NEON FSU Field and Lab Protocol for Ops CPER 2011: Aboveground Plant Vegetation Structure and Biomass

PREPARED BY	ORGANIZATION	DATE
Courtney Meier	FSU	3/25/2011

APPROVALS (Name)	ORGANIZATION	APPROVAL DATE
Becky Kao	FSU	09/29/2011

RELEASED BY (Name)	ORGANIZATION	RELEASE DATE
Anne Balsley	CCB ADMINISTRATOR/DCS	10/03/2011

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1 INTRODUCTION

1.1 Purpose

The primary purpose of this document is to provide a change controlled version of Observatory protocols, and is the version used for external review by subject-matter experts. This document provides the content for training and field-based materials for NEON staff and contractors. Content changes (i.e. changes in particular tasks or safety practices) occur via this change controlled document, not through field manuals or training materials.

This document is a detailed description of the field data collection, relevant pre- and post-field tasks, and safety issues as they relate to this procedure and protocol.

1.2 Scope

This document relates the tasks for a specific field sampling or laboratory processing activity and directly associated activities and safety practices. This document does not describe:

- general safety practices (i.e. how to drive a boat)
- site-specific safety practices (e.g. how to safely walk in a stream)
- general maintenance (i.e. fill the car with gas)

It does identify procedure-specific safety requirements such as safe handling of small mammals or safe use of required chemicals and reagents.

1.3 Acknowledgements

If a protocol is based closely on the work of another program or author, note that here.

2 RELATED DOCUMENTS AND ACRONYMS

2.1 Reference documents

If you want to reference other procedural documents (e.g. associated Protocol document), drawings, etc. then include filenames in the following sections.

RD[01]	NEON.DOC.000008 NEON Acronym List
RD[02}	EHS Safety Policy and Program Manual
RD]03…]	<primary science design docs explaining/justifying this protocol/these procedures>
RD[04]	NEON Sampling Design Document
RD[05]	Training Plan
	QA/PA Plan
	DOORS requirements
	ATBD
	NEON.DOC.000243 NEON Glossary of Terms

2.2 Acronyms

NEON	National Ecological Observatory Network
FSU	The NEON Fundamental Science Unit at Headquarters
P&P	Procedure and Protocol
EP	Ecosystem Productivity [plot]
VS	Vegetation structure
CPER	Central Plains Experimental Range

3 BACKGROUND AND OBJECTIVES

3.1 Background

This document describes the required protocols for conducting field sampling, making a human-mediated field observation, or operating an instrument to make measurements in the field, or any other activity that generates a Level 0 data product.

Briefly describe science rationale for selecting protocol. Specific details of methodology are described in standard operating procedures (SOPs) included as appendices. Recommended length < 1 page.

3.2 Science requirements

This protocol fulfills the following Observatory science requirements:
List science requirements from DOORS that are met by this protocol.

3.3 Data products

List Level 0 data products measured by protocol.

Table 1. A summary of field and related lab measurements and the associated NEON Data Products.

Measurement	Data Product

4 PROTOCOL

Linking ground-based measurements of vegetation structure, biomass and productivity with remotely sensed datasets collected by the NEON Airborne Observation Platform will enable mapping of plant biomass, productivity, and carbon flux at landscape scales.

Properly accounting for grazing, the contribution of different plant growth forms to overall aboveground biomass (sorting biomass to sub-shrubs, grass functional type, etc.), and determining whether biomass was produced in the current year or a previous year are the most important requirements for collecting quality data from this field work.

Vegetation structure measurements and aboveground biomass clip harvests occur in the 4 Ecosystem Productivity (EP) plots (Figure 1) located within the dominant vegetation type found within the tower airshed. There are 5 biomass sampling plots per EP plot (referred to throughout this document as "biomass plots"), and each biomass plot is matched with a grazing exclosure. This design results in 10 measurement/harvest points per EP plot, for a total of 40 measurement/harvest points. In addition to EP plots, vegetation structure and aboveground biomass procedures may also be carried out within the

NEON biodiversity plots. In Figure 1, permanent markers along the transect are represented by concentric circles spaced every 50 meters. These markers are associated with high-resolution GPS coordinates. The red concentric circle shows the EP plot origin. Rectangles represent biomass plots, and short black lines mark the locations of grazing exclosures; VS measurements and aboveground clip harvests occur at both of these locations. Green lines are spaced every 10 m, and mark locations where Leaf Area Index is measured (see additional documents for LAI protocols).

Placeholder for **Error! Reference source not found.**Figure 1: General layout of an EP plot and associated biomass plots at the NEON D10 CPER site.

Within each biomass plot, harvest strips are moved each year to minimize effects of harvest on subsequent biomass data. Grazing exclosures are also moved yearly. There are 3 species of cactus at the CPER site, and biomass from these species is not harvested. Biomass from *Opuntia polyacantha* (Plains Pricklypear) is estimated with a conversion factor after counting the number of pads. The two small species of barrel cactus are not included in biomass estimates.

The draft sampling design for aboveground plant vegetation structure and biomass employed for the D10 2011 Field Operations prototype is based on sampling designs and harvest techniques developed at two Long-Term Ecological Research (LTER) sites also located on grasslands (the SGS-LTER, and the Cedar Cr. LTER). It is NEON's desirement to estimate aboveground vegetation structure, biomass and productivity to within 10% of the mean, and to adequately capture the range of variability of these measurements within the tower airshed. However, at present it is unclear whether the current design will accomplish this goal.

5 QUALITY ASSURANCE AND QUALITY CONTROL

The NEON QA/QC plan for these measurements is under development and TBD.

6 DECISION TREE

Table 2. Decision tree associated with the plant aboveground vegetation structure and biomass clip harvests, indicating how to respond to unanticipated delays in field or lab work, and consequences of these delays.

Delay	**Action**	**Adverse outcome**	**Outcome for Data Product**
Hours	If 1) Delay prevents completion of sub-plot harvest: a) Ensure all small bags of sorted biomass are labeled; b) place small bags into one larger 25# bag and label; and c) resume harvest of same sub-plot asap.	None	None
	If 2) Delay occurs between sub-plot harvests: resume harvest of next sub-plot asap.	None	None
Day	If 1) Delay prevents completion of sub-plot harvest: a) Ensure all small bags of	None	None

Delay	Action	Adverse outcome	Outcome for Data Product
	sorted biomass are labeled; b) place small bags into one larger 25# bag and label; c) oven-dry all biomass as per protocol; d) resume harvest of same sub-plot asap with new labeled bags; and e) combine dried biomass for weighing for sub-plot.		
	If 2) Delay occurs between sub-plot harvests: resume harvest of next sub-plot asap.	None	None
2-7 days	If 1) Delay prevents completion of sub-plot harvest: a) Ensure all small bags of sorted biomass are labeled; b) place small bags into one larger 25# bag and label; c) oven-dry all biomass as per protocol; d) resume harvest of same sub-plot asap with new labeled bags; and e) combine dried biomass for weighing for sub-plot.	None	None
	If 2) Delay occurs between sub-plot harvests: resume harvest of next sub-plot asap.	None	None
8-13 days	If 1) Delay prevents completion of sub-plot harvest: a) Ensure all small bags of sorted biomass are labeled; b) place small bags into one larger 25# bag and label; c) oven-dry all biomass as per protocol; d) resume harvest of same sub-plot asap with new labeled bags; and e) combine dried biomass for weighing for sub-plot.	Aboveground biomass harvested per unit area may change in the field over this length of time.	Increased error in aboveground biomass and NPP estimates.
	If 2) Delay occurs between sub-plot harvests: resume harvest of next sub-plot asap.	Aboveground biomass harvested per unit area may change in the field over this length of time.	Increased error in aboveground biomass and NPP estimates.
2 or more weeks	If 1) Delay prevents completion of sub-plot harvest: a) Ensure all small bags of sorted biomass are labeled; b) place small bags into one larger 25# bag and label; c) oven-dry all biomass as per protocol; d) resume harvest of same	Aboveground biomass harvested per unit area may change in the field over this length of time.	Increased error in aboveground biomass and NPP estimates.

Delay	Action	Adverse outcome	Outcome for Data Product
	sub-plot asap with new labeled bags; and e) combine dried biomass for weighing for sub-plot.		
	If 2) Delay occurs between sub-plot harvests: resume harvest of next sub-plot asap.	Aboveground biomass harvested per unit area may change in the field over this length of time.	Increased error in aboveground biomass and NPP estimates.

7 SAFETY

Personnel working at a NEON site should be familiar with and practice safe field work as outlined in the EHS Safety Policy and Program Manual. 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.

If measuring vegetation structure for trees and woody shrubs, care is required when using the laser rangefinder/clinometer in order to avoid pointing the laser in a person's eyes.

Safety training is required to maintain and to operate the electric shears that are used for the aboveground biomass clip harvests.

For laboratory work, safety training is required to operate the drying ovens, Wiley Mill, and Mixing Mill.

8 PERSONNEL REQUIREMENTS

For the field work, a minimum of two field technicians are required for vegetation structure measurements and aboveground biomass clip harvests. Optimal number of field technicians is four (two teams of 2, with each person working somewhat independently).

- Demonstrated ability to identify to species the dominant cool season and warm season grasses at the CPER site, using diagnostic traits and a dichotomous or polyclave key.

For the labwork associated with biomass harvested from the field, a minimum of two technicians is required to sort biomass following clip harvests; the optimal number of technicians for sorting biomass is four. Preferably, these technicians are the same technicians who harvested the biomass in the field. One technician is required to weigh, grind and sub-sample biomass for shipment to external analytical facilities, though efficiencies in these tasks will be gained if 2 technicians are able to work together.

- Demonstrated ability to identify to species the dominant cool season and warm season grasses at the CPER site, using diagnostic traits and a dichotomous or polyclave key.

9 TRAINING REQUIREMENTS

The NEON training plan associated with these activities is under development and TBD.

10 FIELD STANDARD OPERATING PROCEDURE

10.1 Sampling frequency and timing

At sites with significant cover of trees and shrubs, measurement of tree and shrub vegetation structure may take place at any time following peak biomass. Peak biomass of the vegetation typically occurs between mid-July and mid-August, at the height of the growing season. Site specific NDVI data or other model outputs will be used in the future to estimate the timing of peak biomass.

Measurement of sub-shrub, forb, and grass vegetation structure shall take place immediately prior to the aboveground biomass clip harvest.

In the grasslands of the short-grass steppe, vegetation structure measurements and aboveground biomass clip harvests are performed twice during the growing season. The first bout is timed to capture peak biomass of cool season annual grasses and cool season perennial grasses, and should be initiated between 5/23/2011 and 6/6/2011. The second bout is timed to capture peak biomass of the warm season perennial grasses that dominate CPER, and should be initiated between 8/15/2011 and 8/29/2011. Biomass must be sorted in the lab the same day it is harvested from the field; for a given biomass plot, it is strongly preferable that the field harvest and lab sorting be performed by the same technician on the same day. Sorted aboveground biomass must be placed in a drying oven within 24 hours of harvest from the field.

We have estimated that for each plot requiring vegetation structure measurements and aboveground biomass clip harvest, it will take 2.5 hours of field work (temporary plot delineation, measurements, clip harvest, initial sorting, etc.), and 1.5 hours of lab work (final sorting and drying; performed on the same day as harvest and by the same technicians). As such, one technician should be able to measure, clip, and sort 2 plots in one day. Given that there are 20 grazed plots, and 20 un-grazed plots to measure, clip, and sort, it follows that 4 technicians should be able to process all plots in 10 × 8-hour work days (not including travel time).

Table 3. The approximate sampling dates for vegetation structure measurements and aboveground biomass clip harvests at the NEON CPER site.

Domain, Site	Date(s)	Frequency
D10, CPER	5/23/11 – 6/6/11	1X Spring bout
D10, CPER	8/15/11 – 8/29/11	1X Summer bout

10.2 Contingency decisions

Please see the Decision Tree in Section 6.

10.3 Field procedure

At each NEON site, field technicians will collect vegetation structure (VS) data from two different size classes of trees and shrubs: individuals > 1 m in height with DBH > 5 cm, and tree and shrub individuals with DBH < 5 cm and height ≥ 30 cm. A subset of VS data will also be collected for smaller stature sub-shrubs, forbs, and graminoids at two times during the growing season.

Vegetation structure measurements of small-stature plants (i.e. sub-shrubs, forbs, and graminoids), and clip harvests of these same plants occurs within biomass plot harvest strips (Figure 2). Following vegetation structure measurements, sub-shrub, forb and graminoid biomass is collected from these harvest strips, and in additional strips located in matched grazing exclosures. Biomass sub-plots and matched grazing exclosures are located near the 50 m, 100 m, 150 m, 200 m, and 250 m permanent markers along the EP plot center-line (Figure 1).

Placeholder for Figure 2: Biomass sampling sub-plot located within Ecosystem Productivity plots.

10.3.1 Equipment and materials

The equipment listed here is sufficient for two teams of 2 to simultaneously measure vegetation structure and carry out the aboveground biomass clip harvests in two different biomass plots and their matched grazing exclosures (i.e. 4 harvest strips total – 2 from biomass plots, and 2 from matched grazing exclosures). It is assumed that field technicians are working along the same EP plot at any given time.

Table 4. Materials and supplies required for vegetation structure measurements and aboveground biomass clip harvest procedure.

Item Description	Quantity per sampling event	Hazardous Chemical
100 meter steel tape	1	NA
100 meter fiberglass tape	1 (used only for shrub VS[1] procedure)	NA
Juno SB GPS unit	2 (one per team)	NA
Digital rangefinder/clinometer with foliage filter	1 (used only for shrub VS procedure)	NA
3-inch white bicycle reflector	1 (used only for shrub VS procedure)	NA
30” wire stakes with 4”× 5” vinyl flags	100 (used only for shrub VS procedure)	NA
Roll of flagging tape	2 (one per team)	NA
Pre-marked string and stake sets[2]	20 sets (5 per person)	NA
15 meter fiberglass tape	2 (one per team)	NA
Battery powered electric grass shears (10 cm width)	4 (one per person)	NA
Hand clippers	2 (one per team)	NA
Paper bags, 8# kraft	(100, 25 per person)	NA
Work gloves	(2 pair, one per team)	NA
Pre-printed labels	(100, 25 per person)	NA
Hammer	2 (one per team)	NA
Rite-in-the-Rain datasheets and QC checklists	1 set per person	NA

Item Description	Quantity per sampling event	Hazardous Chemical
Sharpies	4 (one per person)	NA
Mechanical pencils	4 (one per person)	NA

[1]VS = vegetation structure
[2]Pre-marked string and stake sets are used to temporarily mark plot boundaries while carrying out field work, and require fabrication prior to field work. Each set consists of 2 tent stakes connected by fine nylon cord. Each field technician will need 5 sets of pre-marked string and stakes. Because biomass plots are 3 m × 4 m in length, this means 2 sets with 4 m length cord, and 3 sets with 3 m length cord. Nylon cord is measured, cut, and marked such that the biomass plot boundaries, buffer zones, and harvest strips depicted in Figure 2 are clearly visible on the cord. Pre-marked string and stake sets should be clearly labeled so that the length of the string is readily apparent.

10.3.2 Preparation

At least 10 days prior to field work, check to make sure that all consumables required for this field work are available for use (i.e. flagging tape, 8# kraft paper bags, pre-printable labels, and water resistant paper). Re-order these items as necessary prior to field work.

At least 2 days prior to field work, all necessary field equipment for vegetation structure measurements and aboveground biomass clip harvests shall be assembled to ensure that:

- Batteries are charged in the digital rangefinder/clinometer
- Batteries are charged in the electric shears
- Batteries are charged in the GPS unit(s)
- Waypoints, layers, and background images are loaded onto the GPS units.
- Blades on electric shears are sharp, and shears are in good working condition
- Datasheets and QC checklists are printed on water resistant paper and organized into a field binder
- Labels for paper bags used for the biomass harvest are pre-printed, with appropriate space available for data that must be recorded manually in the field
- Pre-marked string and stake sets are fabricated, marked, and labeled

Finally, laboratory bench space (for fine sorting of clipped biomass) and sufficient space within drying ovens shall be available the same day as biomass harvests occur in the field.

10.3.3 Sample collection in the field: Vegetation structure measurements

At each NEON site, field crews will collect vegetation structure data from two different size classes of trees and shrubs: individuals > 1 m in height with DBH > 5 cm, and tree and shrub individuals with DBH < 5 cm and height ≥ 30 cm. A subset of VS data will also be collected for smaller stature sub-shrubs, forbs, and graminoids immediately prior to aboveground biomass clip harvests. At the D10 CPER site, vegetation is dominated by sub-shrubs, forbs, and graminoids, and there are two biomass peaks and associated harvests in the tower airshed: one in late spring, and another in mid/late summer. The following protocols detail how to collect VS data from shrubs, sub-shrubs, forbs, and grasses.

10.3.3.1 Data collection for shrubs

All shrubs greater than 30 cm in height will be measured within the EP plot boundary. If there are > 1000 shrubs ≥ 30 cm in height, a smaller 100 m × 20 m sub-plot may be prescribed for data collection. This decision will be made by the NEON Plant Ecologist or FSU Manager. Small sub-shrubs ≤ 30 cm in height (e.g. *Artemisia frigida*) are included in the forb category with respect to biomass measurements at the CPER site.

1. Locate the EP plot for which shrub vegetation structure data will be obtained. See Figure 1 for a general layout of EP and biomass plots at the site.
2. Run a 100 m steel tape down the center of the plot from the plot origin marker to the 100 m marker. Employing the triangulation method (Figure 3), use 30" wire stakes with vinyl flags to temporarily mark the plot corners, and also use triangulation to mark the left and right sides of the plot boundary (long dashed lines in Figure 4) at the 50 m mark.

Placeholder for Figure 3: Establishing location of plot corners for vegetation structure measurements.

Placeholder for Figure 4: Method for determining the relative location of individual trees and shrubs within EP plots.

3. Next, run a 100 m fiberglass tape along the left long-edge of the plot (long dashed lines in Figure 4), and using the tape as a guide, mark the boundary every 10 m with flags in order to help visualize which shrubs are within/without the plot boundary.
   a. Collect data from the plot in sections, beginning with the area to the left of the plot center-line (i.e. those individuals with a "-X" position; see Figure 4).
   b. When all shrubs left of center have been measured, move the fiberglass 100 m tape to the right long-edge of the EP plot, flag the boundary every 10 m as above, and measure those individuals with a "+X" position.
   c. Only collect data from trees and shrubs that are rooted within the EP plot boundary.
4. For a given individual tree/shrub, record the following data on the "Large stature veg structure" datasheet:
   a. The individual ID # (this may not exist for the CPER prototype).
   b. Species code – the first two letters of the genus combined with the first two letters of the species, often followed by a number (see site specific species list for species codes).
   c. The horizontal distance "X" to the plot center-line to the nearest 0.1 m, using a digital rangefinder (e.g. TruPulse 200 equipped with a brush filter, by LaserTech Inc.). *Note: The "X" and "Y" positions of shrubs will not need to be recorded for individuals that are already permanently marked.* Do the following to record the "X" distance with the TruPulse 200:
      i. One team-member holds the 3-inch reflector at the base of the target shrub. The other team-member holds the rangefinder while standing at the plot center-line, such that the line between the shrub and the center-line is at right-angles to the center-line (Figure 4). Once in position:
      ii. Power on the unit by pressing the "Power/Fire" button.

iii. Press ▲for 4 seconds, and the active Target Mode will be displayed in the viewfinder of the TruPulse 200. It should read “Flt” for “Filter” mode. This mode is used in conjunction with a reflector.

iv. If the viewfinder shows something other than “Flt,” use the ▲ or ▼ buttons to cycle through the target modes, and press the “Power/Fire” button to select “Flt.”

v. Look through the viewfinder at the target shrub, and twist the knurled ring between the objective and the housing to focus the within-viewfinder text.

vi. Press the ▲ or ▼ buttons until **HD** (i.e. Horizontal Distance) appears in the viewfinder.

vii. Look through the viewfinder and aim the crosshairs at the reflector target.

viii. Press and hold the “Power/Fire” button. The word **Laser** should appear in the viewfinder while the laser is active.

ix. Once the measurement is displayed, release the “Power/Fire” button. The measurement will flash once, indicating the datum has been saved to memory.

x. The distance is displayed continuously in the viewfinder until any button is pushed, or the unit powers off automatically.

xi. Record the measured value to the nearest 0.1 meters on the datasheet under the “X” position column.

xii. The unit may be powered off manually to conserve batteries by simultaneously pressing the ▲ and ▼ buttons for 4 seconds.

d. The vertical distance “Y” along the plot center-line, to the nearest 0.1 meters. The “Y” distance is relative to the marker at the origin of the plot center-line (see Figure 4).

i. *Again, note that this step is not required if the individual shrub is already permanently marked.*

e. Average canopy diameter, to the nearest 0.1 m, measured with the 15 m fiberglass tape. Visually inspect the shrub canopy and record data from one measurement that represents the average canopy diameter.

f. Maximum height, to the nearest 0.1 m, using the 15 m fiberglass tape.

g. Status – e.g. alive, dead, obvious herbivore damage, etc.

5. After recording data for a given shrub, temporarily mark it with orange flagging tape to prevent repeat measurements.

6. Measure and mark all individuals ≥ 30 cm height within the initial 100 m × 20 m section of the EP plot.

7. If measuring VS for all shrubs within the EP plot, establish and mark off the next 100 m × 20 m section of the EP plot using the triangulation method described above, and proceed with data collection as described.

8. When finished measuring all shrubs – either from the whole EP plot if shrub cover is relatively sparse, or from a single 100 m × 20 m sub-plot if shrub cover is dense – remove all temporary flagging.

10.3.3.2 Data collection for sub-shrubs, forbs, and graminoids

The average height of sub-shrubs, forbs, and graminoids (i.e. grasses and sedges) is measured within each of 5 biomass plots located within each EP plot, and within the grazing exclosures, for a total of 40 measurement locations within the tower airshed. Height data for sub-shrubs, forbs, and graminoids are collected prior to clipping from the harvest strip to be used for the current year's biomass harvest.

1. Use the "Small stature veg structure" datasheet and/or the "Field biomass QC" checklist to determine which biomass plots require measuring.
2. Using pre-loaded GPS waypoints, locate the biomass plot to be measured that is within the correct EP plot.
    a. The plot corner that is closest to the origin of the plot center-line is hereafter referred to as the "bottom-left" corner of the plot (marked with a circle in Figure 2). See Figure 1 for a general layout of EP and biomass plots at the site.
3. If a grazing exclosure is present, remove the stakes from the grazing exclosure. Use flagging to temporarily mark where the grazing exclosure was placed, and then move the exclosure off to the side of the plot.
4. Find the permanent markers for the sub-plot corners, and use two sets of the pre-marked strings and stakes to temporarily mark from the bottom-left to the bottom-right corners of the plot, and from the top-left to the top-right corners of the plot. Place two more pre-marked string and stake sets along the buffer lines that are parallel to the left and right boundaries of the plot – i.e. the buffer lines marked in red in Figure 2.
    a. Markings on the strings should indicate where plot boundaries, buffer boundaries, and sampling strips are located.
    b. Do not walk in the current year's harvest strip. To avoid trampling the other harvest areas in the plot, walk in the buffer area of the plot during sampling rather than in the harvest area (if possible).
5. Locate the strip assigned to the current year's height and biomass sampling.
    a. If a noticeable and significant disturbance (e.g. gopher mound or ant mound) lies within the assigned strip location, move the location of the sampling strip toward the bottom-left corner of the plot until a strip is found that is not obviously disturbed.
    b. Record the location of the new moved strip.
    c. If a minor disturbance (e.g. rodent activity, some ant activity, etc.) lies within the strip, then record these metadata in the "Notes" column on the datasheet.
6. Run another string/stake set along the harvest strip buffer immediately adjacent to the current year's harvest strip that is to be measured. This will serve as a guide to the area to be measured and then subsequently clipped.

7. Use a fiberglass meter tape to visually estimate the following from the current year's harvest strip to the nearest 0.01 meter. Record all data required in the "Small stature veg structure" datasheet:
    a. Minimum forb height
    b. Maximum forb height
    c. Average forb height
    d. Minimum graminoid height
    e. Maximum graminoid height
    f. Average graminoid height
8. Leave all temporary string/stake markings in place for use with the biomass harvest protocol described in the next section.

10.3.4 Sample collection in the field: Aboveground biomass clip harvests

At the D10 CPER site, sub-shrub, forb and graminoid biomass is collected from harvests conducted twice a year in strips located within five 3 m × 4 m sub-plots per EP plot, and in additional strips located in matched grazing exclosures. Biomass sub-plots and matched grazing exclosures are located near the 50 m, 100 m, 150 m, 200 m, and 250 m permanent markers along the EP plot center-line (Figure 1). Because there are 4 EP plots within the tower airshed, this design results in a total of 40 strips harvested for graminoid, forb and sub-shrub biomass. Within each biomass sub-plot, harvest strips are moved each year to minimize effects of harvest on subsequent biomass data. Grazing exclosures are also moved yearly. There are 3 species of cactus at the CPER site, and biomass from these species is not harvested. Biomass from *Opuntia polyacantha* (Plains Pricklypear) is estimated with a conversion factor after counting the number of pads. The two small species of barrel cactus are not included in biomass estimates.

1. Use the "Field biomass QC" checklist in combination with pre-loaded GPS waypoints to determine which biomass plots require harvesting.
2. Within the correct EP plot, locate the biomass plot that is to be harvested. See Figure 1 for a general layout of EP and biomass plots at the site.
    a. The plot corner that is closest to the origin of the plot center-line is hereafter referred to as the "bottom-left" corner of the plot (see Figure 2).
3. If a grazing exclosure is present, remove the stakes from the grazing exclosure. Use flagging to temporarily mark where the grazing exclosure was placed, and then move the exclosure off to the side of the plot.
4. Find the permanent markers for the biomass plot corners, and use two sets of the pre-marked strings and stakes to temporarily mark from the bottom-left to the bottom-right corners of the plot, and from the top-left to the top-right corners of the plot. Place two more pre-marked string and stake sets along the buffer lines that are parallel to the left and right boundaries of the plot – i.e. the buffer lines marked in red in Figure 2.
    a. Markings on the strings should indicate where plot boundaries, buffer boundaries, and harvest strips are located.

   b. Do not walk in the current year's harvest strip. To avoid trampling the other harvest areas in the plot, walk in the buffer area of the plot during sampling rather than in the harvest area (if possible).

   c. Use the "Notes" section of the "Field biomass QC" checklist to record relevant observations – e.g. trampled grazing exclosure, missing plot markers, vandalization of plots, etc.

5. Locate the harvest strip assigned to the current year's vegetation structure (height) and biomass sampling.

   a. If a noticeable and significant disturbance (e.g. gopher mound or ant mound) lies within the assigned harvest strip location, move the location of the current year's harvest strip toward the bottom-left corner of the plot until a strip is found that is not obviously disturbed.

   b. Record the location of the new moved strip in the "Field biomass QC" checklist.

   c. If a minor disturbance (e.g. rodent activity, some ant activity, etc.) lies within the strip, then record these metadata in the "Notes" column on the "Field biomass QC" checklist.

6. Place a string/stake set along the harvest strip buffer immediately adjacent to the current year's harvest strip that is to be clipped.

7. Label paper bags for the collection of plant biomass. The number and type of plant functional groups that are harvested depends on season. For the Spring harvest, harvested plant groups and codes are listed in (A) below; for the Summer harvest, harvested plant groups and codes are listed in (B) below.

   a. Spring harvest biomass codes:

      i. "CSAG" – cool season annual grasses; live biomass and senesced biomass produced in the current year.

      ii. "CSPG" – cool season perennial grasses; live biomass and senesced biomass produced in the current year.

      iii. "GRAM" – current year biomass from graminoid species other than cool season grasses, i.e. all warm season grass biomass.

      iv. "FORBS" – all cool season and warm season forbs; live biomass and senesced biomass produced in the current year.

      v. "SS" – all cool season and warm season sub-shrubs; live biomass and senesced biomass produced in the current year.

      vi. "OSD" – old standing dead biomass; biomass produced in previous years.

   b. Summer harvest biomass codes:

      i. "BOGR/BODA" – *Bouteloua gracilis* and *Bouteloua dactyloides* (Blue Grama and Buffalograss, respectively); live biomass and senesced biomass produced in the current year. These two species are the codominant grasses of the Shortgrass Steppe, and are therefore counted in their own group.

ii. "FORBS" – all cool season and warm season forbs; live biomass and senesced biomass produced in the current year.

iii. "SS" – all cool season and warm season sub-shrubs; live biomass and senesced biomass produced in the current year.

iv. "WSPG" – warm season perennial grasses; live biomass and senesced biomass produced in the current year from those species other than *B. gracilis* and *B. dactyloides*.

v. "GRAM" – current year biomass from graminoid species other than warm season grasses, i.e. all cool season grass biomass.

vi. "OSD" – old standing dead biomass; biomass produced in previous years.

c. All bags should be labeled with the following information, using a combination of pre-printed label and sharpie. The (*) indicates required information labeled directly on the bag with a sharpie:

i. Date

ii. Site

iii. EP plot #

iv. Biomass plot #

v. *Starting position of harvest strip relative to "bottom" of plot harvest area (do not include buffer, see Figure 4).

vi. *Biomass category; listed in (A) and (B) above. Use the shorthand codes provided.

vii. *Bag # X of Y, where X is the bag #, and Y is the total # of bags within a given biomass group (biomass groups defined above).

8. If cactus biomass is present in the harvest strip, this biomass is treated differently from graminoid and forb biomass. There are 3 species of cactus at the CPER site, and only biomass from *Opuntia polyacantha* (Plains Pricklypear) is estimated. The two small species of barrel cactus are not included in biomass estimates.

a. For *O. polyacantha* rooted within or passing through the harvest strip, count the number of pads within the strip, and record the # of pads in the "Notes" cell of the field datasheet.

i. If only part of a pad lies within the harvest strip, count as a full pad.

b. Remove the *O. polyacantha* biomass from within the harvest strip with clippers. If the individual is rooted within the harvest strip, clip off the cactus plant at the soil level.

c. Dispose of the cactus biomass, and proceed with harvesting graminoid and forb biomass.

9. Once cactus biomass is accounted for (if present), harvest graminoid and forb biomass. Using the cordless electric shears – which have 0.1 m (10 cm) width blades – clip all aboveground vegetation that is rooted in the 1 m × 0.1 m harvest sub-strip (see Figure 2), and sort the clipped biomass into the appropriately labeled bag. Do **NOT** clip vegetation that passes through the harvest strip but is not rooted in the strip.

a. Clip slowly, so as to allow the immediate sorting of clipped vegetation into the categories described above.

b. Clip vegetation as close to the ground as possible (i.e. 1-2 cm above the ground), but do not clip the crowns of perennial grasses, as this will kill the plant (see Figure 5). Crowns are treated separately in the "Belowground biomass" protocol.

Placeholder for Figure 5: Illustration of a perennial grass, and the location of crown material relative to leaves and the soil surface.

c. Use the string/stake set that runs parallel to the harvest strip as a guide for harvesting the correct length strip.

d. Label and use additional paper bags as necessary if you cannot fit all of the vegetation from a given category into one bag.

i. When more than one bag per category is used, label bags within a category as "1 of X", "2 of X", etc., where X is the total # of bags ultimately used for that category.

10. Once all biomass has been harvested and sorted into bags, enter the total number of bags from each plot, as well as the total number of bags within each category into the "Field biomass QC" checklist.
11. If previously removed, replace the grazing exclosure in the same location, re-stake the exclosure into the ground, and remove the temporary flagging that marked where the exclosure was placed.
12. Use the "Field biomass QC" checklist to ensure that height data have been recorded and biomass has been clipped from all current year harvest strips from all biomass plots. There should be data from 40 total harvest strips: 20 from within grazing exclosures, and 20 from outside grazing exclosures.

10.3.5 Sample preservation

Not applicable to this procedure.

10.3.6 Sample shipping

Not applicable to this procedure.

10.3.7 Data handling

Following field work, all information from field QC checklists should be entered and saved as soon as possible to the appropriate MS Access database or Excel spreadsheet.

10.3.8 Refreshing the field sampling kit

Make sure that the following consumable supplies are available in sufficient quantity for the next round of aboveground biomass clip harvests:

- Paper bags, 8# kraft
- Pre-printable labels

- Water resistant paper

10.3.9 Equipment maintenance, cleaning, and storage

Maintain equipment for the next sampling day/bout by doing the following:

- Check blades are sharp on the electric shears
- Recharge batteries for the electric shears
- Check and recharge batteries (if necessary) for the GPS unit(s)
- Check and recharge batteries (if necessary) for the laser rangefinder/clinometer
- Cleaning and drying meter tapes
- Check 30" wire stakes with vinyl flags to determine whether they can be re-used

After completing cleaning and maintenance tasks, return all equipment to the appropriate storage location in the Support Facility laboratory.

11 LAB STANDARD OPERATING PROCEDURE

The following goals are associated with the laboratory procedure: 1) sort, dry, weigh, and record data for the aboveground biomass clip harvest; and 2) grind sorted biomass samples, and create sub-samples suitable for shipment to an external facility for chemical analysis.

11.1 Timing

- Aboveground biomass that is clipped and coarsely sorted in the field must be carefully re-sorted in the lab the same day as harvest, preferably by the same technicians who performed the initial coarse sorting in the field.
- Sorted aboveground biomass must be placed in a drying oven within 24 h of the field harvest.
- Following drying, biomass should be weighed, recorded, and entered into the appropriate MS Access database or Excel spreadsheet within 30 days.
- Grinding and sub-sampling of biomass for bioarchive and shipment to external facilities for chemical analysis may take place as time permits.

11.2 Lab procedure

11.2.1 Equipment and materials

Table 5. Materials and supplies required for processing plant aboveground biomass samples in the laboratory.

Item Description	Quantity per sampling event	Hazardous Chemical
Plant ID book	1	NA
Plant dichotomous or polyclave key	1	NA
Drying ovens	Space as needed	NA
Mass balance (0.01 g accuracy)	1	NA

Large weigh boats	100	NA
Wiley Mini Mill	1	NA
Retsch Mixing Mill	1	NA
20 mL scintillation vials (Wheaton or equivalent)	2000 per harvest bout	NA
Pre-printable labels	2000 per harvest bout	NA
Data sheets and QC checklists	1 set	NA
Sharpies	1 per technician	NA
Pencils	1 per technician	NA

11.2.2 Preparation

At least 10 days in advance, check inventories of all laboratory consumables (i.e. weigh boats, scint vials, pre-printable labels) to ensure that sufficient time is available to re-order items as needed prior to laboratory sample processing.

On the same day as clip harvests occur in the field, bench space for sorting, and space within drying ovens shall be made available.

11.2.3 Sample processing in the lab: Sorting, drying, and weighing

1. ***On the same day as harvest***, return all bags to the support facility lab, and prior to drying, perform the following checks with the “Lab biomass QC” checklist:
    a. Count all bags again.
    b. Compare this independent count of the bag numbers to the field count to make sure no bags have been misplaced.
2. Identify and collate all aboveground biomass bags from a given plot – i.e. group together all bags collected in the field with a unique biomass plot #.
3. Enter the required information on the “Lab biomass QC” checklist:
    a. Name
    b. Time that sorting of bags from a given plot begins
    c. Day
    d. EP plot #
    e. Biomass plot #
    f. Harvest strip location
4. Proceeding with the first bag, empty the contents onto a clean workbench and carefully sort the biomass that was coarsely and quickly sorted in the field. There are two objectives to this additional sorting in the lab:
    a. To remove any “old standing dead” material.
    b. To remove any “live” and “recently senesced” material that belongs in another group. For example, for a Spring harvest, there should not be any “cool season annual grasses

(CSAG)" mixed in with either the "cool season perennial grasses (CSPG)" or the other "GRAM" biomass.

5. After sorting the contents of the bag, do the following:
    a. Place the properly sorted biomass back into its own bag.
    b. Place any "old standing dead" material into the "OSD" bag.
    c. For all other biomass that does not belong in the original bag, create temporary piles on the bench of the other biomass categories that belong in other bags.
    d. Clean the bench of any debris, and proceed to the next bag from the same biomass plot.
    e. Confer with a supervisor to check that all biomass has been sorted correctly.
        i. This step is particularly important early in the season when field techs may be less familiar with local flora. As employees become well-trained, a spot-check of 5%-10% of sorted bags will suffice.
    f. Temporary piles can then be placed in the appropriate bags.
    g. Clean the bench and proceed to the next bag from the same biomass plot. Continue sorting bags from a given biomass plot until all bags are sorted. Bags containing "old standing dead" are not sorted further in the lab.
6. Record the ending time in the "Lab biomass QC" checklist.
    a. Knowing how much time is required to sort all the biomass from a given plot is important for improving both the workflow and the time management of biomass harvesting.
7. ***Within 24 hours of harvest***, place sorted biomass into a 55 ˚C drying oven for 72 h – 120 h (3 d – 5 d).
    a. In order to assess how long each bag has been in the drying oven, label each bag with the date and time it was placed in the oven.
    b. Check that plant material is truly dry by sequentially weighing the same sub-set of 5 samples on day 2, 3, 4, etc.
    c. Use the "Lab biomass QC" checklist to record repeated sample weights during drying.
    d. Samples are dry when weight is constant from one day to the next.
8. Repeat steps (2) – (7) until bags from all biomass plots are sorted and in the drying ovens.
    a. Use the "Lab biomass QC" checklist to make sure bags from all biomass plots have been sorted.
9. Following drying, use a mass balance (0.01 g accuracy) and a weigh boat to weigh the contents of each bag.
    a. Record the mass to the nearest 0.01 g on the "Aboveground biomass" datasheet.
    b. Use the pre-labeled datasheet to make sure that weights are recorded for all bags.
    c. All bags from a given plot are weighed consecutively before weighing begins for another plot.

   d. Weighing should begin immediately after drying is complete, and should take no more than 3 days.

10. Once all weights have been recorded, return the plant material to the appropriate bags, and place into temporary storage. Samples in temporary storage can then be prepared as time permits for bioarchive and tissue chemistry analysis at an external facility (next section).

11.2.4 Sample preservation for bioarchive and chemical analysis

Aboveground biomass samples are first ground to 20-mesh (~0.8 mm) with a Wiley Mill, and this first grind is used for fiber analysis (i.e. lignin, cellulose, hemicellulose). Biomass samples are then re-ground to 0.2 mm with a Mixing Mill, and samples from this second fine grind are used for all other chemical analyses. In order to speed throughput, two people may simultaneously perform three tasks: 1) perform initial coarse grind of biomass samples; 2) perform secondary fine grind of biomass samples; and 3) sub-sample previously ground biomass samples with the splitter. See Figure 6 for a flow diagram of the laboratory processing associated with each aboveground biomass sample.

1. Locate bags of dried biomass in temporary storage that are to be prepared for bioarchive and chemical analyses.
2. For each bag of dried biomass to be ground, prepare eight 20 mL scintillation vials for collection of ground sub-samples. Sub-samples will be used for the following:
   a. Carbon and nitrogen (1 vial); code = “CN”
   b. “Majors” elemental analysis (1 vial); code = “MAJ”
   c. Fiber analysis – i.e. cellulose, hemicellulose, and lignin (1 vial); code = “FIBER”
   d. Ash content (1 vial); code = “ASH”
   e. Isotope analyses (3 vials); code = “ISO”
   f. Bioarchive (1 vial); code = “BIO”
3. Label each vial with a pre-printed label containing the following information:
   a. Date
   b. Site
   c. EP plot #
   d. Biomass plot #
   e. Plant material code (CSAG, CSPG, BOGR/BODA, WSPG, FORB, SS, OSD, etc.)
   f. Analysis code (listed above)
4. To obtain a relatively homogeneous sample suitable for fiber analysis, pass the dried aboveground biomass from each bag through the Wiley Mill one bag at a time, using the 20-mesh (0.841 mm) sieve screen.
   a. Collect ground biomass from each bag in a large beaker or equivalent, and mix thoroughly.

   b. If more than one bag was collected in the field for a given type of plant material (e.g. 2 bags of BOGR/BODA leaves were collected), combine all ground material from the same plant material type and mix thoroughly into one batch.

5. Use a sample splitter with hi-back pans to split out one aliquot of approximately 2 g sample coarsely ground to 20-mesh size.
   a. This is the sub-sample used for fiber analysis. Place the sub-sample into the 20 mL scint vial labeled with “FIBER”.
   b. Clean the splitter between samples using compressed air, or by repeated blowing.
6. Install the 0.2 mm sieve screen into the Retsch Mixing Mill (MM), and pass the remainder of the 20-mesh ground sample through the MM. This will create a fine, homogeneous powder suitable for all other chemical analyses.
7. Use either the sample splitter or a scoopula to divide the 0.2 mm ground sample among the seven remaining 20 mL scintillation vials. The splitter will generate considerable amounts of dust if ground material is poured too quickly.
   a. The “CN” and “ISO” vials will require ~0.25 g ground plant material each.
   b. The remaining ground plant material should be distributed evenly between the “MAJ”, “ASH”, and “BIO” vials.
   c. If there is very little sample to work with, and the “MAJ”, “ASH”, and “BIO” vials ultimately contain < 0.25 g ground material each, then re-distribute the 0.2 mm ground biomass equally among all 7 of the “CN”, “ISO”, “MAJ”, “ASH”, and “BIO” vials.
8. Clean the Mixing Mill with 70% or 95% ethanol, KimWipes, and compressed air in between grinding the contents of each biomass bag (or group of bags of the same plant material type).
9. Use the “Biomass prep QC” checklist to ensure that all bags of dried biomass are processed, and that all sub-samples are accounted for (i.e. 8 scint vials per sample).

11.2.5 Sample shipping

Collate sub-sample vials with the same analysis code, and prepare for shipment to the appropriate external facility for analysis. For example, “CN” vials from all samples should be grouped together, etc. Record the following in the “Biomass prep QC” checklist:

1. The address of the external analytical facility to which samples are shipped.
2. The contact name and telephone number of the sample manager at the external facility.
3. The date of shipment.

The Domain Lab Manager should record in the “Biomass prep QC” checklist the date that data are received from an external analytical facility for a given batch of sub-samples.

11.2.6 Data handling

Enter all data from the “Aboveground biomass” datasheet(s) into the appropriate MS Access database or Excel spreadsheet.

Following initial grinding and sub-sampling, enter data from the "Biomass prep QC" checklist into the appropriate MS Access database or Excel spreadsheet. Once data are received from an external facility, the Domain Manager should coordinate entry of additional QC data (e.g. which datasets have been received from whom, turnaround time, etc.) and sample analysis data into the appropriate MS Access database or Excel spreadsheet. The NEON Plant Ecologist or Biogeochemist is responsible for performing any further QA/QC analysis on the returned data.

11.2.7 Refreshing the laboratory supplies

Make sure that the following consumable supplies are available in sufficient quantity for processing the next round of aboveground biomass samples:

- Large weigh boats
- 20 mL scintillation vials (Wheaton or equivalent)
- Pre-printable labels

Consult with the Domain Manager to re-order consumable supplies as necessary.

11.2.8 Laboratory maintenance, cleaning, storage

At the end of every day, the Wiley Mini-Mill, Retsch Mixing Mill, and sample splitter require cleaning with ethanol (either 70% or 95%) and compressed air. These pieces of equipment also require cleaning between different samples or between different batches of similar samples (as described in the procedure above).

12 DEFINITIONS

Define all protocol specific technical terms in alphabetical format.

13 REFERENCES

Use Ecology style.

APPENDIX A Field data sheets

The following field data sheets serve as a backup procedure for times when electronic data collection devices (PDA) are not available.

Field datasheets to be prepared for the D10 2011 Field Ops prototype include:

- Large-stature vegetation structure
- Small-stature vegetation structure
- Site-specific species list, including species codes, families, and growth forms (e.g. warm season perennial, cool season annual, etc.)

APPENDIX B Lab data sheets

The following data sheets serve as a backup procedure for times when electronic data collection devices (PDA) are not available.

Laboratory datasheets to be prepared for the D10 2011 Field Ops prototype include:

- Aboveground biomass

APPENDIX C Considerations for implementation

Indicate activities that could result in equipment damage, degradation of sample, or possible invalidation of results; listed here and at the critical steps in the procedure.

Describe any component of the process that may interfere with the accuracy of the final product.

Discuss how to avoid common errors in sampling or common ways samples can be contaminated.

Clearly flag things that might impact their work or the scientific data that aren't covered in the procedural pieces (stupid examples: "We're measuring nitrates, if you are exposed to or using nitrates at home on your lawn, trace amounts might contaminate our data"; "If it's raining, sky water getting into the samples before you seal them could alter results")... i.e. call out weird issues and folklore explicitly. See: http://en.wikipedia.org/wiki/Phantom_of_Heilbronn

APPENDIX D Procedure checklist

Field and laboratory QC checklists to be prepared for the D10 2011 Field Ops prototype include:

- Field biomass QC
- Lab biomass QC
- Biomass prep QC

APPENDIX E Figures

Figure 1. General layout of an Ecosystem Productivity (EP) plot and associated biomass plots at CPER.

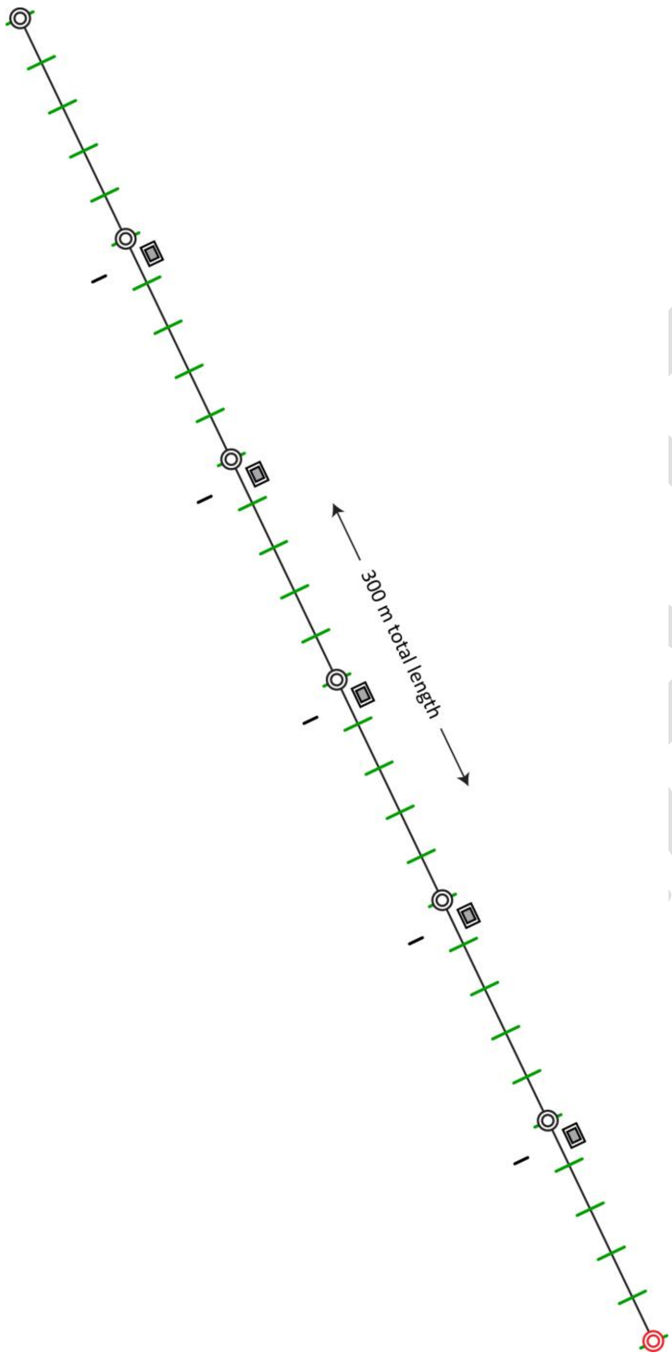

Figure 2. Biomass sampling sub-plot located within Ecosystem Productivity plots.

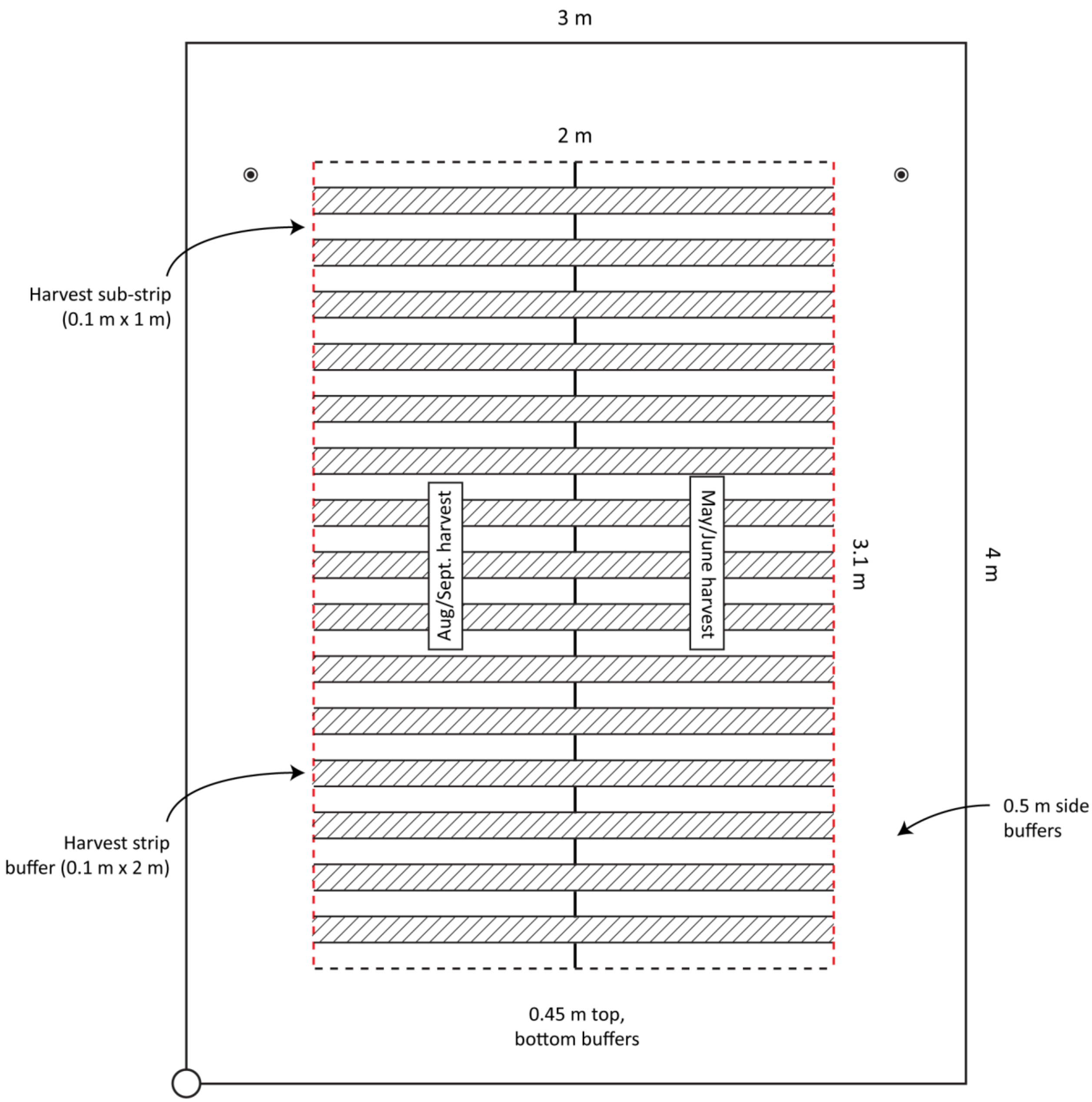

Figure 3. Using the triangulation method to establish the location of plot corners for vegetation structure measurements of trees and woody shrubs.

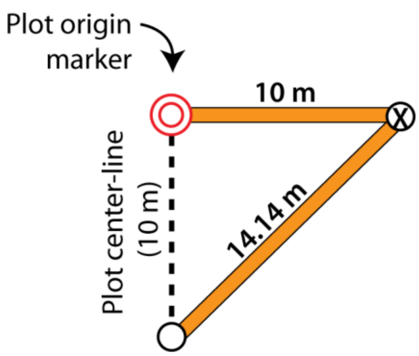

Figure 4. Method for determining the relative location of individual trees and woody shrubs within EP plots.

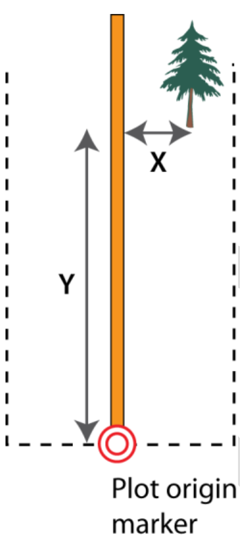

Figure 5. Illustration of a perennial grass, showing the location of crown material relative to leaves and the soil surface.

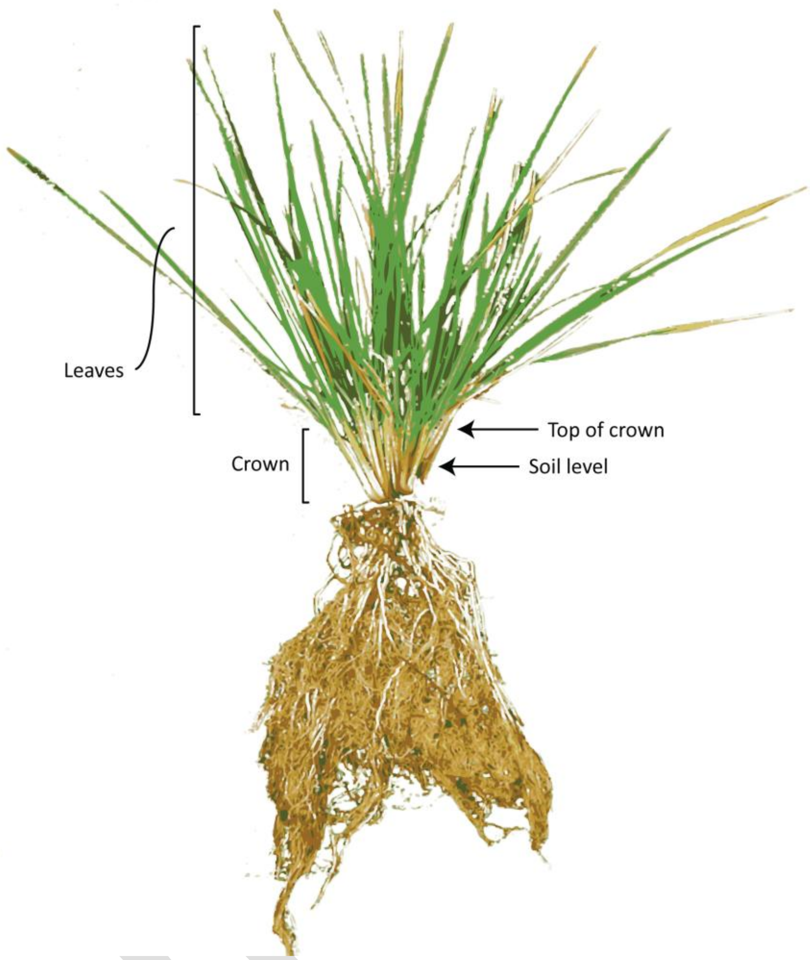

Figure 6. Laboratory sample processing workflow to prepare dried aboveground biomass samples for bioarchive and chemical analysis.