



Environmental Analysis Program

Pomona College
Biogeochemistry Laboratory

SOP-F608

Plant Tissue Sampling

Standard Operating Procedure

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1. Purpose

This SOP describes procedures for collecting, handling, and preserving plant tissue samples in the field for subsequent laboratory analysis. Plant tissue analysis provides data on nutrient concentrations (C, N, P, K, metals), stable isotope ratios ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$), and structural traits such as leaf mass per area (LMA) (Kalra, 1998; Mills & Jones, 2004). Proper sampling technique is essential to ensure that results represent the target tissue and are not contaminated or degraded during collection and transport.

Foliar nutrient concentrations and LMA form part of the “leaf economics spectrum” (LES), a global axis of plant strategy that links leaf structure to function across biomes: species with low LMA and high leaf nitrogen tend toward fast resource acquisition and short leaf lifespans, while species with high LMA and low nitrogen invest in durable, long-lived leaves (Reich, 2014; Wright et al., 2004). Extending this framework, the “fast-slow” plant economics spectrum integrates leaf, stem, and root traits into a whole-plant strategy axis (Reich, 2014; Westoby et al., 2002). Standardized sampling protocols allow EA Program data to be compared with global trait databases (e.g., TRY, BIEN) and regional reference values (Cornelissen et al., 2003; P’erez-Harguindeguy et al., 2013).

2. Scope

- **Applies to:** Collection of leaf, stem, root, and reproductive tissue from vascular plants for nutrient and isotope analysis at all EA Program field sites
- **Does not apply to:** Soil organic matter sampling (see [SOP-F400](#)); algal tissue sampling; plant tissue culture; DNA extraction from plant tissue (see [SOP-L700](#))
- **Location:** All EA Program field sites; initial processing in SGM 133/134

3. Definitions

- **Tissue type:** The specific plant organ sampled (e.g., fully expanded mature leaves, current-year stem growth, fine roots less than 2 mm diameter, seeds).
- **Composite sample:** A sample formed by combining tissue from multiple individuals of the same species within a plot to represent the population average. Compositing reduces analytical cost but eliminates information about individual-level variation.
- **Voucher specimen:** A pressed and identified plant specimen collected alongside tissue samples to verify species identification.
- **Leaf mass per area (LMA):** The ratio of leaf dry mass to one-sided leaf area (g m^{-2}). LMA is a key functional trait that reflects the trade-off between resource capture (photosynthesis per unit area) and leaf durability. High-LMA leaves are thicker or denser, with lower nitrogen per unit mass but greater physical toughness (Poorter et al., 2009; Wright et al., 2004).
- **Specific leaf area (SLA):** The inverse of LMA: $\text{SLA} = 1/\text{LMA}$, expressed as $\text{m}^2 \text{kg}^{-1}$. SLA and LMA contain the same information but are used in different contexts; report whichever is standard for the analysis.
- **Leaf economics spectrum (LES):** A global pattern relating leaf traits (LMA, nitrogen, phosphorus, photosynthetic capacity, and leaf lifespan) along a single axis of variation. High-nitrogen, low-LMA species occupy the “fast return” end; low-nitrogen, high-LMA species occupy the “slow return” end (Wright et al., 2004).

- **Sun leaf / shade leaf:** Leaves developed in high-light (sun) or low-light (shade) conditions. Sun and shade leaves from the same tree can differ by 20–40% in nitrogen concentration and by a factor of two or more in LMA (Cornelissen et al., 2003; P’erez-Harguindeguy et al., 2013). Sampling must standardize canopy position to avoid confounding light environment with species-level traits.
- **Field blank:** An empty labeled sample container opened and exposed at the field site, then sealed and processed identically to real samples. Blanks detect contamination introduced during collection and transport.

4. Responsibilities

Role	Responsibility
PI / Field Lead	Approve sampling design and target species
Trained Personnel	Collect and process tissue per this SOP
New Students	Complete training before independent sampling

5. Safety and Hazards

Hazards

- **Biological:** Contact with allergenic plants (poison oak, stinging nettles). Some target species may have thorns or irritating sap.
- **Physical:** Use of pruning shears and scissors poses a laceration risk.
- **Chemical:** If using liquid nitrogen for snap-freezing, follow cryogenic safety procedures (gloves, face shield, ventilated area).

5.1. Required PPE

- ☐ Nitrile gloves
- ☐ Closed-toe shoes or field boots
- ☐ Sun protection
- ☐ Leather gloves (for thorny species)
- ☐ Cryogenic gloves and face shield (if using liquid nitrogen)

5.2. Emergency Procedures

For cuts from pruning shears, apply direct pressure and clean the wound. For poison oak exposure, wash with cold water and soap as soon as possible. Refer to [SOP-F001 Field Safety](#).

6. Required Materials

Item	Notes
Stainless steel pruning shears or scissors	Clean between species
Paper coin envelopes or labeled paper bags	For drying samples; avoid plastic for fresh tissue intended for drying
Ziplock bags (gallon size)	For fresh tissue requiring freezing
Cooler with ice packs	For transport of fresh tissue
Permanent markers (Sharpie)	For labeling
Pre-printed labels	Site, species, tissue type, date, collector
DI water squeeze bottle	For rinsing contamination
Ethanol (70%) in a spray bottle	For decontaminating tools between species
Paper towels	For drying tools after decontamination
GPS unit	For recording sampling locations
Plant identification guides	For species verification
Plant press (if collecting vouchers)	See SOP-F603 Section 10
Field data sheet	Pre-printed
Liquid nitrogen dewar and cryo-vials	Only if snap-freezing is required
Analytical balance (field-portable, optional)	For recording fresh mass
Leaf area meter or scanner (optional)	For measuring fresh leaf area in the lab before drying (needed for LMA calculation)

7. Estimated Time

Task	Time
Site setup and species identification	15–30 minutes
Tissue collection (10 samples)	30–60 minutes
Labeling and packing for transport	15 minutes
Lab processing (cleaning, drying)	Variable (see Section 9)

8. Procedure

8.1. Sampling Design

1. Define the target species, tissue type, and number of replicates in the sampling plan before going to the field
2. Determine whether individual or composite samples are needed. For nutrient analysis where individual variation is important, collect tissue from individual plants. For site-level averages, composite tissue from 5–10 individuals per species per plot.
3. Select individuals randomly within the sampling area. Avoid plants at plot edges, obviously damaged or diseased individuals, and plants with heavy herbivory or epiphyte loads (unless these are the target condition).

8.2. Leaf Tissue Collection

1. Put on clean nitrile gloves. Change gloves between species to avoid cross-contamination.
2. Select fully expanded, mature leaves from the outer canopy (sun leaves) unless the project specifically targets shade leaves or another position. Standardizing canopy position is critical for comparability: sun and shade leaves from the same individual can differ by 20–40% in nitrogen concentration and by a factor of two in LMA (Cornelissen et al., 2003; P'erez-Harguindeguy et al., 2013). For trees, sample from the sunlit portion of the mid- to upper crown; for herbs and grasses, sample the most recently fully expanded leaf.
3. Using clean pruning shears, clip the petiole at its base. For grasses, clip the most recent fully expanded blade at the ligule.
4. Collect enough tissue mass for the planned analyses. As a guideline:

Analysis	Minimum dry mass
C, N, S by EA-IRMS	2–5 mg
$\delta^{13}\text{C}$, $\delta^{15}\text{N}$	2–5 mg
Total P, metals (acid digestion)	100–500 mg
LMA (requires leaf area scan)	3–5 leaves per individual
Multiple analyses from one sample	2–5 g fresh weight recommended

5. For composite samples, collect an approximately equal mass of tissue from each individual and combine in a single bag.
6. Place tissue immediately into a labeled paper envelope (if air-drying) or a labeled Ziplock bag (if freezing or transporting fresh). For frozen samples, place bags into the cooler with ice packs.

8.3. Measuring Leaf Area (for LMA/SLA)

If LMA or SLA is needed, leaf area must be measured on fresh tissue before drying (P'erez-Harguindeguy et al., 2013):

1. Transport fresh leaves to the lab within 6 hours of collection, keeping them in sealed bags in the cooler to minimize wilting
2. Scan leaves on a flatbed scanner (600 dpi) or measure with a leaf area meter. Record total one-sided area (cm^2) for each sample.
3. Dry and weigh as described in Section 9.1
4. Calculate $\text{LMA} = \text{dry mass (g)} / \text{area (m}^2\text{)}$, or $\text{SLA} = \text{area (m}^2\text{)} / \text{dry mass (kg)}$

8.4. Stem, Root, and Reproductive Tissue

- **Stems:** Clip current-year growth. Record the diameter and length of the stem segment.
- **Fine roots (less than 2 mm diameter):** Excavate carefully around the base of the target plant using a trowel. Separate live roots from dead roots and soil. Rinse briefly with DI water in the field or at the first opportunity in the lab.
- **Seeds/fruits:** Collect from a minimum of 5 individuals. Record the phenological stage (e.g., immature, mature, dehiscent).

8.5. Decontamination Between Species

1. Spray pruning shears with 70% ethanol
2. Wipe dry with a clean paper towel
3. Allow to air-dry for 30 seconds before cutting the next species
4. For metal analysis, use stainless steel tools only (no galvanized or painted tools) and rinse with DI water after ethanol to remove residual salts

8.6. Labeling

Every sample must be labeled with:

- Site name and plot/transect ID
- Species (use the 6-letter code from [SOP-F603](#) Section 12.2)
- Tissue type (leaf, stem, root, seed)
- Individual or composite (if composite, record the number of individuals)
- Date (YYYY-MM-DD)
- Collector initials

Write on both the bag/envelope and a separate tag inside (in case the exterior label smudges).

8.7. Field Data Sheet

Record the following for each sample:

Field	Entry
Sample ID	Unique sequential number
Site / Plot	
Species	
Tissue type	
Number of individuals	(if composite)
Canopy position	Sun / shade / mixed
GPS coordinates	
Fresh mass (g)	(if field balance available)
Notes	Condition, herbivory, phenology

9. Sample Preservation and Lab Processing

9.1. Drying

The default preservation method for nutrient and isotope analysis (Jones, 2001):

1. Transport paper envelopes to the lab within 24 hours

2. Dry at 60 C in the drying oven ([SOP-L105](#)) for at least 48 hours or until a constant mass is reached
3. Record the dry mass
4. Store dried tissue in labeled coin envelopes in a desiccator or sealed container until grinding

! Temperature Matters

Do not exceed 60 C for tissue intended for nitrogen or carbon isotope analysis. Higher temperatures can volatilize nitrogen compounds and alter $\delta^{15}\text{N}$ values (Kalra, 1998). For samples intended only for total C and N by combustion (not isotope analysis), drying at 70 C is acceptable and reduces drying time.

9.2. Freezing

For analyses requiring fresh tissue (enzyme assays, gene expression, volatile compounds):

1. Transport in cooler with ice packs (or snap-freeze with liquid nitrogen in the field)
2. Transfer to the -80 C freezer ([SOP-L106](#)) within 6 hours of collection
3. Record in the freezer inventory

9.3. Grinding

1. Grind dried tissue to a fine powder using a ball mill or mortar and pestle
2. Clean the grinding equipment between samples (brush, then wipe with ethanol)
3. Store ground tissue in labeled microcentrifuge tubes or coin envelopes

10. Quality Control

- Collect at least one field blank per sampling event: open an empty labeled bag at the site, expose it to the same conditions as the samples, then seal and process identically
- For metal analysis, verify that tools are stainless steel and DI-rinsed
- Record the time between collection and drying/freezing on the data sheet; flag any samples exceeding the 24-hour (drying) or 6-hour (freezing) window
- For composite samples, verify that approximately equal mass was contributed by each individual
- When interpreting foliar nutrient results, compare against published reference ranges for the target species or functional group (Mills & Jones, 2004; Reuter & Robinson, 1997). Values outside the expected range may indicate contamination, analytical error, or genuine ecological signal (e.g., nutrient deficiency or toxicity).
- For LMA, verify that leaf area was measured on fresh, fully turgid tissue; wilted leaves underestimate area and inflate LMA (Pérez-Harguindeguy et al., 2013)

11. References

- Cornelissen, J. H. C., Lavorel, S., Garnier, E., D'iaz, S., Buchmann, N., Gurvich, D. E., Reich, P. B., Steege, H. ter, Morgan, H. D., Heijden, M. G. A. van der, Pausas, J. G., & Poorter, H. (2003). A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. *Australian Journal of Botany*, 51(4), 335–380. <https://doi.org/10.1071/BT02124>
- 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.
- P'erez-Harguindeguy, N., D'iaz, S., Garnier, E., Lavorel, S., Poorter, H., Jaureguiberry, P., Bret-Harte, M. S., Cornwell, W. K., Craine, J. M., Gurvich, D. E., et al. (2013). New handbook for standardised measurement of plant functional traits worldwide. *Australian Journal of Botany*, 61(3), 167–234. <https://doi.org/10.1071/BT12225>
- Poorter, H., Niinemets, U., Poorter, L., Wright, I. J., & Villar, R. (2009). Causes and consequences of variation in leaf mass per area (LMA): A meta-analysis. *New Phytologist*, 182(3), 565–588. <https://doi.org/10.1111/j.1469-8137.2009.02830.x>
- Reich, P. B. (2014). The world-wide “fast–slow” plant economics spectrum: A traits manifesto. *Journal of Ecology*, 102(2), 275–301. <https://doi.org/10.1111/1365-2745.12211>
- Reuter, D. J., & Robinson, J. B. (1997). *Plant analysis: An interpretation manual* (2nd ed.). CSIRO Publishing.
- Westoby, M., Falster, D. S., Moles, A. T., Vesk, P. A., & Wright, I. J. (2002). Plant ecological strategies: Some leading dimensions of variation between species. *Annual Review of Ecology and Systematics*, 33, 125–159. <https://doi.org/10.1146/annurev.ecolsys.33.010802.150452>
- Wright, I. J., Reich, P. B., Westoby, M., Ackerly, D. D., Baruch, Z., Bongers, F., Cavender-Bares, J., Chapin, T., Cornelissen, J. H. C., Diemer, M., et al. (2004). The worldwide leaf economics spectrum. *Nature*, 428, 821–827. <https://doi.org/10.1038/nature02403>

12. Training Record

Before collecting plant tissue independently, personnel must:

1. Read this SOP and [SOP-F001 Field Safety](#)
2. Demonstrate species identification for the target community
3. Demonstrate proper collection technique (glove changes, tool decontamination, labeling, canopy position standardization)
4. Process one set of samples through drying and grinding under supervision
5. Be signed off by the PI or field lead