# TOS STANDARD OPERATING PROCEDURE: MEASUREMENT OF ABOVEGROUND PRODUCTIVITY FOR AGRICULTURAL CROPS

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# Change Record

| REVISION | DATE | ECO # | DESCRIPTION OF CHANGE |
|---|---|---|---|
| A | 09/15/2016 | ECO-04141 | Initial release |
| B | 07/12/2017 | ECO-04624 | Major changes from initial release:<br>• Section 4, Timing: Clarified that 2+ harvests per plot are required if plot is planted with multiple crops per season, or is fallow for part of year and then planted.<br>• Section 6, Equipment: Added folding ruler, loppers, and spring scales.<br>• SOP B: Weighing of total fresh mass, creation of sub-sample, and weighing of subsample fresh mass now carried out in the field with spring scales, rather than lab, and can be employed for all crop-types. Prevents transport of large volumes of clipped mass. |
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**TABLE OF CONTENTS**

**LIST OF TABLES AND FIGURES**

# 1 DESCRIPTION

## 1.1 Overview

The Standard Operating Procedure (SOP) described in this document is an extension of the TOS Protocol and Procedure: Measurement of Herbaceous Biomass (RD[04]). For crop clip-harvesting in the field described here, the dimensions and orientation of typical Clip Strips used for 'wild-type' herbaceous biomass clipping are modified. In contrast to Clip Strips for 'wild-type' vegetation, crop Clip Strips adopt wider dimensions, and are oriented perpendicular to crop rows in order to account for the spatial structure introduced by crop row-planting. The field procedure for crops is also modified from RD[04] to include a subsampling routine. Because more area is sampled in the field, and crops can produce large amounts of biomass, the subsampling routine allows smaller amounts of biomass per sample to be transported back to the laboratory and placed into the drying ovens. Subsampling requires that additional fresh weight data are collected in the field so that subsample dry weights can be scaled back up to the entire sample collected in the field.

## 1.2 Purpose

This document outlines the procedure for measuring aboveground biomass in plots that have been planted with:

- Corn
- Soybean
- Sorghum, or
- Cereal crops (Wheat, barley, triticale, rye, oat, etc.)

Other crops, such as squash, tomatoes, peppers, etc., are outside the scope of this SOP, and must be addressed on a case by case basis via NEON's problem tracking software (currently JIRA).

## 1.3 Scope

This document provides a change-controlled version of an Observatory procedure. Documentation of content changes (i.e. changes in particular tasks or safety practices) will occur via this change-controlled document, not through field manuals or training materials.

## 1.4 Applies To

The procedure described in this document is used in the following protocols:

| Doc # | Title |
|---|---|
| NEON.DOC.014037 | TOS Protocol and Procedure: Measurement of Herbaceous Biomass |
| | |

### 1.5 Acknowledgments

Quadrat dimensions for corn, soybean, and graminoid crops were taken from the Kellogg Biological Station LTER protocol for Aboveground Net Primary Productivity.

## 2 RELATED DOCUMENTS AND ACRONYMS

### 2.1 Applicable Documents

Applicable documents contain higher-level information that is implemented in the current document. Examples include designs, plans, or standards.

| | | |
|---|---|---|
| AD[01] | NEON.DOC.004316 | Operations Field Safety and Security Plan |
| AD[02] | NEON.DOC.004300 | EHSS Policy, Program and Management Plan |
| AD[03] | NEON.DOC.050005 | Field Operations Job Instruction Training Plan |
| AD[04] | NEON.DOC.000914 | TOS Science Design for Plant Biomass, Productivity, and Leaf Area Index |

### 2.2 Reference Documents

Reference documents contain information that supports or complements the current document. Examples include related protocols, datasheets, or general-information references.

| | | |
|---|---|---|
| RD[01] | NEON.DOC.000008 | NEON Acronym List |
| RD[02] | NEON.DOC.000243 | NEON Glossary of Terms |
| RD[03] | NEON.DOC.002652 | NEON Level 1, Level 2 and Level 3 Data Products Catalog |
| RD[04] | NEON.DOC.014037 | TOS Protocol and Procedure: Measurement of Herbaceous Biomass |
| RD[05] | NEON.DOC.001574 | Datasheets for TOS Protocol and Procedure: Measurement of Herbaceous Biomass |
| RD[06] | NEON.DOC.001717 | TOS Standard Operating Procedure: TruPulse Rangefinder Use and Calibration |
| RD[07] | NEON.DOC.001025 | TOS Protocol and Procedure: Plot Establishment |
| RD[08] | NEON.DOC.001271 | TOS Protocol and Procedure: Manual Data Transcription |

### 2.3 Acronyms

| Acronym | Definition |
|---|---|
| NA | NA |

### 2.4 Definitions

**Brace roots:** (Also called prop roots) Aerial roots originating along a corn stalk, above the soil surface (**Figure 1**).

**Ear:** Pollinated female corn inflorescence comprised of cob, kernel, husk leaves and silk (style).

**Pod**: The elongated seed vessel of the soybean plant.

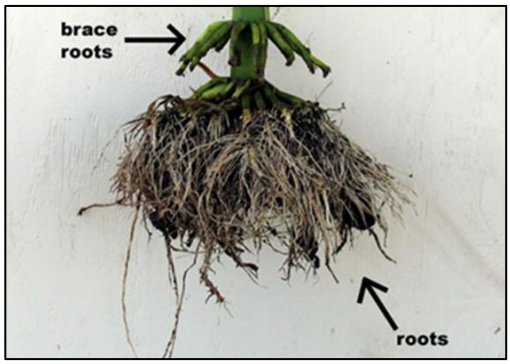


**Figure 1**. Base of corn stalk, including belowground roots and aboveground brace roots; the latter are also known as 'prop' roots.

## 3 METHOD

A combination of Distributed, Gradient, and Tower Plots may be used for collecting biomass and productivity data from agricultural crops. The timing of agricultural biomass sampling is managed on a per plot basis, is targeted to peak crop biomass, and is constrained by crop senescence, anticipated harvest date, or both. In addition, multiple clip harvests per season are required to capture the combination of fallow cover and agricultural crop productivity if farmers employ multiple crop rotations within a growing season (see Section 4 for more details).

In the field, the primary distinction between the procedure for agricultural crops described here, compared to that presented in RD[04] for 'wild' herbaceous vegetation, is that crops require larger, variable Clip Strip dimensions, and Clip Strips are oriented perpendicular to crop rows. In contrast, 'wild' herbaceous vegetation uses a Clip Strip with fixed dimensions that is always oriented North/South. Variable Clip Strip dimensions are required because planting in rows introduces spatial structure to the biomass that is not adequately captured with a narrow Clip Strip.

Because agricultural plots may also support other herbaceous plants besides the crop of interest (subsequently referred to in this document as 'non-crops'), clipped vegetation within these larger Clip Strips must still be sorted to functional group as in RD[04] if non-crops are present. For example, if a Clip Strip contains wheat, as well as other plants belonging to several of the herbGroups defined in RD[04], clipped wheat biomass will be separated from non-crop biomass, and clipped non-crop biomass will be further sorted to herbGroup (i.e., CSG, WSG, LFB, etc.).

Because agricultural crops may attain relatively large stature, the laboratory procedure in this SOP differs from RD[04] by introducing a subsampling method prior to oven-drying clipped biomass of high-volume crops. This subsampling step allows for more efficient drying and processing of large sample volumes. Low-volume crops are processed identically to 'wild' herbaceous vegetation. In addition, data collected via this SOP are entered via the same data ingest mechanism as RD[04].

# 4 SAMPLING SCHEDULE

## 4.1 Sampling Frequency and Timing

Agricultural sites often practice crop rotation, which results in NEON plots located in different parcels, with each parcel supporting a crop-specific planting and harvest schedule that changes from year to year. Each crop that matures within the given sampling year will need to be sampled for biomass in order to estimate the productivity of the site, and multiple sampling bouts are therefore required to capture the productivity of crops planted and harvested in different seasons. Moreover, plots left fallow or planted with cover crops will also need to be sampled in order to adequately capture site-level productivity. For these reasons, communicating and collaborating with the site host and/or farmer to determine and coordinate harvest dates and sampling activities is highly advantageous in agricultural systems. **Table 1** provides an overview of spatial and temporal sampling strategy for agricultural sites.

> ***Example:*** A north temperate site has 10 plots planted with cover-crop, 10 plots planted with winter wheat, and 10 plots planted with corn. Plots planted with winter wheat would be harvested for bout1 (likely late May or early June); cover-crop and corn plots would be harvested for bout2 (likely mid to late August). Cover-crop plots may be harvested on a different schedule than corn if the cover-crop reaches peak biomass later than the corn harvest.

> ***Example:*** A plot (Distributed or Tower) is fallow for the spring (i.e., nothing planted) and has mixed non-crop plants growing opportunistically in it. The plot is then plowed and planted with a crop that matures in late summer or early autumn. Plots such as this require a first bout for harvesting non-crop biomass in the spring, and a second bout for quantifying crop biomass in the autumn.

> ***Example:*** A plot (Distributed or Tower) is planted with winter wheat that is harvested early to mid-summer. The plot is then left fallow, and opportunistic non-crop plants grow, or is planted with a cover crop, and these plants senesce in the autumn. Plots such as this require a first bout for quantifying early-season crop biomass, and a second bout for assessing peak non-crop biomass (scheduled when greenness begins to decrease – i.e., when plants first begin to senesce).

**Table 1**. Summary of clip harvest sampling frequency and timing guidelines by plot type for agricultural sites.

| Plot Type | Plot Number | # Events per Sampling Year | Yearly Interval | Sampling Start | Sampling Stop |
|---|---|---|---|---|---|
| Distributed or Gradient | n=10 (min) n=20 (max) | Once per crop cycle or fallow period | Annual | Beginning of crop senescence | Within 14d of sampling start |
| Tower | n=10 | | | | |

### 4.2 Criteria for Determining Onset and Cessation of Sampling

*Sampling onset:* Crop biomass typically peaks some time before plants begin to senesce, and a fraction of total peak biomass mass is lost as senescence proceeds. Peak greenness data provided in Appendix D of RD[04] may guide scheduling of sampling onset, but because multiple rotations are often planted within a site, MODIS data should be considered as a guide only. A more reliable indicator for sampling onset is to monitor plants for the beginning of senescence, and begin clipping as soon as the first leaves begin to dry or yellow. For corn and soybeans, drying and yellowing usually begins with the lower, older leaves.

*Sampling cessation:* As indicated in **Table 1**, sampling should be completed within 2 weeks of sampling onset. In the event of unforeseen delays, sampling must be completed before the farmer harvests the crop; if the farmer removes crop biomass before NEON clip harvest is complete, estimates of productivity for the site will be severely compromised. **Table 2** gives the earliest dates that farmers typically harvest specific crops within NEON sites (data provided by USDA, http://usda.mannlib.cornell.edu/usda/current/planting/planting-10-29-2010.pdf).

**Table 2**. Anticipated earliest harvest dates for unique site by crop combinations likely at NEON agricultural sites. Data come from long-term USDA records aggregated at the state level. Sites with crops other than those listed here are covered in the site-specific modification appendix at the end of this document (e.g., D04 LAJA).

| Domain | Site(s) | Crop | Harvest begin date | Harvest most active dates |
|---|---|---|---|---|
| D02 | BLAN | Corn for grain | 8/25 | 9/5 – 10/25 |
| | | Corn for silage | 8/5 | 8/30 – 10/1 |
| | | Soybeans | 10/5 | 10/25 – 11/25 |
| | | Wheat, winter | 6/5 | 6/20 – 7/15 |
| | SERC | Soybeans | 10/5 | 10/18 – 11/15 |
| D03 | JERC | Corn for grain | 7/25 | 8/15 – 9/5 |
| | | Soybeans | 10/1 | 11/1 – 11/25 |
| | | Wheat, winter | 5/20 | 6/1 – 6/15 |
| D06 | KONA | Barley, fall | 6/10 | 6/15 – 7/1 |
| | | Barley, spring | 6/20 | 6/25 – 7/1 |
| | | Corn for grain | 9/5 | 9/20 – 10/20 |
| | | Sorghum for grain | 9/15 | 10/10 – 11/5 |
| | | Soybeans | 9/20 | 10/5 – 10/25 |
| | | Wheat, winter | 6/15 | 6/20 – 7/10 |
| D09 | NOGP | Barley, spring | 7/30 | 8/8 – 8/23 |
| | | Corn for silage | 8/31 | 9/12 – 9/28 |
| | | Corn for grain | 9/29 | 10/10 – 10/27 |
| | | Soybeans | 9/16 | 9/26 – 10/11 |
| | | Wheat, durum | 8/9 | 8/21 – 9/9 |
| | | Wheat, spring | 8/4 | 8/14 – 9/1 |
| D10 | STER | Barley, spring | 6/10 | 7/25 – 9/5 |
| | | Corn for silage | 9/1 | 9/10 – 9/30 |

| Domain | Site(s) | Crop | Harvest begin date | Harvest most active dates |
|---|---|---|---|---|
| | | Corn for grain | 10/1 | 10/15 – 11/10 |
| | | Sorghum for grain | 9/25 | 10/10 – 11/15 |
| | | Sorghum for silage | 9/1 | 9/5 – 9/20 |
| | | Wheat, winter | 6/25 | 7/10 – 7/20 |

Dates listed in **Table 2** may be used for hiring and high-level scheduling purposes if Field Operations obtains information from the farmer with respect to which crops will be planted in the coming year. However, with respect to scheduling the exact week in which clip harvests will take place for a particular crop once the growing season is underway, bear in mind that weather can cause significant deviations from the averages presented in **Table 2**.

### 4.3 Timing for Laboratory Processing and Analysis

Because clipped biomass continues to be biologically active after clipping and before drying (i.e. plant cells continue to respire and therefore lose mass), it is important to place clipped samples into the drying oven as soon as possible after clipping occurs. For Herbaceous Biomass (RD[04]), clipped plants are kept in cold storage after clipping and until samples can be placed in the drying oven. Similar to RD[04], freshly clipped crop samples or subsamples must be placed in the drying oven within 5 d of clipping in the field, and crop biomass must be kept in cold storage, same as in RD[04]. Once samples are dry, timing considerations for weighing are identical to RD[04].

## 5 SAFETY

This document identifies procedure-specific safety hazards and associated safety requirements. It does not describe general safety practices or site-specific safety practices.

Personnel working at a NEON site must be compliant with safe field work practices as outlined in the Operations Field Safety and Security Plan (AD[01]) and EHSS Policy, Program and Management Plan (AD[02]). Additional safety issues associated with this field procedure are outlined below. The Field Operations Manager and the Lead Field Technician have primary authority to stop work activities based on unsafe field conditions; however, all employees have the responsibility and right to stop their work in unsafe conditions.

***Agricultural Chemicals***

Common agricultural chemicals include pesticides, insecticides, fungicides, and herbicides and residues of these chemicals may be found on active agricultural sites. Their hazards may remain after fields have been treated. These chemicals can be on plants, in soil, and sometimes in irrigation systems used to apply hazardous chemicals. Chemical residue cannot always be seen, and may also be carried by the wind.

It is important to determine if it is safe to enter a field where pesticides have been applied prior to beginning work. Attempts should be made to determine pesticide application schedule and the restricted entry interval (REI) of applied pesticides prior to entering agricultural fields or areas where pesticide use is expected. When entering agricultural fields or other areas, check for posted warning signs at access roads, field borders adjacent to public areas and established walking routes where agricultural workers enter the area.

The following precautions must be followed when re-entering areas within 30 days after expiration of restricted entry interval (REI):

- Wear full-length pants, long-sleeved shirts, a hat, socks, and non-leather gloves and work shoes. Leather can absorb chemicals from plants and the soil.
- Wash gloves and work boots/shoes prior to removing, if possible.
- Wash work clothes separately from other laundry using hot water and laundry detergent. Always wash clothes, boots, and gloves before wearing them again.
- Wash face, neck, hands, and arms as soon as possible, after potential exposure to agricultural chemicals.
- NEVER enter a field that has been posted with a “Re-Entry” warning sign!
- DO NOT smoke while working in a treated field, regardless of when the area was sprayed with the hazardous chemical.
- DO NOT carry lunch into treated work area.
- If exposed or if exhibiting any signs or symptoms of exposure, seek Safety Data Sheet for the hazardous chemical that was used on the field. If medical assistance isn’t immediately available, follow the first-aid directions on the label or included with the Safety Data Sheet.

***Laser Rangefinder***

A laser rangefinder/hypsometer/compass instrument may be used to navigate to clip cells within plots. Safety considerations for this instrument include:

- Avoid staring directly at the laser beam for prolonged periods. The rangefinder is classified as eye-safe to Class 1 limits, which means that virtually no hazard is associated with directly viewing the laser output under normal conditions. As with any laser device, however, reasonable precautions should be taken in its operation. It is recommended that you avoid staring into the transmit aperture while firing the laser.
- Never attempt to view the sun through the scope. Looking at the sun through the scope may permanently damage the eyes.

***Sharp Blades***

Sharp-bladed pruners and/or loppers may be used to clip and subsample crop plants. Safety considerations for these tools include:

- Select the correct tool for the job.
- Use personal protective equipment, to include gloves, safety glasses, and work boots to minimize injuries in the field.
- Assure all personnel working in the area are aware of the use of the sharp tools, and keep all sharp blades safely away from others.
- Maintain good posture and do not twist or stretch body awkwardly while making cuts with a pruner or lopper.
- When cutting overhead, a hardhat should be worn.

# 6 PERSONNEL AND EQUIPMENT

## 6.1 Equipment

The following equipment is needed to implement the procedures in this document. Equipment lists are organized by task. They do not include standard field and laboratory supplies such as charging stations, first aid kits, drying ovens, mass balances, ultra-low refrigerators, etc.

**Table 3**. Equipment needed to prepare for sampling.

| Item No. | R/S | Description | Purpose | Quantity | Special Handling |
|---|---|---|---|---|---|
| **Durable Items** | | | | | |
| MX102549 | S | GPS receiver, decimeter accuracy (e.g. Trimble GEO XH 6000, Trimble GEO 7X, or equivalent) | Navigate to sampling locations at sites where plot markers are absent | 1 | N |
| MX100703 | S | GPS receiver, recreational accuracy | Navigate to sampling location at sites with plot markers | 1 | N |
| MX100322 | S | Laser Rangefinder, ± 30 cm accuracy | Delineate plot boundaries, determine clip cell location | 1 | N |
| | R | USB Cable | Transfer data to GPS unit. | 1 | N |
| **Consumable Items** | | | | | |
| | R | All weather paper | Printing field datasheets | 1 | N |

R/S = Required / Suggested

**Table 4**. Equipment needed for a 2-person team to perform agricultural clip harvest sampling at one plot.

| Item No. | R/S | Description | Purpose | Quantity | Special Handling |
|---|---|---|---|---|---|
| **Durable Items** | | | | | |
| MX102549 | S | GPS receiver, decimeter accuracy (e.g. GEO XH 6000, 7X) | Navigate to sampling locations at sites where plot markers are absent | 1 | N |
| MX100703 | S | GPS receiver, recreational accuracy | Navigate to sampling location with plot markers | 1 | N |
| MX100722 | R | Measuring tape, minimum 30 m | Locate clip-harvest strips within plots/subplots; measure and delineate dimensions of clip harvest strip. | 1 | N |
| MX100320 | R | Compass with mirror and declination adjustment | Locate clip-harvest strips (with measuring tape) | 1 | N |
| MX100322 | S | Laser Rangefinder, ± 30 cm accuracy | Delineate plot boundaries, determine clip cell location | 1 | N |
| | R | Pruning shear | Clip plants | 2 | N |
| | S | Loppers, long-handled | Clip and cut corn | 1 | N |
| | R | Spring scale, 10 kg capacity, tareable | Weigh total fresh mass of high-volume crops (e.g., corn) | 1 | N |
| MX100705 | R | Spring scale, 5 kg capacity, tareable | Weigh subsample fresh mass of high-volume crops (e.g., corn) | 1 | N |
| | R | Cooler | Chill perishable samples in field | 1 | N |

| Item No. | R/S | Description | Purpose | Quantity | Special Handling |
|---|---|---|---|---|---|
| MX100358 | R | Cold packs | Chill perishable samples in field | | N |
| | S | Pre-marked string and stake sets; see RD[04] for more details | Delineate clip harvest strip for small-stature crop types | 2 | N |
| MX105823 | S | Measuring stick, 2 m folding | Delineate clip harvest strip for tall-stature crop types | 1 | N |
| MX104361 | R | Chaining pins or other suitable anchor | Anchor measuring tapes | 2 | N |
| | R | Survey marking flag, PVC or fiberglass stake | Delineate sampling area | 4 | N |
| | S | Work gloves | Protect hands | 2 | N |
| MX106656 | R | Magnifier hand-lens, 10X | Aid in species identification (particularly for non-crops) | 1 | N |
| MX103211 | R | Magnifier hand-lens, 20X | Aid in species identification (particularly for non-crops) | 1 | N |
| MX105089 | R | Paper bags, #8[1] | Contain clipped herbaceous biomass, sorted to functional group | 6[2] | N |
| | R | Large paper bags, yard waste type or equivalent (e.g., 30 gallon capacity) | Contain clipped crop biomass | 1-4[2] | N |
| | R | Permanent marker | Label paper bags | 2 | N |
| | S | CR123A battery | Spare battery for laser rangefinder | 2 | N |

| Item No. | R/S | Description | Purpose | Quantity | Special Handling |
|---|---|---|---|---|---|
| | S | AA battery | Spare battery for GPS receiver | 2 | N |
| | R | Field notebook | Calculation of seed:fresh ratio when using paper data sheets. | 1 | N |
| **Resources** | | | | | |
| RD[05] | R | Herbaceous Biomass Field Datasheets | Record sampling metadata | | N |
| | R | Per plot or subplot Clip Lists | Identify random Clip Strip locations | As needed | N |
| | R | Field guide, regional flora reference guide and/or key | Identify leguminous forbs and graminoids to species | 1 | N |

R/S = Required / Suggested

[1] Bag size may be adjusted as necessary based on size/bulk of plants being clipped. For example, sites with tall grasses will require larger bags.
[2] Quantity may be adjusted as necessary based on field experience at a given site.

**Table 5**. Equipment needed for post-field sampling tasks.

| Item No. | R/S | Description | Purpose | Quantity | Special Handling |
|---|---|---|---|---|---|
| **Durable Items** | | | | | |
| MX103211 | S | Magnifier hand-lens, 20X | Aid in non-crop species identification | 1 | N |
| MX106656 | S | Magnifier hand-lens, 10X | Aid in non-crop species identification | 1 | N |
| | R | Cooler, size dependent on anticipated crop volume | Chill perishable non-crop and low-volume crop samples | As needed | N |
| | R | Cold packs | Chill perishable non-crop and low-volume crop samples | Variable | |
| **Resources** | | | | | |
| RD[05] | R | Completed Herbaceous Biomass Field Datasheets | Contains field-collected sampling metadata | Variable | N |
| | R | Field guide, regional flora reference guide and/or key | Aid in distinguishing morphologically similar species to functional groups | 1 | N |

R/S = Required / Suggested

**Table 6**. Equipment needed for processing agricultural biomass clip harvest samples in the laboratory.

| Item No. | R/S | Description | Purpose | Quantity | Special Handling |
|---|---|---|---|---|---|
| **Durable Items** | | | | | |
| MX100230 | R | Drying oven | Dry samples at 65 ˚C | 2 (typically) | N |
| MX100265 | R | Balance, 0.01 g accuracy | Weigh dried subsamples | 1 | N |
| | R | Large capacity scale, 10 g accuracy; shipping or spring type | Weigh total fresh weight of clipped crop samples up to 20 kg total weight | | |
| MX100689 | S | Weigh boats, large | Contain dried non-crop samples while weighing | Variable | N |
| MX103931 | R | Plastic tray or equivalent | Contain crop samples and subsamples while weighing | 1 | N |
| **Consumable Items** | | | | | |
| RD[05] | R | Datasheets:<br>• Lab Drying QC Datasheet<br>• Lab Weighing Datasheet | Recording dry weight of herbaceous biomass | As needed | N |

R/S = Required / Suggested

### 6.2 Training Requirements

All technicians must complete required safety training and procedure-specific training for safety and implementation of this procedure as required in Field Operations Job Instruction Training Plan (AD[03]).

For the field component of this protocol, technicians must be trained in navigating to points in the field with a GPS and manual methods. Most critically, technicians must be trained to quickly identify common herbaceous species that inhabit agricultural fields within the region. Because different herbaceous functional groups can be sensitive indicators of ecosystem responses to global change (e.g. N deposition, warming, rising $CO_2$), it is very important that field technicians within a domain can accurately and quickly identify C3 and C4 graminoids as well as identify leguminous and non-leguminous forbs within that domain.

Training for both the field and laboratory work must emphasize the importance of consistent, detailed labeling of all samples. ***Improper or inconsistent labeling is the most common and problematic error associated with this work!***

### 6.3 Specialized Skills

The lead plant technician must possess the demonstrated ability to identify crops to species, and to identify non-crop species inhabiting cropped plots to functional group – either via visual inspection, or via visual inspection in combination with a dichotomous or polyclave key.

- Identification of all leguminous forbs to functional group, in the absence of flowers, is required.
- Identification to species is not required for non-leguminous forbs and woody stemmed plants.
- Identification to species is required for cool-season (C3) and warm-season (C4) graminoid functional groups. Technicians should be able to identify graminoids vegetatively.

To identify non-crop species, ideally each team member should know how to use diagnostic traits and a dichotomous or polyclave key.

### 6.4 Estimated Time

An experienced two-person team will require approximately 1 hour to perform clip-harvest sampling of an agricultural crop within a given Clip Strip. This time includes identifying an acceptable clip-harvest location, delineating the Clip Strip, and clipping the crop and any non-crop plants present. Lab work for an agricultural Clip Strip should require approximately 1 hour, and this time includes effort required for sorting, drying, and weighing clipped agricultural biomass.

# 7 STANDARD OPERATING PROCEDURES

## SOP A Preparing for Sampling

### A.1 Sampling Equipment Preparation and Checklist

- See RD[04] for equipment preparation.
- Use of the laser rangefinder is only necessary at agricultural sites if plot markers are not present, and full plot delineation is required.

### A.2 Early-season Preparation at Agricultural Sites

- Check with the Domain Manager to ensure that the farmer (or site host) has approved implementation of this SOP within target plots.
- If density of mature vegetation precludes easy navigation and Clip Strip delineation (e.g., in mature corn crops), marking of plot corners and delineation of Clip Strips may occur earlier in the season while plants are young and line-of-sight is not obscured.
    - Temporarily record GPS coordinates for locations marked in this manner to enable re-location during sampling.

## SOP B Field Sampling Aboveground Biomass of Agricultural Crops

At agricultural sites, clip harvesting produces estimates of total crop biomass production on a per crop basis, as well as the biomass production of any non-crop herbaceous plants growing with the crop. Crop biomass is separated from non-crop biomass, and non-crop biomass is further sorted to herbGroup as in RD[04].

In the procedure below, it is assumed that technicians have a working knowledge of SOP B in the TOS Protocol and Procedure: Measurement of Herbaceous Biomass (RD[04]).

### HELPFUL HINT: COORDINATING WITH OTHER PLANT PROTOCOLS

***Plant Diversity:***

- If plant diversity sampling is scheduled to occur prior to crop clip-harvest sampling in a given sampling year, it may be helpful to identify and demarcate a suitable Clip Strip prior to performing diversity sampling. This will ensure that the Clip Strip is not trampled during diversity sampling.
- Should clip-harvest occur before diversity sampling, take care to avoid trampling 1 $m^2$ nested subplots used for Plant Diversity % cover measurements.
- Reject the clip cell if rotation required for sampling causes the desired clip strip to overlap with nestedSubplots used for Plant Diversity (applicable only in Distributed Plots and those Tower Plots that support Plant Diversity).

***Belowground Biomass Core:***

- Belowground Biomass Core samples should be collected according to the standard protocol, in the usual designated north/south sampling areas, regardless of whether the clip strip was rotated due to crop rows.

### B.1 Sample Collection in the Field

1. Navigate to the selected plot, using the GPS if necessary.
    - Should the farmer harvest the crop prior to scheduled Agricultural Clip Harvest sampling, record 'Crop harvest preceded NEON sampling' in the **remarks** field.
    - Perform subsequent steps below if live green material persists in the plot (e.g., weeds are still present and growing). Else, proceed to the next plot.
2. As described in RD[04], use the plot- or subplot-specific Clip List to identify and locate the target clip cell for sampling (steps 2 – 7 in RD[04]).
    - When these steps are complete, you should have placed a pin flag at what is defined as the SW corner of the Clip Strip in wild-type vegetation. This flag is referred to as 'Flag1' below (**Figure 2**), and will NOT necessarily be the SW corner in an Ag Clip Strip.

- On the Clip List, record the clip cell that was used as the starting point for clip cell delineation. Because agricultural clip strips are often oriented off the typical north/south axis, they will typically intersect > 1 clip cell, and there is no need to determine exactly which clip cells an angled clip strip intersects.

3. Record required plot-level field sampling information:

    - **boutNumber**; use *XX* format, where XX is the bout number assigned by Field Operations, e.g. '01'. Values of **boutNumber** reset every year (typically the end of the farming season). Multiple bouts per season will be required if crop rotation is practiced at the site.
    - **clipCellNum**; as provided in Clip List; use last 3 numbers from the clipID, e.g. for BLAN_001_0126, record '126'.
    - **clipDate**; use *YYYYMMDD* format.
    - **exclosure**; this is typically 'N' for agricultural clip harvests.

4. Select the appropriate crop-specific dimensions for the Clip Strip (**Table 7**), and record:

    - **clipDimension**: The length (L) and width (W) of the Clip Strip, in meters; e.g., *'1.5 x 0.65'*.

5. Delineate the accepted Clip Strip for harvesting. Clip Strip orientation **is not the same as in RD[04]**. For agricultural crops, clip strips are oriented perpendicular to crop rows in order to consistently account for the spatial structure introduced by row planting (see **Figure 2**). Crop row orientation, and therefore Clip Strip orientation, may change from year to year.

    a. Stand over the top of 'Flag1' facing North, and rotate clockwise until you are perpendicular to the crop rows.

    b. Extend a meter tape or measuring stick outward to the required 'Length' (L) distance specified in **Table 7**, and mark this distance with another pin flag – 'Flag2'.

    c. Stretch a string and stake set between Flag1 and Flag2 to mark one long edge of the Clip Strip.

    *Note:* For crops in **Table 7** that require Clip Strips with length < 2 m, use the folding measuring stick instead of a string/stake set.

    d. Standing over Flag1 again, look down the length of the string toward Flag2, and use the meter tape or measuring stick to place two more pin flags X cm to the right of Flags1 and 2, where X is the 'Width' (W) distance (**Table 7**, **Figure 2**).

    e. Stretch a second string and stake set between these two new flags. The second string should be parallel to the first, and the strings and flags should delineate a rectangular Clip Strip with the dimensions shown in **Table 7**.

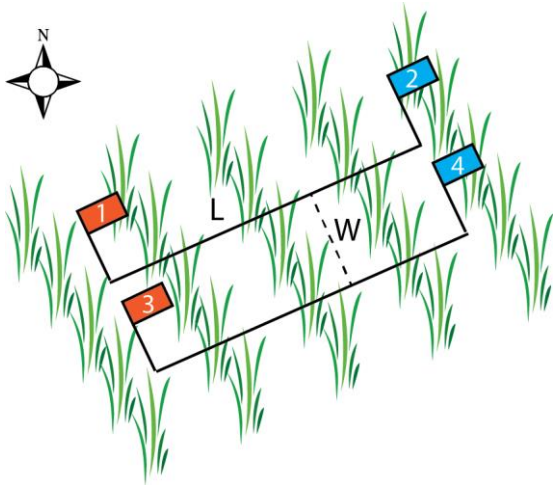


**Figure 2**. Clip Strip establishment in an agricultural row crop. Numbered flags correspond to those referenced above in step (5); 'Flag 1' corresponds to the SW corner of the Clip Strip that is provided in the Clip List. Values for the length (L) and width (W) of the Clip Strip are crop-specific and are provided in the table below.

**Table 7**. Clip Strip dimension required for crops expected at NEON agricultural sites. Row spacing is for typical plantings, and may vary from listed value.

| Crop | Row spacing | Quadrat dimensions, L x W (m) |
|---|---|---|
| Barley | 18 cm (7 in) | 2.0 x 0.5 |
| Corn | 38 or 76 cm (15 or 30 in) | 1.5 x 0.65 |
| Sorghum | 38 or 76 cm (15 or 30 in) | 1.5 x 0.65 |
| Soybeans | 38 cm (15 in) | 1.5 x 0.65 |
| Wheat | 18 cm (7 in) | 2.0 x 0.5 |

### ROW SPACING

- If row spacing differs from that listed in **Table 7** for a given crop by > 25%, submit a JIRA ticket to determine whether a different quadrat dimension is warranted.
- An early season reconnaissance may be helpful to detect potential crop spacing, and address Clip Strip dimension issues prior to scheduled sampling. For example, you may check on crop rows during plant phenology sampling.

6. If there is no biomass in a Clip Strip (i.e., neither crop nor non-crop biomass is present), AND the Clip Strip is deemed representative of the plot:
    - Record 'targetTaxaPresent = N' for the plotID in question.

7. Using a permanent marker, label an appropriately sized paper bag with the information below (use large yard-debris paper bags for high-volume crops like corn). Remember that you will need to clip and sort any non-crop biomass into the **herbGroups** listed in **Table 8** of RD[04], so label additional smaller 8# or 25# craft paper bags for this biomass, if present.

    - **boutNumber**
    - **date**; use *YYYYMMDD* format
    - **clipID**; e.g. BLAN_001_126
    - **herbGroup**; for crops, this will simply be the crop type (e.g., 'corn', 'soy', etc.), and for non-crop biomass, herbGroup values are identical to those in RD[04].
    - **bagCount**; the total # of bags generated from a given clipID (for Field Ops tracking purposes only).

8. Clip and sort all crop and herbaceous non-crop biomass rooted within the Clip Strip. Recall that an individual must be ≥ 50% rooted within the Clip Strip to be counted as 'in' for clipping.

    General guidelines:

    - For corn brace roots (**Figure 1**), clip all brace roots for an individual if it is ≥ 50% rooted within the Clip Strip.
    - Do **NOT** clip vegetation that passes through/leans over the Clip Strip but is not rooted in the strip.
    - **DO** include leaves and stems in the harvest that exit the strip, but originate from stems rooted within the strip.

    For high-volume crops (e.g., corn, soybeans, some cereals): Create a representative sub-sample to bring back to the lab:

    ***Step-by-Step Sub-sampling Procedure:***

    a. Attach a large bag to the 10 kg spring-scale that will accommodate the entire fresh sample, and tare the scale.

    b. Separate ears or seed pods from the stalk/leaves, weigh the ears or seed pods, and note the mass to the nearest 100 g; this is the **seedFreshMass.**

    c. Remove ears/seeds from the large bag and temporarily set aside in a second bag. If harvesting corn, leave ears intact.

    d. Use hand pruners or long-handled loppers to break up large stems and leaves into roughly 12" sections, and collect all clipped vegetative mass (stalk/leaf biomass) into the large bag.

    e. Place **seedFreshMass** from step (b) into the large bag on top of the vegetative mass, and weigh the total clipped mass with the spring-scale.

i. This is the total **freshMass**; record to the greatest precision afforded by the spring-scale (e.g., if the spring-scale has 100 g sub-divisions at the finest resolution, record to the nearest 50 g).

f. Divide the **seedFreshMass** by **freshMass**, and note in your field notebook; this is the **seed:fresh** ratio.

g. Attach the bag labeled in step (7) to the 5 kg spring-scale, and tare. Select a representative ear or handful of seed pods from the large bag, place into the labeled bag, and note the mass to the nearest 100 g.

i. This is the **subsampleSeedFreshMass**.

h. Divide the **subsampleSeedFreshMass** by the **seed:fresh** ratio.

i. This is the target **subsampleFreshMass** (vegetative mass + **subsampleSeedFreshMass**)

i. Add representative handfuls of vegetative biomass from the large bag to the labeled bag until the desired **subsampleFreshMass** is achieved (± 100 g).

i. Record **subsampleFreshMass** to the greatest precision afforded by the spring-scale, as above.

j. Place the subsample into cold storage as soon as possible (e.g., cooler with re-usable cold packs), and transport back to the laboratory for drying.

***Example:***

The **freshMass** of a corn Clip Strip is 5000 g (vegetative + cobs), and the **seedFreshMass** is 2000 g (cobs only). The **seed:fresh** ratio is 2000/5000 = 0.4

One representative cob is selected for the subsample, with **subsampleSeedFreshMass** = 500 g. The target **subsampleFreshMass** = **subsampleSeedFreshMass** / **seed:fresh** = 500/0.4 = 1250 g.

Add vegetative mass to the existing cob until the total mass is 1250 ± 100 g. This is the **subsampleFreshMass** to place in the cooler and take back to the laboratory.

<u>For low-volume crops (some cereals, etc.): Do not create a sub-sample</u>

a. Place the entirety of the clip-harvested crop sample into the bag from step (7).

b. Place the bagged sample into cold storage as soon as possible, and transport back to the laboratory for drying.

c. Leave **freshMass** and **subSampleFreshMass** blank.

9. When clipping is finished, group any small 8# bags from non-crop samples from the clipID together into a 25# bag, label with the clipID and date, and place in cold storage.
    - If cold storage space is insufficient for the crop volume, seal paper bags containing the sample, and keep at ambient temperature while still in the field.
10. Record the total number of bags from the Clip Strip in the **bagCount** field, and if any bags were placed in the cooler, record the **time** in the "Field" ingest table.
    - Do not record **time** if there are no samples placed in the cooler.
11. If there is no biomass associated with one or more herbGroups defined in RD[04], record which herbGroups were absent in the **remarks** field so it is clear that all herbGroups have been assessed.
    - In the **remarks** field, record 'absent = XXX, YYY' etc., where XXX and YYY are the 3-letter codes for the herbGroups that were absent.
12. If a high-volume crop was subsampled, dispose of any excess fresh biomass that will not be brought back to the laboratory at the edge of the crop field.
13. Return to step (1) for the next plotID.

### B.2 Points of Confusion

**Table 8**. Additional guidelines for field conditions that required special handling or consideration.

| Field condition | Guidelines |
|---|---|
| Plot planted in more than one crop type | Accept a random Clip Strip that falls within the dominant crop, as visually assessed by % cover. Reject other Clip Strips. Note that rejected Clip Strips are not rejected permanently, as are un-representative Clip Strips in RD[04], due to the fact that crop cover changes regularly. |
| Wind thrown corn with multiple root points, at least one of which is in the Clip Strip | Only clip biomass associated with rooting points located within the strip. |

### B.3 Sample Preservation

- Keep paper bags with clipped vegetation in a cooler with cold packs to minimize wilting and biomass loss.
- Change cold packs for fresh ones every 12 h or transfer to a 4 ˚C refrigerator if a drying oven is not immediately available.
- Return to the laboratory for drying within 24 h of clipping, if possible.

- Transfer bags of clipped biomass to the drying ovens as soon as possible after field sampling, and monitor drying progress with the "Lab Drying" data sheet.
- Remember that samples must be dried within 5 days of clipping.

**IMPORTANT**: Record the **clipDate** and **time** in the "Field" ingest table AND **ovenInDate** and time on both the sample bags and the "Lab Weighing" ingest table so that the number of hours the bags were stored cold can be automatically calculated.

## SOP C Post-Field Sampling Tasks

### C.1 Refreshing the Sampling Kit

- Make sure the following consumables are available in sufficient quantity for the next round of clip-harvests:
    - Large 'yard waste' style paper bags, 30 gallon capacity (high volume crops)
    - Paper bags, 8# and 25# kraft (or the necessary size given site vegetation stature)
    - All-weather paper for printing field datasheets
    - Permanent markers for labeling bags in the field
- Return cold packs to the -20 ˚C freezer to refresh.

### C.2 Equipment Maintenance and Cleaning

- Clean blades of hand clippers with an appropriate solvent (oil, ethanol, water), and dry thoroughly.
- Recharge batteries for the GPS unit (if necessary).
- Recharge batteries for the TruPulse (if applicable).

## SOP D Laboratory Processing of Agricultural Biomass Samples

***Overview***

Oven dried 'dryMass' values are generated for crops and herbaceous non-crop plants from the same clip strip. Drying and weighing of clip-harvested crop biomass is very similar to that described for herbaceous biomass in RD[04], with the exception that high-volume crop biomass is subsampled prior to oven drying. A subsampling approach ensures the drying ovens are not monopolized by a small number of high-volume samples. Because of the subsampling approach used for high-volume crops, data are entered into the 'Lab Weighing' ingest table differently for high-volume crop versus low-volume crop and non-crop herbaceous biomass:

- High-Volume Crop biomass (SOP D.1):
    - Values for **freshMass**, and **subsampleFreshMass** are recorded in the field (SOP B).
    - Record **subSampleDryMass** in the 'Lab Weighing' ingest table.
    - Values of **dryMass** are calculated automatically.
- Low-Volume Crop and Non-crop Herbaceous Biomass (SOP D.2):
    - Fields for **freshMass, subsampleFreshMass ,** and **subsampleDryMass** are left blank.
    - Record measured **dryMass** of entire clipped sample.

### D.1 Drying and Weighing Clipped High-Volume Crop Biomass

Most of the crops to which this SOP applies will be high-volume crops, and clipped biomass from all plots will therefore not readily fit into NEON's drying ovens. Certain cereals may be low-volume crops; if all of the clipped crop samples can fit into the drying ovens, use the low-volume crop biomass approach (SOP D.2, below).

1. Place labeled 25# subsample bags into a 65 ˚C drying oven for 48h – 120h (2d – 5d).
2. After placing all bags from one clipDate in the oven, check the drying progress of clipped biomass using a subset of 10 bags, and the "Lab Drying" datasheet.
    a. Check the weight of the same selected subset of 10 bags per clipDate after day 1, 2, 3, etc. Record these weights each day on the "Lab Drying" datasheet.
    b. Calculate the difference in weight between the latest two time points for each bag.
    c. Subsamples are dry when the average weight difference between the last two time points = 0 (averaged across all 10 bags, ± 0.1 g or ± 0.5% of the previous timepoint mass, whichever is larger).

### DRYING TIPS

- A spreadsheet calculator is useful for calculating the average weight difference. A link is provided in the 'Supporting Documents' section of the Field Ops Sampling Support Library on Sharepoint.
- To save time, plant material may be weighed WITH the bag.
- Focus on the heaviest bags, as these will likely take the longest to dry.

3. Remove bags of dried biomass from the drying oven, and label bags with **ovenOutDate**/Time.
    - Dried plant material should be weighed immediately after removing from the drying oven, as it will absorb moisture from the air if left in ambient room conditions (particularly in humid environments).
        - If using this method, it is helpful to remove bags from the oven and weigh one at a time.
    - Dried subsamples may also be stored for up to 30 days in ambient room conditions prior to weighing. Subsamples treated in this manner must be returned to the drying oven for 24 h prior to weighing, and must be weighed as above after removal from the oven.
4. Weigh crop subsamples from each clipID using an electronic scale, and a plastic tray or weigh boat.
    - Record **subsampleDryMass** in the "Lab Weighing" ingest table; nearest 0.01 g, plant material ONLY (without the bag).
    - Avoid splitting the subsample into subgroups for weighing, as uncertainty values from weighing must be added each time a subgroup is created.
    - Do NOT record **dryMass**. This will be calculated automatically by the Fulcrum ingest application.
5. Record required metadata for each subsample in the "Lab Weighing" ingest table:
    - **weighDate**; date subsample was weighed in the laboratory, *YYYYMMDD* format
    - **plotID**; *SITE_XXX* format
    - **clipCellNum**; last three digits of the clipID
    - **clipDate**; date sample was clipped in the field, *YYYYMMDD* format
    - **ovenInDate / Time**; date and time (24-h format) subsample was placed in oven
    - **ovenOutDate / Time**; date and time subsample was removed from oven
    - **herbGroup**; select 'corn', 'wheat', 'soy', 'sorghum', etc.

### D.2 Drying and Weighing Clipped Low-Volume Crop and Non-crop Herbaceous Biomass

Low-volume crop biomass and non-crop herbaceous biomass is dried and weighed as described in RD[04]. In contrast to high-volume crop biomass processing above (SOP D.1), no subsampling is employed here: Dry and weigh the entire sample, as in RD[04].

1. In the "Lab Weighing" ingest table, record:
    - **herbGroup;** for low-volume crop, select 'wheat', 'barley', etc.; for non-crop biomass select the appropriate herbGroup described in RD[04] (i.e., cool-season graminoid, leguminous forb, etc.)
    - **dryMass**; total dry mass of the entire sample, nearest 0.01 g, plant material ONLY (without the bag).

### D.3 Data Verification and QA

- QA sample weighing is not performed for high-volume crop samples.
- For low-volume crop samples and non-crop herbaceous biomass, QA sample weighing is performed as in RD[04].
    - Record QA weight data to the nearest 0.01 g in the **qaDryMass** field of the "Lab Weighing" ingest table.

## SOP E Data Entry

Data entry uses the Herbaceous Biomass ingest, and is identical to that described in SOP G of RD[04].

# APPENDIX A SITE-SPECIFIC CROP SAMPLING

## A.1 D04 LAJA

**Crops present (Distributed Plots):**

- Tomatoes
- Peppers
- Squash
- Sunflowers
- Mangoes
- Others

**Distributed Plot sampling strategy:**

- Many of the plots planted with the above crops are located in small-scale experimental parcels of an Agricultural Experimental Station, and support Master's experiments, etc.
- Disrupting small-scale academic research is not desired, and plots located within these crop types should not be clip-harvested.
    - Record `targetTaxaPresent = Y` for the plot/clipID.
    - In the **remarks**, record 'Experimental Crop: X, not sampled', where X is one of the crops listed immediately above.

**Crops present (Tower Plots):**

- None. Tower Plots are grazed.