**Overview:**

This protocol is used for 2 main data collection points.

**1. BIOMASS:** First, we harvest 1 m2 area of biomass, and will use the fresh weight of biomass recorded in the field, and a subsample of this dried back in the lab to estimate the total biomass, and biomass N contributed by the cover crop. This biomass information is communicated to growers, and used in cover crop models, including the water sensor model.

**2. DECOMPOSITION:** Second, we use the harvested fresh biomass in decomposition or litter bags to create a model of decomposition rate of the cover crop over the following growing season.

Decomp B****ag Logic: You will be harvesting cover crop biomass from a 1 m2 area - twice - in each subplot to provide the biomass needed for all 12 litter bags in each subplot. These 2 areas are then averaged in the subplot. This value of average biomass per 1m2 is divided by six to give the mass/ decomp bag.

**IMPORTANT: READ ME FIRST. The “In Field Protocol” begins as close as possible to the date a grower terminates the cover crop.**

Go to the Database Tab Field Hist\_CC\_Crop\_N - scroll over to the CC Termination Date column. Record the date that the cover crop you are harvesting is actually terminated by the grower. You will need to communicate with the grower that you need them to contact you when they are getting ready to terminate a cover crop. Giving them phone calls when the weather warms will help them give you an estimate of when they’ll kill their cover.

In the Database Tab Bag\_Dates - In Time 0 deploy, enter the actual date you went to harvest biomass and put decomp bags in the field.

Note: **Ideally you are harvesting biomass Time 0 deploy immediately prior to CC Termination** and Time 0 deploy is **same or before** CC Termination Date . But in reality they will be close to each other, and sometimes you will have to harvest biomass after a grower has terminated. Record the herbicide used in termination in the Field Hist\_CC\_Crop\_N and use proper re-entry periods and safe handling methods when harvesting biomass with herbicides. Always use gloves when handling biomass treated with herbicides, and evaluate whether you can use safe grinding procedures when processing biomass later.

**Step 1 Prep BEFORE FIELD Bag\_Pre Tab**

1. Print Barcode\_bag for your field sites
2. Calculate how many 1mm mesh “decomp” bags you need by multiplying # of sites by 20.
3. Sort 24 barcodes per field code: T0 to T5 = 6 time points x 2 subplots (1 and 2) in field x 2 crop rows (A and B).
4. Attach metal tags to litter bags.
5. On 20 mesh bags attach T1 to T5 time point barcodes.
6. On 4 paper bags stick the T0 time point barcodes
7. DO NOT WASTE THE MESH BAG WITH T0. Use pre-weighed and barcoded T0 on paper bags not mesh.
8. On the mesh bags, use a paint marker to write an additional number system in case the metal barcode tags fall off and are lost.
9. In Bag\_Pre Tab, scan the Barcode\_bag, RECORD the written number, and RECORD pre-weight.
   1. Note about weighing: This pre-weight should be to at least 1 decimal place (2 is better), using the same scale, or very similar (ie: same model, accuracy, calibration, etc) that you will use for the fresh weight and dry weight of the same bag later on in the process.
10. Use large rubber bands or bags to organize each site’s 12 bags per subplot for ease of finding in the field.
11. ~~Print Barcode\_biomass (different than Barcode\_bag) for using to record whole 1 m2 fresh biomass in the field. There are 2 Barcode\_biomass per site - one for each subplot.~~

**Materials for In Field:**

**Supplies:**

* Laptop and charger that can go to the field
* Barcode scanner
* Nitrile Gloves
* Google Database sheet active for being able to edit offline, or hotspot to pull up Google sheets in the field
* Barcoded and Pre-weighed 1 mm mesh litter bags (26cm x 60 cm) and paper bags (24 total per site - 20 mesh and 4 paper - details later)
* Lots of staplers and extra staples (because you are going to lose a stapler in the truck)
* Field Scale, large (for weighing whole m2 harvest area) ~ 10 to 20 lb max
* Field Scale, smaller (for weighing fresh weight of decomp bags) with at least one decimal place - and is equal to the scale that you use for pre-weighing the bags and taking dry weights after retrieving the decomp bags)
* 2 to 4 Meter sticks, Quadrats of 1 m2 area , or similar measuring device
* 2 Large Totes for weighing total biomass
* 1 Small tote for weighing fresh wt. Decomp bags
* Landscape staples
* Electric clippers or Hand shears
* Extra pairs of scissors and clippers
* 2 to 4 Extra large plastic bags
* Brown paper bags for time zero
* Glyphosate and *Metolachlor* and small sprayer
* GPS unit

**Protocol: In the Field**

**Step 1 Choosing your Subplots:**

1. Refer to the Field Level Schematic and scout out biomass in Subplot 1 and 2 - the same where soil samples are taken.
2. The biomass 1 m2 areas need to represent the biomass that will be present where a sensor will be installed.

Note: Generally Biomass in litter bags in Subplot 1 *will not match the amount of biomass* in Subplot 2. Also note, having lots of variation between subplots is good. Make sure your Subplot 1 area inside cover crop treatment is representative of the Subplot 1 area inside the bare ground treatment. Variation within subplots (subsample A and B) is bad.

**Step 2 Harvest Biomass:**

1. First note method of cover crop planting, broadcast or drill line spacing.
2. Open Database tab Biomass\_INFIELD.
   1. RECORD drill line spacing Use chart to accurately harvest a meter square area. - See Appendix at end
3. Take 2 plastic bags (labeled A and B) into subplot 1. You will be harvesting subsamples A and B for each subplot.
4. Walk into treatment area with cover crop, lay measuring device in representative area for Subplot 1. Avoid center 15ft for moisture sensor area. Avoid edges by about 7ft. (See “CIG field level schematic diagram”)
5. When harvesting cover crop, remove or avoid previous cash crop litter. Clip biomass near base but excluding decomposed material.
6. Put harvested cover crop material from 1m2 into large plastic bag labeled A
7. Repeat harvest of 2nd m2 area for subplot , bag labeled B.
8. Return bags to truck weigh fresh biomass.
9. In tab Biomass\_INFIELD. SCAN barcode\_biomass (Ex: B FFF 1). Now put this barcode inside the paper bag for the subplot in A0. We will use it later.
10. Place bag A on scale and RECORD fresh weight of biomass subsample A, then in next column RECORD fresh weight of biomass subsample B.
11. RECORD the empty weight of a plastic bag from either A or B (they should be very similar), and allow calculation in sheet to divide by 6 for “Target fresh wt. per decomp bag” to create in field for Subplot 1.
12. In large tote empty the contents of subsample A and B bags and *mix well.*  Take scissors and trim stems to 25 cm fit in decomp bags.
13. Observe the contents of the tote - RECORD if it is greater than 40% legume.
14. Once our fresh weights of the first subplot has been recorded, collect a few handfuls of extra cover crop material from a region nearby and mix it into tote.
    1. This is to ensure ease of making bags as some material gets lost in the process.
    2. While storing this material and preparing to make decomp bags be sure to keep all materials in the shade and work quickly as to not lose water weight, and alter the intended fresh weights going into the decomp bags
    3. If you cannot make decomp bags immediately after taking fresh weights, store samples in plastic bags in the shade of the truck.

1. Repeat all these steps for biomass harvest for Subplot 2.

**Step 3 Fill Decomp bags:**

1. Find the corresponding stack of pre-weighed mesh bags for subplot 1 A’s and B’s T0 to T5. There should be 12 total, (10 mesh and 2 paper). For good science, mix them up right now. Don’t distribute fresh biomass in chronological order.
2. Check the Biomass\_INField Tab for the Column “Target fresh wt. per decomp bag” of that subplot. What is that number? Memorize it. You are going to try to hit that target 12 times. You can go over or under by a gram or so, but it’s important to record the actual fresh weight accurately.
3. In Bag\_Fresh Tab, scan barcode\_bag of the first bag you grab.
4. You will distribute the fresh material into each bag (60cm x 26cm). Fill the bag with “Target fresh wt. per decomp bag” fresh weight by cutting biomass pieces to 25 cm (good enough to fit in the bag the shorter dimension) in length as you fill the litter bags.
5. Fold bag over one time, small fold just ½ inch or so, and staple 3 times across to secure.
   1. Important: Be aware that the surface area of 6 bags (26 x 60 cm) is important, and has been designed to represent an area on the ground that would contain cover crop. Make sure that the fold in the bag to staple closed is not too large, and that the cover crop inside the mesh is very well distributed across the whole bag, representing how the cover would look in the field.
   2. The size (26 x 60 cm) times 6 is actually total 0.936% of the total 1 m2. That means we are stuffing about 7% more biomass into each bag than was grown per area. This has been accounted for.
6. RECORD IN THE FIELD BagFreshWt(g) Bag+sample - combined total weight of bag plus biomass plus staples.
7. When grabbing biomass to fill bags, take random, representative samples.
8. Make sure that the T0 paper bags are very representative of the subsamples, since those ones will be used to estimate moisture for every decomp bag in the subset. I like to fill my paper bags near the beginning of the set of 12.
9. Retain Time Zero brown paper bags to be brought back to the laboratory. For each site you will bring home 4 paper bags and leave 20 mesh bags in the field.

**Step 4 Placing litter bags in plots:**

*Note: There are two subplots and two sets of six bags (A and B) going in each subplot. In plots that have fertilizer broadcast, you don’t have to be concerned about placement in the field. For bags receiving fertilizer every other inter-row, each set of two litter bags will be split within the subplot to fertilized and unfertilized inter-rows. Record whether row A or B resided in the row with or without fertilizer*

**If Cover Crop is Terminated Prior to Crop Planting::**

1. Put bags in bare ground in the headlands of the field.
2. **Make some bare ground with your cutters if you need to and/ or bring a backpack sprayer to kill a patch for the temporary placement of the bags**.
3. Be cautious of weeds growing into the bags during this time when they are on field edge. *A pre-emergent herbicide mixed with roundup is the key to keeping decomp bags weed free when growers terminate prior to planting.*
4. The bags will need to be moved in between the corn rows according to procedure below.
5. Place bags T1 to T5, A and B, each subplot (20 of them) all together for now…
6. Use metal landscape staples around the corners to secure them to the ground.

**Placing Litter Bags in Rows::**

1. Following crop planting (typically after your next trip to retrieve decomp bags at T1 or at sensor installation), and after emergence
2. Separate decomp bags into Subplot 1 and 2 (Decomp bags that will be in the same subplot all begin with the same number *prior* to A or B)
3. Then further divide decomp bags into A and B - A and B represent a crop row in a subplot.
   1. Ex: A row: 1 A1; 1 A2; 1 A3; 1 A4; 1 A5
4. Line up remaining decomp bags in the center of the crop row, in a representative area of subplot 1, near the water sensor, but Litter-bag placement should be **at least 5 feet from water sensors** and previous soil sampling holes.
5. Remove cover crop residue from the surface where decomp bags will be placed so that they are touching soil surface.
6. Align the bags so that they are skinny end to end (long ways).
7. Pin down bags on four corners with metal pins.
8. Place a flag near the bags in row A and row B. Then place a flag at the front of the crop rows, of these rows, so that you will be able to find your bags further in the season.



**Step 5 Litter bag collection:**

1. Go to the Bag\_Dates Tab, and record the Time 0 deploy date on the day you harvested biomass and created litter bags in the field. By entering the T0 date, you will pre-generate the dates on which you will need to return to collect the remaining bags. The T5 Target date is determined after you enter the Crop Planting Date in the FieldHist\_CC\_Crop\_N tab.
2. **Bag retrieval times in DAYS AFTER DEPLOYMENT:**
   1. **T0: 0 - returned to lab day of biomass harvest**
   2. **T1: 14**
   3. **T2: 30**
   4. **T3: 60**
   5. **T4: 90**
   6. **T5: Harvest ~ 120 days depending on crop**
3. **How many bags do I collect?**
   1. **Everytime you retrieve decomp bags you will collect a time point for Subplot 1 and 2, row A and B - so each site will have 4 bags to retrieve EACH COLLECTION TIME**
   2. **Carefully check each label when in the field an example collection for Time2 should read:**
      1. **DAA 1 A2**
      2. **DAA 1 B2**
      3. **DAA 2 A2**
      4. **DAA 2 B2**
   3. **It is important that you track the actual recovery date of each bag, so check labels carefully and make notes.**
4. Bare ground left from litter bag removal should be covered with nearby material to reduce edge effect.
5. Upon removing them from the field, remove any weeds growing into the bags, **shake off soil from outside of bag**, place the litter bags in a brown grocery bag.
6. **Return to the lab and put decomp bags IMMEDIATELY in the dryer at 60C to halt decomposition.**
7. Dry bags for 2 days/ 48 hours at 60C until dry, checking samples periodically. Don’t over stuff the dryer, to allow air flow.

**Step 6 Back in the Lab - and Grinding:**

1. When bags are dry, remove from oven and weigh hot.
2. In Tab Bag\_DWT- scan barcode\_bag and RECORD dry weight hot. IMPORTANT - RECORD bag retrieval dates here in this tab.
3. Store decomp bags to be ground for %C and %N analysis.

**For T0 paper bags:**

1. Record the dry weights in In Tab Bag\_DWT as soon as possible in the season. These T0 dry weights are being used in calculating the total biomass for the field.
2. **Once dry weight is recorded - Combine T0 A and B to represent 1 subplot. This is for T0 bags only.**
3. **Grind T0 biomass ASAP - this is for use in predicting nitrogen decomposition in the current cash crop season. This data will go in NIR tab.**
4. Put the barcode\_biomass (that you put inside the A0 bag) from “B - Farmcode -subplot1or2” on a plastic ziplock bag, about quart size and put contents of ground biomass in ziplock bag.
5. Send 10 grams (at least 5 grams) goes to Julia Gaskin for NIR analysis. Archive remainder of sample.

1. **In Laboratory: Ashing protocol:**

**Materials/equipment:**

1. Muffle furnace
2. Dessicator with fresh desiccant
3. Analytical scale
4. Crucibles
5. Tongs

**Procedure:**

1. Wash a set of crucibles with distilled water, mark with number. If needed, heat at 550 C for 4 hours. Cool in desiccator. (dry desiccant in oven at 65C, if needed).
2. Weigh crucible, record weight to nearest 0.0001g.
3. Thoroughly mix sample. Place 1 gram of ground litter bag material into a crucible.
4. Place crucibles with litter sample in oven at 60-70C for four hours. Cool in desiccators. Record crucible and litter dry weight to nearest 0.0001 g.
5. Transfer crucibles with litter sample in furnace. Set at 550 C for four hours to overnight. Then set to 100C and allow to cool. When temperature is down to 100C, place in desiccators to cool.
6. Record crucible and ash weight to 0.0001 g.
7. Clean the crucibles.

APPENDIX: How to represent 1 meter square area

Harvesting Biomass in 1 m2 area depending upon drill spacing.

When choosing drill lines, scout out the subplot first, and choose the drill lines to harvest that have the same average gappy-ness in stand and representative cover crop size.

|  |  |  |  |
| --- | --- | --- | --- |
| Drill spacing  In inches | Drill Spacing in cm | **# of Drill lines to Harvest** | **Cm long length per drill line to Harvest** |
| Broadcast | Broadcast | none | 100 cm x 100 cm |
| 6” | 15.24 | 7 | 93.74 cm |
| 6.5” | 16.51 | 6 | 100.95 cm |
| 6.5” | 16.51 | 7 | 86.53 cm |
| 7” | 17.78 | 6 | 93.74 cm |
| 7.5” | 19.05 | 6 | 87.49 cm |
| 8” | 20.32 | 5 | 98.43 cm |
| 10” | 25.4 | 4 | 98.42 cm |