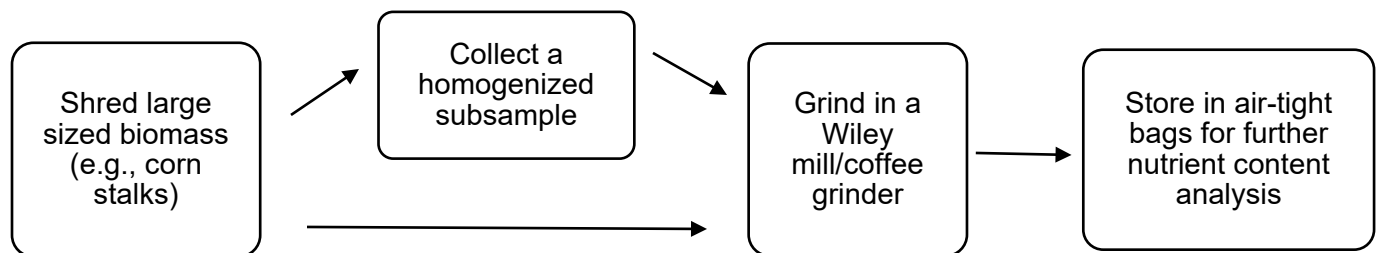


SOP: Tissue Grinding

Overview:

This standard operating procedure (SOP) describes a protocol for plant tissue processing post in-field sample collection and drying to subsequently quantify above (shoot), below (root) and total tissue nutrient (e.g., N, P) concentrations for corn/soybean. The protocol is modified from Jones et al (1991). For the previous steps on sample collection, drying and dry biomass/yield measurement, refer to Above-ground Biomass: Corn/Soybean SOP. Key instruments and accessories include shredder, Wiley mill, coffee grinder, resealable Ziploc bags and sharpies.



Safety:

All standard safety protocols and online safety training via UIUC [Division of Research Safety \(DRS\)](#) are required.

- Safety goggles
- 3M N 95 masks (rated for PM 2.5 pollution)

Instrumentation:

Shredding

- Earthwise GS70015 15-Amp Garden Corded Electric Chipper, Collection Bin (https://www.amazon.com/Earthwise-GS70015-Electric-Shredder-Collection/dp/B00W874Y46/ref=asc_df_B00W874Y46/?tag=hyprod-20&linkCode=df0&hvadid=241967373029&hvpos=&hvnetw=g&hvrnd=14641157928944103808&hvpone=&hvptwo=&hvqmt=&hvdev=c&hvdvcmid=&hvlocint=&hvlocphy=9022196&hvtargid=pla-395303946696&psc=1)

Tissue grinding

- Wiley mill
- Blade coffee grinder

- 1 gallon size Ziploc bags
- Sharpies

Detailed Procedure:

I. Shredding

Note: Usually shredding is required for corn biomass (except V7) as the volume of the biomass and stalks are difficult to be ground when directly passed through a Wiley mill.

1. Oven dried (dried to 60 °C) corn biomass is passed through a shredder to reduce the volume of biomass to be ground through the Wiley mill.
2. Pass biomass through shredder in small bundles at a time to avoid clogging shredder.

Note: The shredding and subsequent grinding should be done as soon as possible after removing from oven as dry samples will be easier to grind than those that have had time to reabsorb moisture.

Note: liquid N

3. A homogenized subsample is used for further grinding.

II. Tissue grinding

1. Oven-dried biomass (and shredded corn biomass subsample) is ground to through a Wiley mill or a coffee grinder. This can also be done for smaller size and volume of samples in the lab (e.g., V7 corn and soybean, wheat early vegetative stages, tomato fruit).

Note: Make sure to wear a full-face respirator or a 3M N95 mask and safety goggles as this step generates a large amount of temporarily airborne solid particulate matter.

2. The sieve size (2-, 1- or 0.5-mm hole diameter) in the Wiley mill can be adjusted depending on the size of tissue required for nutrient content analysis. For tissue N and P content analysis, dried plant tissue is ground to 1 mm. Material to be ground is fed at the top of the mill and is carried around inside the mill between revolving and stationary blades until the material is sufficiently fine to drop through the holes of the sieve at the bottom. The finely ground tissue is then collected below the sieve in a plastic container.
3. A homogenized subsample (~100 g) of the ground tissue is collected and stored in clearly labelled air-tight Ziploc bags for further nutrient content analysis. 10-20 g of ground tissue is usually required when outsourcing for

tissue nutrient content analysis, whereas 1-2 g of ground tissue is more than enough for even replicate tissue nutrient content analysis in lab (*100 – 200 mg of tissue sample is used in wet acid digestion method; refer to SOP for Total P digestion*).

III. Precautions

1. Steel mills should not be used when Fe is to be determined in the tissue, to prevent contamination. Use mills made of non-ferrous alloys.
2. Brass contamination (e.g., from brass sieves) should be avoided if Cu or Zn is to be determined in the tissue.

References:

Jones Jr., J.B., Wolf, B. and Mills, H.A. (1991) Plant Analysis Handbook: A Practical Sampling, Preparation, Analysis, and Interpretation Guide. Micro-Macro Publishing, Athens.

Citation:

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