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USDA LTAR Common Experiment measurement: Aboveground biomass

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We use this protocol and it's working

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Abstract

The most important ecosystem service from agriculture is the provision of food, fiber, feed, and fuel. These outputs from the system are almost always a function of the amount of biomass accumulated above the soil surface (except with root crops). Measuring the aboveground biomass is necessary for determining the amount of plant material allocated to vegetative and reproductive organs. The amount of biomass allocated to these organs will vary to some degree each year due to interactions among the genetics, environment, and stressors. Measuring the vegetative-to-reproductive biomass ratio across treatments, landscapes, and years is an important metric and this ratio is termed the “harvest index.” The harvest index provides insight into improving management and cost efficiency in response to positive and negative impacts incurred from local (e.g., soil quality, pest and disease pressure, and weather), technological (e.g., genomic enhancement, precision inputs, and remote sensing/AI), or systematic (e.g., market, policy, and climate) sources. The amount of biomass remaining in the field is additionally important for determining the amount of C, N, P, K, S, and other nutrients returned to the soil. These data, paired with nutrient export and off-site losses, enable the construction of nutrient balances critical in evaluating tradeoffs among ecosystem services provided by agricultural landscapes.

This protocol describes the methods used to collect aboveground biomass which includes everything that:

- *Stays in the field:* such as stem, stalk, branches, leaves, grain enclosures (such as pod shells and husk leaves), and grain support structures (such as cob and ear shank), and
- *Leaves the field:* such as grain, hay, fiber, and plant material for bioenergy production.

Materials

Equipment and supplies needed will vary among sites, but several items may be useful:

- Plants may be cut using electric, gas, and manual tools such as a hand pruner, tree shearer, cordless trimmer, or cutter bar
- Mesh or paper bags for sample collections
- Woodchipper
- Thresher
- Large tubs, garbage cans, tins, and/or bags for processing samples
- Large scale for weighing whole samples
- Small, precise scale for weighing subsamples
- Airtight vials for shipping and archiving. Consider plastic vials for shipping and glass vials for archiving.
- Forced air oven that can heat to 60°C
- Mill for grinding
- Appropriate personal protective equipment for eyes, face, hearing, and respiratory hazards

Overview

- 1 This protocol describes methods for collecting whole plant samples taken by hand from random or predetermined sampling areas in plots and fields. These samples constitute the collection of all plant material (including non-crop species). Following the collection process, the individual will then separate the biomass into two categories: staying versus leaving the field. These samples provide the data and material for biomass, plant diversity, and nutrient analyses.

There are four protocols that are dependent on the physical aboveground biomass samples collected in this protocol; these are the first three bulleted items. The fourth protocol in the bulleted list is as an additional protocol to carry out in tandem with this protocol; see Step 13 below.

- USDA LTAR Common Experiment measurement: Concentration of carbon and nitrogen in aboveground biomass (Cavigelli and Strickland, 2024)
- USDA LTAR Common Experiment measurement: Concentration of phosphorus, potassium, and sulfur in aboveground biomass (Kovar and Fortuna, 2024)
- USDA LTAR Common Experiment measurement: Obtaining quality metrics in forage aboveground biomass (Cassida and Collins, 2024)
- USDA LTAR Common Experiment measurement: Cropland plant diversity (Wilke, 2024)

Type of plant

- 2 There are important considerations to account for based on the type and timing of the plant material (crop) being sampled.
- 3 **Annual grains**

These grains include maize, soybean, wheat, rice, and grain sorghum. Collect biomass during maximum vegetative biomass and maximum reproductive biomass. The crop may not reach maximum vegetative and reproductive at the same time or may not maintain the vegetative structures to totality throughout the reproductive developmental stages. Therefore, it may be necessary to sample not just once but twice a season. Determine whether your crop of interest has "leaf drop" before maximum reproductive development, such as with indeterminate soybean. If yes, additional documentation may be needed on your method. Two approaches are typically used when collecting aboveground biomass from crops with leaf drop:

- 3.1 Collect samples right before leaf drop occurs and remove the grain by threshing. Use all non-grain components for the maximum vegetative biomass component. The grain can be discarded because only the vegetative biomass is needed. The grain will be collected when

sampling occurs again at maximum reproductive biomass (typically referred to as physiological maturity).

- 3.2 Collect whole-plant samples slightly before or during harvest and collect leaves that fell on the ground within the sampling area. It is likely that some leaves are from neighboring plants with this approach. Do not collect leaves from crops that were grown prior to the current crop of interest. This combined sample of standing biomass plus leaves on the ground make up the entirety of the aboveground biomass sample.

4 **Root crops**

These crops include potatoes and sugar beet. Although this protocol is for aboveground biomass, a similar approach should be utilized for belowground biomass when it is the marketable, economic component. Collect biomass during maximum vegetative biomass and maximum reproductive biomass.

5 **Weeds**

Collect weed biomass (if present) during aboveground biomass sampling. Use the same sampling area used for collecting crop biomass but collect weed biomass in a separate bag for weight and analysis. Weed biomass is valuable for aboveground net primary productivity (ANPP) calculations and cropland plant diversity, as described in other protocols.

6 **Forages**

If the crop has multiple cuttings, site personnel need to collect biomass before each cutting, process, analyze, and then sum the values for a total per calendar year.

7 **Biofuel/energy crops**

Same as forages except only one cutting typically occurs in the fall.

8 **Fiber crops**

This category includes cotton and hemp. It is likely that separation of the fiber components (such as cotton bolls) will be needed from the rest of the plant.

9 **Non-cash crops, such as cover crops**

Collect biomass at 1 to 5 days before termination in the spring. If the cover crop is not terminated but simply has the cash crop planted into the green stand, wait until the maximum biomass of the cover crop is reached before sampling. If the crop typically experiences “winter kill,” sampling should occur in the fall before the first hard freeze.

10 **Prairie and conservation plantings**

Collect biomass once at physiological maturity of the dominant plant community, typically in late summer or early fall. If possible, collect twice per year, aligning with maximum growth for cool-season plants in the spring and warm-season plants in the fall.

11 Intercropping

Collect biomass at maximum vegetative and reproductive biomass based on the crops grown which will require multiple samplings throughout the season. If sampling crops together, plant and seed separation will be necessary in-field or during processing.

Sample collection and processing

- 12 The suggested area (referred to as “quadrat”) for sampling is 1 m², but the dimensions of the sampling quadrat may vary with treatment and plot size. For crops planted in 76 cm (30") rows, the ideal quadrat size is 1.5 m × 0.65 m (4' 11" × 2' 2"). For crops planted in narrow rows, drilled, or broadcast, quadrats equal to 2.0 m × 0.5 m (6' 7" × 1' 8") work well. Record the quadrat dimensions for subsequent area-based calculations. Quadrats are oriented with the long side parallel to the direction of crop planting and allow for the inclusion of row and inter-row plant communities in the sampling area.
- 13 Determine the location for a quadrat based on random or preselected stations in each treatment replicate. The number of quadrats will depend on the experiment based on the scale of the plot or field and how best to account for spatial variability. After placing the quadrat, count and record the number of individual plants or reproductive stems within that area. All plants rooted within the boundary of a sampling quadrat are hand-clipped at ground level (or just above the crown of perennial grasses). Remove all standing plant material from within the quadrat. If any plant material from the intended crop is dead and lying on the soil surface (termed “surface litter”), collect it and add it to the bagged sample. However, if the surface litter is heavily soiled or compromised do not add it to the sample due to the presence of minerals that will impact nutrient analyses later conducted on the sample. Also, do not collect surface litter if it is from past seasons and unassociated with the current crop.

Note

To measure and record plant species diversity, implement the *USDA LTAR Common Experiment measurement: Cropland plant diversity* protocol at this point.

- 14 Separate plant samples into vegetative and reproductive components (or “fractions”) before the processing steps below. In mature crops, grain removal is fairly easy, which allows your tissue and grain samples to be distinct before drying. However, in non-mature crops (such as before leaf drop), it may be easier to separate the grain following drying.
- 15 Depending on oven space, it may be necessary to dry a subsample instead of whole plants.

- 15.1 If subsampling, chop the vegetative components and reproductive components separately. Chop each sample into pieces a few inches in size and mix fully in a large pail/can (Figure 7). Collect a series of handfuls randomly from the pail/can to fill a one (1) gallon container or paper bag. This specimen is your subsample. Put it in the dryer. Collect the fresh weight of the whole sample and the subsample, as these weights are needed later for Calculation 1a (see below).
 - 15.2 If oven space is not a limitation, chopping is unnecessary. If grain threshing occurs after drying, briefly re-dry tissue and grain to remove ambient moisture gained during the threshing process.
 - 15.3 If considerable weed seed is in the sample or two crops were grown together in an intercropping scenario, separate the seed for each plant type.
- 16 Dry the plant and grain samples at 60°C or lower in a forced air oven (Figure 11). Temperatures higher than 60°C will degrade sample quality, which could negatively impact nutrient analyses performed later. Depending on the size of the samples and drying temperature, drying times should range from 2-4 days. Remove the sample from the dryer when the sample weight has reached equilibrium. If unsure, it is best to leave it in the dryer for an extra day so that all samples are dried consistently. Aboveground biomass weight data are to be reported to the LTAR centralized data portal as oven-dry weight.
 - 17 Collect the dry weight (g) of the samples. Ensure the bag weight is not recorded in the final biomass weight.
 - 18 Calculate total dry weight (g) and the total dry weight per area (kg/ha) using the equations (1a, 1b, 2) below.
 - 19 Once weight data has been collected, this marks the completion of this protocol for collecting data on aboveground biomass. The next steps are to prepare the sample for archiving and the likely analysis of nutrient content on it. This is explained under the *Archiving* Section below.

Calculations

- 20 Calculation 1a. Weight basis (g) for when a subsample is dried:

$$\text{Total biomass}_{\text{DW}} = \text{Total biomass}_{\text{FW}} \times (\text{Subsample biomass}_{\text{DW}} / \text{Subsample biomass}_{\text{FW}})$$

DW = dry weight, FW = fresh weight

Calculation 1b. Weight basis (g) for when the whole sample is dried:

No calculations are needed. Simply collect the dry weight after removing it from the dryer.

21 Calculation 2. Area basis (kg/ha):

$$\text{Total biomass (kg/ha)} = \text{Total biomass}_{\text{DW}} \times (\#\text{ m}^2 \text{ sampling area}) \times 10$$

= the area used to collect the sample, likely quadrat dimensions multiplied

Concurrently sampled covariate metrics

- 22
- Plant population. Count the number of plants within the quadrat for the area calculation below.
 - Management data. Provide planting and harvest dates, tillage, fertilizer/manure applications, pesticide applications, and other field operations as appropriate in the farm management entry interface.
 - Collect grain density (test weight) and moisture content at the time of harvest of the grain sample. This information could also come from the combine when mechanically harvesting.
 - Elemental concentration of nitrogen, phosphorus, potassium, sulfur, and carbon from the ground tissue samples.

Quality assessment and quality control

- 23
- We recommend a “calibration” for all members of sampling teams before sample collection so that everyone follows the same approach to sample collection. This uniformity is important when personnel vary in tenure and perhaps experience. Address quadrat area selection, quadrat orientation, counting techniques, safe clipping practices (across electric, gas, and manual tools), sorting criteria, labeling samples, and transport to chopping or drying facilities.

Archiving

- 24
- Collect a minimum of a 300 gram representative subsample of each plant component (grain, stems/stalk/leaves, weeds, cover crops, etc.) harvested from each plot. Coarsely chop and grind samples through a 1 mm screen, using a mill such as in Figure 12. If samples have not recently been dried to 60°C, place into a dryer for a day to remove any ambient moisture prior to long-term storage. Store 150 grams of ground sample in a labeled plastic container (Figure 8) for utilization in elemental (2 grams) and/or forage NIR (100 grams) analysis. Utilize the remaining ground sample to fill a labeled 4 ounce glass container with a screw-top metal lid, which will be archived at room temperature for future analyses. Archived samples can also be stored as whole seed, rather than as a ground sample, if preferred.



Figure 1. Example of ground tissue in plastic vials ready for submission to a laboratory or for archiving (best if stored in glass vials though). Photo courtesy of L. Abendroth, USDA ARS.

Note

Review the following protocols to ensure your ground samples are ready for nutrient analyses as outlined in:

- USDA LTAR Common Experiment measurement: Concentration of carbon and nitrogen in aboveground biomass (Cavigelli and Strickland, 2024)
- USDA LTAR Common Experiment measurement: Concentration of phosphorus, potassium, and sulfur in aboveground biomass (Kovar and Fortuna, 2024)
- USDA LTAR Common Experiment measurement: Obtaining quality metrics in forage aboveground biomass (Cassida and Collins, 2024)

Illustrative information

- 25 Equipment and supplies needed will vary among sites, but the items shown here are often used by researchers when collecting, transporting, chopping, drying, weighing, and storing biomass samples.
- 26 Tools for cutting plants; this can include electric, gas, and manual tools such as a hand pruner, tree shearer, cordless trimmer, or cutter bar.





Figure 2. Tree shearer. Photo courtesy of L. Abendroth, USDA ARS.



Figure 3. Summer Bjorn, USDA ARS intern, using a gas-powered cutting bar for collecting a hay sample. Quadrat is visible in photograph. Photo courtesy of L. Abendroth, USDA ARS.

- 27 Mesh and paper bags for sample collections. It is not always possible to process samples immediately so keeping them aerated is important. Large mesh bags like this are often termed "onion bags" or "cabbage bags" because of their use in the food industry for transporting these products.



Figure 4. Mesh or paper bags that allow air in and out are ideal for biomass samples.
Photo courtesy of L. Abendroth, USDA ARS.



Figure 5. Stationary wood-chipper mounted on the PTO of a small tractor. This setup is helpful if needing to chop large plants into smaller subsamples for drying. Photo courtesy of L. Abendroth, USDA ARS.

29 Thresher



Figure 6. Thresher for separating vegetative fraction from grain fraction of plants. Screen sizes will differ based on the crop type and needs to be evaluated prior to running research samples through. Some loss typically occurs when threshing so it is recommended to obtain total weight of the whole plant and then subtract one fraction that had the least loss to get the weight of the other fraction. Photo courtesy of L. Abendroth, USDA ARS.

30 Large tubs, garbage cans, tins, and/or bags for processing samples



Figure 7. Large garbage cans are used to catch and transport chopped plant material. After the plant sample is chopped, a subsample is collected and the rest of the material is disposed of. Photo courtesy of L. Abendroth, USDA ARS.

- 31 Airtight vials for shipping and archiving. Consider plastic vials for shipping and glass vials for archiving.



Figure 8. Samples from a set of plots, with 2 vials for each plot. One sample is stored in a plastic vial for sending to a laboratory for analysis and the other sample is stored in a glass vial for long-term archiving. Photo courtesy of M. Maile, USDA ARS.

32 Scales for large and small samples



Figure 9. Large, flat scale for weighing whole samples. Photo courtesy of M. Maile, USDA ARS.



Figure 10. Small, precise scale for weighing subsamples. Photo courtesy of B. Wilke, Michigan State University.

33 Forced air oven



Figure 11. Forced air oven capable of drying at high temperature. For biomass samples, it is recommended to always dry at 60°C to ensure integrity for nutrient analyses performed later. Photo courtesy of M. Volkmann, USDA ARS.

34 Mill for grinding



Figure 12. Wiley mill. Photo courtesy of B. Wilke, Michigan State University.

Recommendations for data collection

35 Table 1. Summary of recommendations for the measurement of aboveground biomass staying in the field and leaving the field.

A	B	C	D
Attribute	Preferred	Minimum	Comments
Spatial scale	Field and plot	Plot	
Frequency	Maximum vegetative and reproductive biomass	Every crop harvest	Harvest is annual for some crops (e.g., corn) but more frequent for other crops (e.g., alfalfa)
Covariate metrics	Moisture content at harvest, grain density, farm management data, plant population, and elemental concentrations of NPKS and	Moisture content at harvest, grain density, farm management data, plant population, and elemental concentrations of NPKS and	

A	B d C in tissue samples	C C in tissue samples	D
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Protocol references

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