential oils, or lipids (Kalra, 1998).

4. Remove dried samples from the oven and cool in a desiccator for at least 30 minutes before weighing or grinding.
5. Record the dry mass if gravimetric moisture content is needed.

7.3. Coarse Grinding (Wiley Mill)

1. Inspect the mill: confirm the 1 mm (20-mesh) screen is installed and the cutting blades are sharp and properly seated.

2. Turn on the mill and allow it to reach full speed before feeding material.
3. Feed dried tissue into the hopper in small amounts, pushing material gently with the wooden plunger. Do not force tissue into the blades.
4. Collect the ground material passing through the screen in a clean, labeled container or bag.
5. Between samples, disassemble the grinding chamber and clean thoroughly:
 - Brush all surfaces with a stiff brush to remove residual material
 - Wipe with 70% ethanol
 - Run a small amount of acid-washed quartz sand through the mill as a blank grind if cross-contamination of trace metals is a concern
 - Allow to dry before the next sample

7.4. Fine Grinding (for IRMS or Homogeneity-Critical Analyses)

For EA-IRMS analysis, the Wiley-milled material (less than 1 mm) must be further ground to a fine, homogeneous powder (less than 250 μm):

1. Transfer approximately 1–2 g of coarsely ground tissue to a clean ball mill vial with grinding balls.
2. Grind for 2–3 minutes until the material is a uniform fine powder.
3. Alternatively, use an agate mortar and pestle, grinding until no gritty texture remains.
4. Transfer the powder to a labeled vial or sample bag.
5. Clean the ball mill vial and balls between samples with 70% ethanol and Kimwipes.

7.5. Weighing into Tin Capsules (for EA-IRMS)

1. Using the analytical balance, tare a tin capsule on the balance pan.
2. Weigh 3–5 mg of finely ground tissue into the capsule (Brodie et al., 2011). Record the mass to 0.001 mg.
3. Fold the capsule into a tight pellet using forceps, ensuring no material escapes.
4. Place the pellet in a labeled well of a microplate or sample tray.
5. Include blanks (empty tin capsules) and reference materials (e.g., acetanilide, USGS-40) in the run sequence per [SOP-L414](#).

8. Quality Control

- Grind and analyze duplicate subsamples at a rate of at least 1 per 10 samples to assess preparation variability. Analytical duplicates should agree within 5% RPD for C and N concentrations.
- Include a laboratory reference material (e.g., NIST SRM 1515 Apple Leaves or NIST SRM 1547 Peach Leaves) in each analytical batch to verify accuracy (Kalra, 1998).
- Document all cleaning steps in the laboratory notebook, especially the blank grind between samples when trace metal analysis is planned.
- Confirm drying completeness by re-weighing a subset of samples after an additional 4 hours of drying.

9. Waste Disposal

Waste Type	Disposal Method
Plant tissue waste	Standard trash (non-hazardous)
Ethanol cleaning rinses	Evaporate in fume hood or flammable waste
Used tin capsules (unfired)	Standard trash
Quartz sand (blank grind)	Standard trash

10. References

- Brodie, C. R., Casford, J. S. L., Lloyd, J. M., Sherlock, S. C., Sherlock, S. C., Sherlock, S. C., & Sherlock, S. C. (2011). A comparison of GasBench II and elemental-analyser-based methodologies for measuring the stable carbon isotope composition of soil organic carbon. *Rapid Communications in Mass Spectrometry*, 25(16), 2413–2421. <https://doi.org/10.1002/rcm.5149>
- Jones, J. B. (2001). *Laboratory guide for conducting soil tests and plant analysis*. CRC Press. <https://doi.org/10.1201/9781420025293>
- Kalra, Y. P. (1998). *Handbook of reference methods for plant analysis*. CRC Press. <https://doi.org/10.1201/9781420049398>
- Mills, H. A., & Jones, J. B. (2004). *Plant analysis handbook II: A practical sampling, preparation, analysis, and interpretation guide*. Micro-Macro Publishing.

11. Related SOPs

SOP	Title	Relationship
L002	Laboratory Safety	Required safety training
L102	Using Balances	Balance operation
L406	Loss on Ignition	Related preparation for SOM
L414	Flash EA Combustion (IRMS)	Downstream analytical method
L410	Acid Digestion	Downstream digestion for metals

12. Training Record

Before processing plant tissue independently, personnel must:

1. Read this SOP in full
2. Complete [SOP-L002 Laboratory Safety](#) training
3. Demonstrate safe Wiley mill operation (startup, feeding, cleaning, shutdown) under supervision
4. Process at least five samples under supervision, including decontamination steps between samples

5. If preparing samples for IRMS, demonstrate fine grinding and accurate weighing into tin capsules (target mass 3–5 mg, recorded to 0.001 mg)
6. Be signed off by the PI or lab manager