

<i>Title:</i> TOS Protocol and Procedure: Measurement of Herbaceous Biomass		<i>Date:</i> 04/10/2014
<i>NEON Doc. #:</i> NEON.DOC.014037	<i>Author:</i> C. Meier	<i>Revision:</i> E

TOS PROTOCOL AND PROCEDURE: MEASUREMENT OF HERBACEOUS BIOMASS

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Change Record

REVISION	DATE	ECO #	DESCRIPTION OF CHANGE
A_DRAFT	03/25/2011	ECO-00280	Initial Draft Release
B_DRAFT	01/13/2014	ECO-01140	Updates from 2013. Will be finalized in next rev.
C	03/24/2014	ECO-01664	Production release, template change, and other changes as detailed in Appendix C (rev C only)
D	04/10/2014	ECO-01792	Updated Appendix D with site-specific information
E	10/01/2014	ECO-02309	Migration to new protocol template

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1 OVERVIEW

1.1 Background

Herbaceous vegetation is operationally defined in this protocol as non-woody plants (i.e. grasses, sedges, forbs, some bryophytes, and non-woody vines such as *Convolvulus spp.* and certain *Rubus spp.*), as well as woody-stemmed plants with diameter at decimeter height (ddh) < 1 cm at the time of sampling. The net primary productivity (NPP) of this plant group dominates the total NPP of grassland sites, and can contribute significantly to NPP in savannahs and some forests, even though total herbaceous biomass is low relative to that of large woody stems.

Understanding long-term trends in herbaceous community structure and biomass is very important in grazed ecosystems where these plants constitute a critical food source for wildlife and livestock. In addition, members of the herbaceous plant community can respond relatively rapidly to various global change drivers. For example, it is predicted that cool-season C3 graminoids may decrease in abundance relative to warm-season C4 graminoids in more northern latitudes as global temperatures and CO₂ concentrations continue to rise, and water availability becomes more variable.

It is standard practice for herbaceous biomass and productivity to be assessed via clip harvests, followed by sorting clipped material into current-year and previous years' growth in order to estimate annual NPP for this plant growth form. Current-year growth is often sorted by species into additional categories based on plant functional traits – e.g. cool-season vs. warm-season graminoids, or leguminous vs. non-leguminous forbs. In order to engender cross-compatibility with existing research, NEON will sort clipped biomass into functional categories that are broadly similar to those employed by the global Nutrient Network research group (http://www.nutnet.umn.edu/exp_protocol).

In sites where grazing is an important part of the management practice, or where grazing pressure on herbaceous plants from wild grazers is significant, it is standard practice to use small, portable grazing exclosures to estimate the productivity that is consumed by grazing. NEON will employ a standard approach where clip-harvests are performed with paired grazed/exclosed areas per plot.

1.2 Scope

This document provides a change-controlled version of Observatory protocols and procedures. 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.

1.2.1 NEON Science Requirements and Data Products

This protocol fulfills Observatory science requirements that reside in NEON's Dynamic Object-Oriented Requirements System (DOORS). Copies of approved science requirements have been exported from DOORS and are available in NEON's document repository, or upon request.

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Execution of this protocol procures samples and/or generates raw data satisfying NEON Observatory scientific requirements. These data and samples are used to create NEON data products, and are documented in the NEON Scientific Data Products Catalog (RD[03]).

1.3 Acknowledgments

Thanks to Daniel Milchunas of Colorado State University and Mary Ashby of the Central Plains Experimental Range USDA-ARS for valuable advice and insight.

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.004300	EHS Safety Policy and Program Manual
AD[02]	NEON.DOC.004316	Operations Field Safety and Security Plan
AD[03]	NEON.DOC.000724	Domain Chemical Hygiene Plan and Biosafety Manual
AD[04]	NEON.DOC.001155	NEON Training Plan
AD[05]	NEON.DOC.050005	Field Operations Job Instruction Training Plan
AD[06]	NEON.DOC.000914	NEON Science Design for Plant Biomass and Productivity
AD[07]	NEON.DOC.014051	Field Audit Plan
AD[08]	NEON.DOC.000824	Data and Data Product Quality Assurance and Control Plan

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.005003	NEON Scientific Data Products Catalog
RD[04]	NEON.DOC.001271	NEON Protocol and Procedure: Manual Data Transcription
RD[05]	NEON.DOC.001574	Datasheets for TOS Protocol and Procedure: Measurement of Herbaceous Biomass
RD[06]	NEON.DOC.005005	Level 0 Data Products Catalog
RD[07]	NEON.DOC.000987	Measurement of Vegetation Structure
RD[08]	NEON.DOC.001788	Grazing Exclosure Assembly Instruction
RD[09]	NEON.DOC.001920	NEON Raw Data Ingest Workbook for TOS Herbaceous Plant Biomass

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2.3 Acronyms

Acronym	Definition
ddh	Diameter at decimeter height
NPP	Net Primary Productivity

2.4 Definitions

N/A

3 METHOD

Herbaceous biomass clip-harvests occur within randomly located clip-harvest strips located in 20m x 20m plots or subplots. The goal of the clip-harvesting procedure is to estimate the amount of herbaceous biomass produced within the delineated clip-strip area. This means that only those herbaceous plants whose stems enter the ground within the clip-strip are clipped (exceptions to this are woody-stemmed plants with diameter at decimeter height [ddh] < 1 cm; the SOPs describe in more detail how to deal with these plants). There will typically be one clip-harvest per plot or subplot per sampling event, although grazed sites receive more than one clip per plot or subplot per bout; see SOP C.

There are two types of plots where clip-harvests will occur: Distributed and Tower plots. Clip-harvests in 20m x 20m Distributed are organized into 0.5 m x 3m gridded, numbered “cells” that cover the available sampling area within the plot. Within each cell, technicians perform clip-harvests in north/south-facing strips with dimensions of 0.1 m x 2 m (Figure 1). Relative coordinates are assigned to the Southwest corner of each clip-strip, which enable technicians to find the desired clip-strip location for a given sampling bout. For reference, the Southwest corner of each plot is defined as (0,0), and the Northeast northeast corner of the plot or subplot is (20,20) (Figure 1).

Clip harvests in 20m x 20m Tower plots will be carried out identically to those performed in Distributed plots, but the clip harvest protocol is implemented in Tower plots 40m x 40m and larger within each of the 20m x 20m subplots that make up the plot. For example, in a 40m x 40m Tower plot with qualifying vegetation, one clip-strip would be harvested per subplot, for a total of four clip-strips per plot per sampling bout (Figure 2). Again, similar to clip-harvesting in Distributed plots, 1 m² and 10 m² nested subplots are not clip-harvested in Tower plots, but larger sized nested subplots within 40m x 40m and larger Tower plots may support clip-harvesting.

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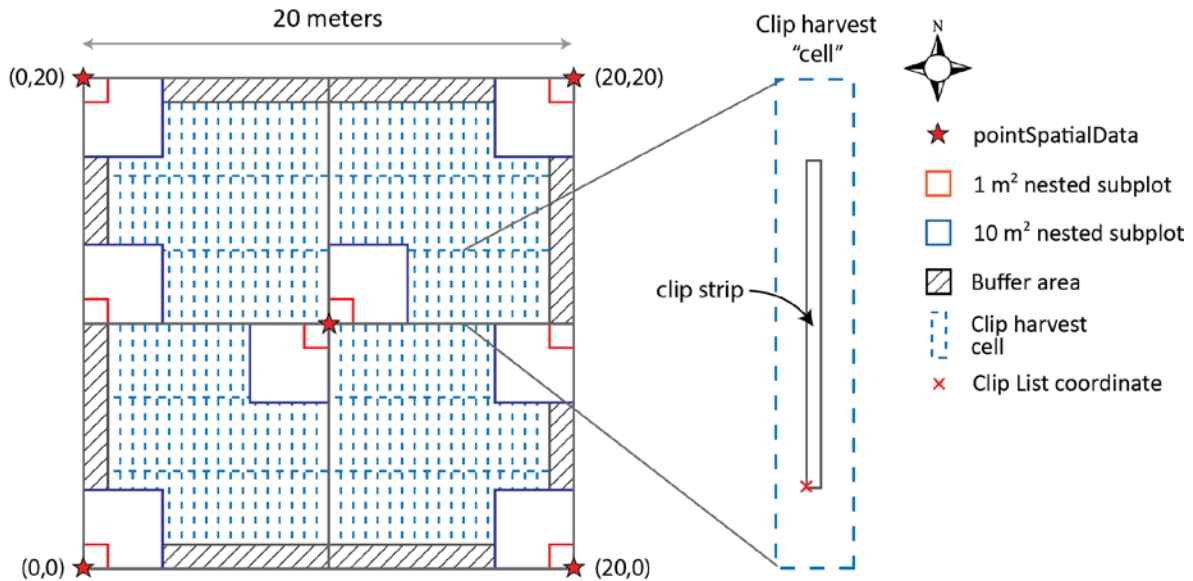


Figure 1. A Distributed plot showing the locations of 0.5m x 3m clip-harvest “cells” (dashed blue lines) that contain potential 0.1m x 2m clip-harvest strips. Coordinates corresponding to the SW corner of the clip-strip (red ‘X’) are provided to technicians in plot-specific Clip Lists.

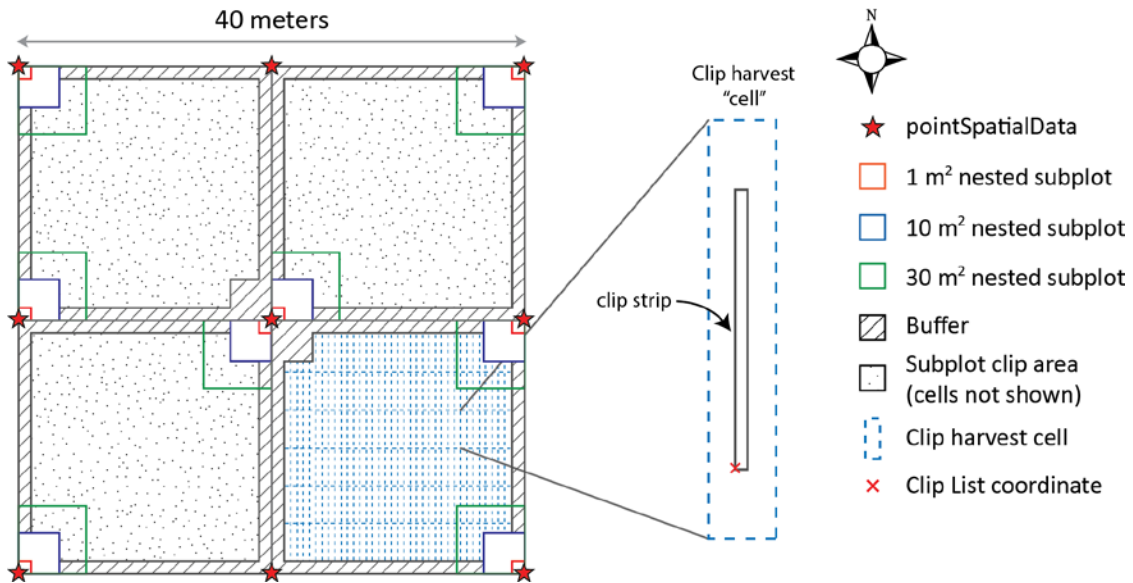


Figure 2. A 40m x 40m Tower plot showing the location of 0.5m x 3m clip-harvest cells (dashed blue lines) within a 20m x 20m subplot. The remaining three subplots support one clip-harvest each per sampling bout, but cells are omitted for clarity. The clip-strip coordinates provided to technicians (red ‘X’) are supplied on a per subplot basis.

To determine a clip-harvest location within a given sampling bout, technicians are provided with a randomized list of potential clip-strip coordinates for each 20 m x 20 m plot or subplot (referred to as “Clip Lists” hereafter). An excess number of potential clip-harvest locations within a particular plot or subplot are randomly determined a priori by NEON Science Operations, with the knowledge that not all

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potential locations will be suitable for clip-harvesting. That is, there may be obstacles such as rocks, trees, ant nests, etc. at any given location that will prevent carrying out a clip-harvest. Technicians should work down this list through time on a per plot or subplot basis, crossing off harvested strips on the list as work progresses from bout to bout, so that re-sampling of a given clip-strip over the lifetime of the Observatory is minimized or eliminated.

Upon arriving at a plot, it is the field technician’s responsibility to first locate the proposed random clip-harvest location, assess its suitability (rejecting and moving on to the next location if necessary), delineate the area for harvesting, and then perform the clip-harvest and biomass sorting. Within each plot or subplot, harvest strips are moved each year to minimize effects of harvest on subsequent biomass data. Additional harvests are required if grazing exclosures are employed at the site. Instructions for utilizing exclosures are provided in SOP C.

Once field work is complete at the plot, technicians must keep harvested biomass cold until sort checking is performed. Best practice is to place clipped biomass into a cooler containing -20 °C cold packs immediately after clipping: keeping clipped biomass cold is critical to prevent wilting, so that species’ diagnostic features are preserved. Within 24-h of harvest, the same technicians who harvested and sorted the biomass in the field must then check each bag of clipped material to make sure that sorting was done properly, and in particular, that no previous years’ biomass is mixed with current-year biomass. Sorted biomass is then either returned to the cooler with fresh -20 °C cold packs, or oven-dried as soon as possible in the laboratory and weighed.

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

Standard Operating Procedures (SOPs), in Section 7 of this document, provide detailed step-by-step directions, contingency plans, sampling tips, and best practices for implementing this sampling procedure. To properly collect and process samples, field technicians **must** follow the protocol and associated SOPs. Use NEON’s problem reporting system to resolve any field issues associated with implementing this protocol.

The value of NEON data hinges on consistent implementation of this protocol across all NEON domains, for the life of the project. It is therefore essential that field personnel carry out this protocol as outlined in this document. In the event that local conditions create uncertainty about carrying out these steps, it is critical that technicians document the problem and enter it in NEON’s problem tracking system.

The procedures described in this protocol will be audited according to the Field Audit Plan (AD[07]). Additional quality assurance will be performed on data collected via these procedures according to the NEON Data and Data Product Quality Assurance and Control Plan (AD[08]).

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A combination of hot, cold, and blind checks may be used to ensure that equipment is used properly in the field, that plant species are identified and sorted properly into functional groups, that samples are dried properly in the lab, and that dried samples are processed according to the protocol.

4 SAMPLING SCHEDULE

4.1 Sampling Frequency and Timing

A given sampling bout should ideally be concluded within 10-14 days of initiation (Table 1), so that the plant community does not change appreciably during the time that all target plots are sampled. This guideline ensures that data collected across all plots within a given bout are comparable. The number of field technicians assigned to the clip-harvesting task should be optimized so that this goal is feasible.

After herbaceous plants are clipped from a given clip-strip, the following points are critical with respect to timing:

- Place clipped biomass immediately into a cooler, and keep stored cool until it can be placed in a drying oven.
- Check field-sorted biomass for sorting accuracy within 24 h of harvest.
- In the “storageHours” column of the “Field Datasheet”, record the number of hours clipped biomass was stored in the cooler before it was placed in the drying oven.

The frequency and timing of herbaceous biomass clip-harvests depends on the type of site being sampled, as well as the type of plots sampled within the site (Table 1).

Herbaceous clip-harvests must be performed within Tower Plots on an annual basis, and sampling these plots is a priority.

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Table 1. Sampling frequency and timing guidelines for herbaceous biomass clip-harvesting based on site and plot type

Site Type	Plot Type	Plot Number	Sampling Events	Yearly Interval	Sampling Start	Sampling Stop
Agricultural	Distributed, Gradient	n=5	1X per sampling year	Annual	2-4 weeks before crop harvest	Within 14d of sampling start
	Tower	n=5	1X per sampling year	Annual		
Grazed ¹	Distributed, Gradient	n=20	1X per sampling year	Every 3y (one site per domain per year)	Peak biomass (Appendix D provides site-specific dates)	Within 14d of sampling start
	Tower	n=30 ²	Every 4 weeks	Annual	10-14d before animal stocking	<ul style="list-style-type: none"> • Senescence, or • Final removal of livestock
Ungrazed	Distributed, Gradient	n=20	1X per sampling year	Every 3y (one site per domain per year)	Peak biomass (Appendix D provides site-specific dates)	Within 14d of sampling start
	Tower	Variable	1X or 2X per sampling year	Annual	Peak biomass ³ (Appendix D provides site-specific dates)	Within 14d of sampling start ⁴

¹ Grazed ecosystems are defined as those actively managed for livestock grazing.

² Plot number may be reduced following initial data collection at a given site.

³ When two clip-harvests are performed per year, peak biomass refers to temporally distinct peaks in biomass associated with particular components of the herbaceous plant community. For example, an early season peak in cool-season graminoid biomass, followed by a late-season peak in warm-season graminoid biomass.

⁴ When two clip-harvests are performed per year, "Sampling Stop" is per bout.

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4.2 Criteria for Determining Onset and Cessation of Sampling

Site-specific sampling start windows are provided in Appendix D. It is incumbent upon Field Operations to select sampling onset dates within these windows. Sampling the herbaceous biomass at agricultural sites poses an additional issue because sampling must be timed such that it occurs before crop harvest takes place. Field Operations Managers responsible for agricultural sites must determine anticipated harvest dates that will influence when sampling should take place.

4.3 Timing for Laboratory Processing and Analysis

Because herbaceous 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. Ideally, samples will be placed in the drying oven within 24 h of clipping in the field, and must be kept in cold storage the entire time between clipping in the field and drying in the laboratory. Keeping samples in cold storage mitigates mass loss by slowing cellular activity. However, when it is not possible to dry samples in the laboratory within 24 h of clipping, it is acceptable to keep samples in cold storage for up to a maximum of 5 days following clipping.

Once samples are dry, they may be placed in temporary storage prior to weighing, grinding, and subsampling for chemical analysis and bioarchive. There are no scientific limits on the time oven-dried samples may be placed in temporary prior to weighing and processing.

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4.4 Sampling Timing Contingencies

Table 2. Contingent decisions

Delay/ Situation	Action	Outcome for Data Products
Hours	If delay prevents completion of clip-harvest strip: 1. Ensure all small bags of sorted biomass are labeled, 2. Place small bags into a 25# bag and label, Resume harvest of same clip-harvest strip ASAP	No adverse outcome.
	If delay occurs between plots, resume harvest of next clip-harvest strip ASAP.	
1-7 days	If delay prevents completion of clip-harvest strip: 1. Ensure all small bags of sorted biomass are labeled, 2. Place small bags into a 25# bag and label, 3. Store already clipped biomass in a cooler/refrigerator (okay), or oven-dry as per protocol (best), 4. Resume harvest of same clip-harvest strip ASAP with new labeled bags, and 5. Combine dried biomass per functional group for weighing when all biomass is dry.	May create potential change in observed NPP, and may increase uncertainty in consumption estimates at grazed sites. May also be difficult to complete clip harvest of all plots in 10-14 day window if delay approaches 7 days.
	If delay occurs between clip-harvest strips, resume harvest of next strip ASAP.	
8-13 days or longer	If delay prevents completion of clip-harvest strip: 1. Ensure all small bags of sorted biomass are labeled, 2. Place small bags into a 25# bag and label, 3. Store already clipped biomass in a cooler/refrigerator (okay), or oven-dry as per protocol (best), 4. Resume harvest of same clip-harvest strip ASAP with new labeled bags, and 5. Combine dried biomass per functional group for weighing when all biomass is dry.	More uncertainty in biomass and NPP estimates, especially in grazed systems. Aboveground biomass per unit area may change in the field over this length of time.
	If delay occurs between clip-harvest strips, resume harvest of next strip ASAP	

For QA/QC of the weighing and data entry portion of the laboratory work, the Field Operations Manager selects 10% of the previously dried, weighed samples for QA/QC per sampling bout. Technicians re-weigh and record the QA mass in the “herbaceousMassQA” field of the “Lab Weighing Datasheet”, and then transcribe QA mass data to the “herbaceousMassQA” field in the “herbaceous_biomass” ingest sheet.

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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[02]) and EHS Safety Policy and Program Manual (AD[01]). 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.

Additional safety issues associated with this field procedure include potential exposure to *Toxicodendron* oils (discussed in Appendix F).

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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, ultra-low refrigerators, etc.

Table 3. Equipment list – Herbaceous biomass clip harvesting at a site with no grazing management (quantities are for two technicians)

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
Durable Items						
	R	Region-specific plantID book	To identify graminoids to species in order to sort into cool-season (C3) and warm-season (C4) categories To identify leguminous forbs	Unknown species expected; early-season on-the-job training	1	N
	R	GPS unit	Navigate to plots/subplots	All	1	N
	R	Compass, mirror-sight	Used with a meter tape to locate clip-harvest strips within plots/subplots.	All	1	N
MX100322	R	TruPulse 360R laser rangefinder and clinometer	Locate clip-harvest strips within plots/subplots when a meter tape and compass are problematic (or unavailable).	Sloped or brushy plots that make finding clip-strips with a meter tape difficult	1	N
	R	Hand clippers	Clipping plants	All		N

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Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
	R	Re-usable cold packs	Keep clipped biomass cool after harvest, preserve species' diagnostic features, reduce unintended mass loss.	All		N
	R	Pre-marked string and stake sets	Delineation of accepted clip-harvest strip	All		N
	R	Per plot or subplot Clip Lists	Identify random clip-strip locations	All	As needed	N
	R	Hand-lens magnifier, 20X	Plant identification	All	1	N
	R	Hand-lens magnifier, 10X	Plant identification	All	1	N
MX100322	R	TruPulse 360R laser rangefinder, with current declination entered	Locate clip-harvest strips within plots/subplots when a meter tape is problematic (or unavailable).	Plot slope > 10 deg; Brushy	1	N
MX103218	R	Foliage filter for laser rangefinder	Facilitates use of TruPulse in very brushy conditions.	Brushy vegetation	2	N
	R	Reflective surface (3-in white reflector or reflective tape on back of field notebook/clipboard)	Aids in accurate location of clip-harvest strips with TruPulse in "FLT" mode, particularly in brushy vegetation.	Used with TruPulse	1	N

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Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
MX104361	R	Chaining pins, steel	Stretching tapes, plot boundary delineation	All	2	N
	R	Fiberglass meter tape (30m or longer)	Locate clip-harvest strips within plots/subplots .	Plot slope < 10 deg; grassland, savannah	1	N
	R	4" x 5" pin flags with PVC stakes	Aids in accurate location of clip-harvest strips; PVC required to avoid magnetic interference with compass or TruPulse.	All	4	N
	R	Pre-marked string and stake sets	Delineation of accepted clip-harvest strip	All	2 sets	N
	R	Ruler, 30cm length, with cm demarcations	Delineation of accepted clip-harvest strip.	All	1	N
	R	Hand clippers, fine tip	Clipping plants	All	2	N
MX100661	R	Dissecting forcep	Plant identification and sorting	All	2	N
	R	Sharpies	Label paper bags	All	2	N
	R	Large chest-style cooler, with frozen cold packs	Keep clipped biomass cool after harvest, preserve species' diagnostic features, reduce unintended mass loss.	All	1	N

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Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
	R	Per plot or subplot Clip Lists	Identify random clip-strip locations	All	Variable	N
	S	GPS unit, pre-loaded with plot locations	Navigate to plots/subplots	All	1	N
	S	Extra battery for TruPulse (CR123A type)	Battery backup	Used with TruPulse	2	N
	S	Work gloves	Hand protection (if necessary)	Coarse, sharp vegetation	2	N
Consumable Items						
	R	Paper bags, 8# kraft ¹	Storage of clipped herbaceous biomass, sorted to functional group	All	50 ²	N
	R	Paper bags, 25# kraft ¹	Storage of smaller bags from a given clipID	All	10 ²	N
RD[05]	R	Herbaceous Biomass Field Datasheets	Record sampling metadata	All	Variable	N
	R	Pencils	Record sampling metadata	All	2	N

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.

R/S=Required/Suggested

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Table 4. Equipment list – Herbaceous biomass clip harvesting at a site with active grazing management (quantities are for two technicians)

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
Durable Items						
	R	Region-specific plantID book	<ul style="list-style-type: none"> To identify graminoids to species in order to sort into cool-season (C3) and warm-season (C4) categories To identify leguminous forbs 	Unknown species expected; early-season on-the-job training	1	N
	R	Hand-lens magnifier, 20X	Plant identification	All	1	N
	R	Hand-lens magnifier, 10X	Plant identification	All	1	N
	S	GPS unit, pre-loaded with plot locations	Navigate to plots/subplots.	All	1	N
MX100322	R	TruPulse 360R laser rangefinder, with current declination entered	Locate clip-harvest strips within plots/subplots when a meter tape is problematic (or unavailable).	Plot slope > 10 deg; Brushy	1	N
MX103218	R	Foliage filter for laser rangefinder	Facilitates use of TruPulse in very brushy conditions.	Brushy vegetation	2	N
	R	Reflective surface (3-in white reflector or reflective tape	Aids in accurate location of clip-harvest strips with TruPulse in “FLT” mode, particularly in brushy	Used with TruPulse	1	N

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Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
		on back of field notebook/clipboard)	vegetation.			
	S	Extra battery for TruPulse (CR123A type)	Battery backup	Used with TruPulse	2	N
MX104361	R	Chaining pins, steel	Stretching tapes, plot boundary delineation	All	2	N
	R	Fiberglass meter tape (30m or longer)	Locate clip-harvest strips within plots/subplots.	Plot slope < 10 deg; grassland, savannah	1	N
	R	4" x 5" pin flags with PVC stakes	Aids in accurate location of clip-harvest strips; PVC required to avoid magnetic interference with compass or TruPulse.	All	4	N
	R	Pre-marked string and stake sets	Delineation of accepted clip-harvest strip.	All	2 sets	N
	R	Ruler, 30cm length, with cm demarcations	Delineation of accepted clip-harvest strip.	All	1	N
	R	Hand clippers, fine tip	Clipping plants	All	2	N

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Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
MX100661	R	Dissecting forcep	Plant identification and sorting	All	2	N
	S	Work gloves	Hand protection (if necessary)	Coarse, sharp vegetation	2	N
	R	Hammer	Remove/pound stakes for grazing enclosure	Grazed sites	1	N
	R	Per plot or subplot clip-strip coordinate lists	Identify random clip-strip locations	All	Variable	N
	R	Sharpies	Label paper bags	All	2	N
	R	Large chest-style cooler, with frozen cold packs	Keep clipped biomass cool after harvest, preserve species' diagnostic features, reduce unintended mass loss.	All	1	N
Consumable Items						
	R	Paper bags, 8# kraft ¹	Storage of clipped herbaceous biomass, sorted to functional group	All	50 ²	N
	R	Paper bags, 25# kraft ¹	Storage of smaller bags from a given clipID	All	10 ²	N

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Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
RD[05]	R	Herbaceous Biomass Field Datasheets	Record sampling metadata	All	Variable	N
	R	Pencils	Record sampling metadata	All	2	N

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.

R/S=Required/Suggested

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Table 5. Equipment list – Post-field sampling tasks

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable Items					
	R	Region-specific plant ID book	Aid in distinguishing morphologically similar species that should be in different functional groups	1	N
MX103211	R	Hand-lens magnifier, 20X	Plant identification	1	N
	R	Hand-lens magnifier, 10X	Plant identification	1	N
	R	Region-specific dichotomous or polyclave key to plant ID	Aid in distinguishing morphologically similar species that should be in different functional groups	1	N
	R	Laptop	Digitization of information in field datasheets	1	N
	R	Clean 2ft x 2ft work surface	Checking the sorting accuracy of biomass clipped and sorted in the field	1	N
	R	Large chest-style cooler or refrigerator	Keep clipped biomass cool	1	N
	R	Re-usable cold packs	Keep clipped biomass cool	Variable	
Consumable Items					
RD[05]	R	Completed Herbaceous Biomass Field Datasheets	Contains field-collected sampling metadata	Variable	N

R/S=Required/Suggested

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Table 6. Equipment list – Processing herbaceous biomass clip-harvest samples in the laboratory

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
Durable Items						
MX100230	R	Drying oven	Dry field-clipped biomass for weighing	All	2 (typically)	N
	R	Large plastic bag (black trash bag or equivalent); e.g. Uline # S-5111	Temporary storage of oven dried samples before they are weighed	All	Box of 100	N
	R	Mass balance (0.01g accuracy)	Weigh oven-dried samples	All	1	N
	R	Large weigh boats	Hold dried sample during weighing	All	Variable	N
	R	Desiccator	Prevents hygroscopic mosses from absorbing water from the air after being dried	Mosses present	1	N
	R	Grinding mill	Grinding dried, weighed samples for archiving and chemical analysis	All	1	N
	R	Sample microsplitter (small capacity)	Creates identical sub-samples from relatively small volumes of ground sample	Relatively little mass clipped per biomassCode per clipID	1	N
	R	Sample splitter (large capacity)	<ul style="list-style-type: none"> Creates identical sub-samples from relatively large volumes of ground sample 	Relatively large mass clipped per biomassCode per clipID	1	N

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Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
			<ul style="list-style-type: none"> Useful with fibrous graminoids 			
	R	Hi-back pans for sample splitter (sized to match splitter size)	Receives sub-samples generated by splitter	All	2 per splitter	N
	R	Scintillation vials with caps	Containers for storing ground split samples for shipment to archive or chemical analysis	All	As needed	N
	R	Sharpie, extra fine tip	Labeling scintillation vials	All	2	N
Consumable Items						
	R	Pencils	Recording dry weight of herbaceous biomass	All	2	N
	R	Datasheets: <ul style="list-style-type: none"> Lab Drying QC Datasheet Lab Weighing Datasheet 	Recording dry weight of herbaceous biomass	All	As needed	N

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Table 7. Equipment list – Team of two minimizing exposure to toxic oils from *Toxicodendron spp.* that should be clip-harvested.

Item No.	R/S	Description	Purpose	Quantity	Special Handling
	R	Small paper bags, pre-weighed, labeled with bag weight. 8# or lunch-sack type.	<i>Toxicodendron</i> biomass never handled directly again after it is placed in pre-weighed bag.	Variable	N
	R	Labeled hand clippers, dedicated to <i>Toxicodendron</i> clipping. Same item type as indicated in main equipment lists	Minimize spread of oils to other equipment.	1	N
	R	Cotton gloves, single use (example)	Prevent oil contact with skin.	Box of 12	N
	R	Disposable PPE outer-wear (example)	Prevent oil contact with skin, normal clothing.	Case of 24	N
	R	Large, single-use plastic bags (trash bag or large Ziploc-type bag)	Transport used gloves and PPE and minimize toxic oil transfer.	Box	N
	R	Cleanser, urushiol-specific. Tecnu or equivalent (example)	Clean clippers after use.	1	N

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6.2 Training Requirements

All technicians must complete required safety training as defined in the NEON Training Plan (AD[04]). Additionally, technicians must complete protocol-specific training for safety and implementation of this protocol as required in Field Operations Job Instruction Training Plan (AD[05]).

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 at the sites within the region of employment. Because different herbaceous functional groups can be sensitive indicators of ecosystem responses to global change (e.g. N deposition, warming, rising CO₂), 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.



For both the field and laboratory work, training 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 required plants 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 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.

Ideally, each team member should know how to use diagnostic traits and a dichotomous or polyclave key to identify unknown species.

Preferably, the technicians sorting biomass are the same technicians who harvested the biomass in the field.

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6.4 Estimated Time

The time required to implement a protocol will vary depending on a number of factors, such as skill level, system diversity, environmental conditions, and distance between sample plots. The timeframe provided below is an estimate based on completion of a task by a skilled two-person team (i.e., not the time it takes at the beginning of the field season). Use this estimate as framework for assessing progress. If a task is taking significantly longer than the estimated time, a problem ticket should be submitted.

An experienced two-person team will require between 1-3 hours to perform clip-harvest sampling within a given clip-strip when biomass is sorted to functional group. This time range includes identifying an acceptable clip-harvest location, delineating the clip-harvest strip, and clipping qualifying herbaceous biomass. The clip-harvest may take less time if herbaceous vegetation is sparse.

For those harvests at sites managed for grazing that are not sorted to functional group, the time required to clip-harvest herbaceous biomass from a clip-strip should be < 1 hour (including all the tasks described above).

7 STANDARD OPERATING PROCEDURES

SOP A Preparing for Sampling

A.1 Sampling Equipment Preparation and Checklist

Table 8. Sampling equipment preparation checklist

✓	Item	Action
	GPS unit	Charge Load target locations
	Compass, mirror-sight	Check/set correct declination ¹
	TruPulse 360R	<ul style="list-style-type: none"> • Check battery, charge (if possible) • Clean lenses with lens cloth or lens tissue (if necessary) • Check/set correct declination¹. See Appendix G.1 for details. Calibrate TruPulse tilt-sensor (only necessary after severe drop-shock; see Appendix G.2 for details).
	Hand clippers	Clean and sharpen blades (if necessary)
	Re-usable cold packs	Place in -20 °C freezer
	Pre-marked string and stake sets ²	Fabricate if necessary: <ol style="list-style-type: none"> 1. Cut 2.5m of 1/8" diameter nylon cord 2. Mark cord at each end with a sharpie so that the center section of the cord measures exactly 2m between the markings. 3. Tie each end to an 8" or longer tent stake.
	Herbaceous Biomass Field Datasheets (RD[05])	Print on all-weather paper
	Per plot or subplot Clip Lists ³	Print on all-weather paper

¹ Declination changes with time and should be looked up annually per site: <http://www.ngdc.noaa.gov/geomag-web/>

² Pre-marked string and stake sets are used to temporarily delineate clip-strip boundaries, and require fabrication prior to field work. Each set consists of two tent stakes connected by nylon cord.

³ Provided separately by Science Operations once plot establishment has been completed.

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A.2 Early-season preparation at a grazed site

Grazing exclosures are deployed only within Tower plots, and one exclosure should be constructed for each 20m x 20m plot or subplot.

- Inspect existing grazing exclosures for wear and damage.
- Construct additional grazing exclosures as needed according to plans outlined in RD[08].
 - The drawings in RD[08] depict two different styles of grazing exclosure, with heights optimized for low-stature grassland vegetation (plants ~ 30cm height), and mid-stature grassland vegetation (plants ~ 1 m height).
 - Choose the exclosure height so that the exclosure height is approximately equal to or just greater than the height of the vegetation.
 - Provide feedback to Science Operations if the assembly document requires updating to include an expanded range of exclosure heights.
- Deploy exclosures within Tower plots or subplots prior to the onset of grazing.
 - The Field Operations Manager must communicate with the site host to ascertain when grazing begins in a given growing season.
 - For each Tower plot or subplot, place an exclosure over the first suitable clip-strip, and stake the exclosure to the ground.

Follow steps in SOP C to locate clip-harvest strips and assess suitability.

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SOP B Field Sampling Sites Not Managed for Grazing

At non-grazed sites, which are defined here to mean those that are not actively managed for grazing, one clip-strip per plot or subplot per bout is harvested. Distributed plots are harvested once per year at peak biomass, and Tower plots are harvested either once or twice per growing season, depending on whether a particular plant community shows seasonally distinct biomass peaks (e.g. C3 peak in spring, C4 peak in summer).

At non-grazed sites, sorting clipped biomass to functional group is required for one harvest per growing season - which may be the only harvest per growing season. If two harvests are performed in Tower plots, the harvest that is sorted to functional group should be selected to coincide with the highest biomass peak.

B.1 Sample Collection in the Field

1. Navigate to the plotID to be sampled (using the GPS if necessary).
2. Use the plot- or subplot-specific Clip List ([plotID]_clipList.csv) to identify the first potential clip-strip location that has not already been sampled or rejected. Where relevant, subplot number is included in the file name and is also provided as a field in the spreadsheet.
 - The Clip List provides the randomized list of potential clip-harvest strips per plot or subplot.
 - The Clip List also indicates which clip-strips have already been harvested or rejected.
3. Locate the relative X,Y-coordinates of the clip-strip SW corner within the plot or subplot. The procedure used to locate the X-coordinate depends on the value of the relative Y-coordinate and the different procedures are detailed below:

If the Y-coordinate is < 10:

- a. Run a tape East/West along the south edge of the plot or subplot between the (0,0) → (20,0) plot markers (Figure 1), and stretch the tape taut.*
- b. Place a pin flag at the desired relative X-coordinate.
- c. Standing directly over the pin flag that was just placed at the X-coordinate, use the TruPulse in **HD** mode with a reflective surface to locate the Y-coordinate.
 - Make sure the azimuth is 0° (True North) when shooting the TruPulse to find the Y-coordinate (see Appendix G).
 - Avoid trampling the potential clip-harvest strip as much as possible.
- d. Place a pin flag at the clip-strip (X,Y) location.

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If the Y-coordinate is > 10:

- a. Run a tape* East/West from the plot centroid (10,10) to either the (0,10) position or the (20,10) position (Figure 1):

X-coordinate	Tape Layout ¹
1 < X < 10	From (10,10) to (0,10) ¹
10 < X < 20	From (10,10) to (20,10) ¹

¹ Use the TruPulse in **AZ** mode to guide the tape along the correct azimuth

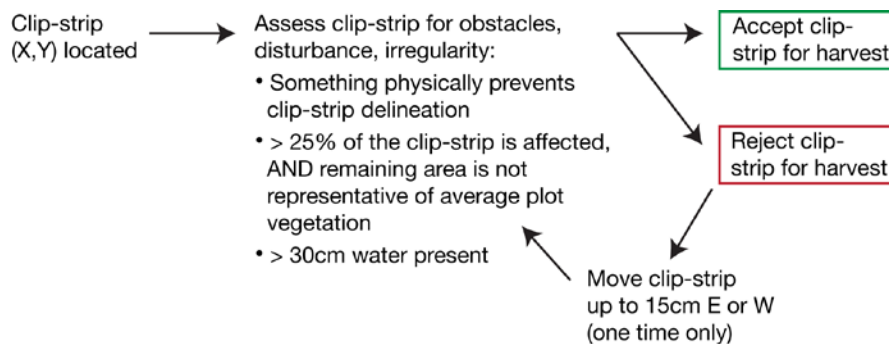
- b. Place a pin flag at the desired relative X-coordinate.
- c. Standing directly over the pin flag that was just placed at the X-coordinate, use the TruPulse in **HD** mode with a reflective surface to locate the Y-coordinate.
- Make sure the azimuth is 0° (True North) when shooting the TruPulse to find the Y-coordinate (see Appendix G).
 - Avoid trampling the potential clip-harvest strip as much as possible.
- d. Place a pin flag at the clip-strip (X,Y) location.

BEST PRACTICE TIPS



- If the plot slope is > 10 %, or there is significant brush or obstacles that prevent accurately stretching a tape, the TruPulse laser rangefinder can be used in **HD** mode to place the initial pin flags relative to the plot markers.
- Plot slope can be quickly estimated using the inclinometer in the TruPulse (**INC** mode).

4. Assess whether the clip-strip location is suitable, and accept or reject the location. Obstacles, disturbances, and/or irregularities may include trees, large rocks, ant nests, etc. Strips should also be rejected if clipping a particular plant specimen in the strip would influence plot-level diversity. That is, the plant in question exists nowhere else in the plot or subplot.



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5. If the clipID is rejected, record why in the “status” column on the clip-strip coordinate list, select the next clipID on the list, and return to step (3) above. Otherwise, update the “status” column and proceed to step (6). Update the “status” column in the clip-strip coordinates list using codes in Table 9.

Table 9. Codes to document acceptance/rejection of clip-harvest strips on the list of clip-strip coordinates.

Code	Definition
0	Rejected; disturbance, obstacle, and/or irregularity encountered within the clipID cell
1	Accepted, no enclosure
2	Accepted, enclosure
3	Rejected temporarily, inundated
4	Rejected temporarily, uncommon plant

6. Delineate the accepted clip-strip for harvesting.
- Using one of the pre-marked string and stake sets, line up one of the marks with the pin flag, and push one stake into the ground.
 - Stretch the string and second stake from the South to the North end of the clip-strip, using the compass or the TruPulse to orient the string in a North/South direction.
 - Keep the compass or TruPulse at least 50 cm from non-aluminum metal plot markers, eyeglasses, wristwatches, etc.
 - Use a ruler to place the second string-and-stake set 10 cm to the right of the first set. Check that the distance between the two strings is exactly 10 cm at both ends of the clip-strip.
 - The two sets of marks on the two string-and-stake sets now clearly delineate a 0.1 m x 2 m area for clip-harvesting.
7. Record salient observations about the clip-strip in the “remarks” field of the “Field datasheet”.
- Example:* Record the estimated % area of the clip-strip disturbed by a gopher mound or the extent of minor ant nesting activity within the strip.
 - If there is no herbaceous biomass in a clip-strip, AND the clip-strip is deemed representative, record “No herbaceous biomass” in the “remarks” column of the Field Datasheet.
8. Using a sharpie, label 8# kraft paper bags with the following information (use larger bags if vegetation is large-stature; if vegetation is wet, cloth bags may be used):
- boutID;** use *[siteID]XX* format, where siteID = 4-letter site code, and XX is the bout number assigned by Field Operations, e.g. CPER01
 - date;** use *YYYYMMDD* format
 - clipID;** as provided in Clip List; use *“plotID_XXX”* format, e.g. CPER_001_126
 - biomassCode;** use 3-letter codes (Table 10)
 - bagNumber;** use *X of Y* notation, where X is the bag # and Y is the total # of bags within a given biomassCode.

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Table 10. Herbaceous clip-harvest biomass codes, descriptions of herbaceous groups, and clipping guidelines.

Biomass Code	Description	Clipping Guidelines
BRY	Bryophytes; lichens are not part of this group, and are ignored	Clip only species that show distinct annual growth, and only clip current year growth
CSG	Cool-season graminoids; includes all grasses, sedges, rushes, etc. with the C3 photosynthetic pathway	Clip 1-2 cm above the ground; DO NOT clip the crowns of perennial graminoids, as this will damage or kill the plant (Figure 3)
WSG	Warm-season graminoids; includes all grasses, sedges, rushes, etc. with the C4 photosynthetic pathway	Clip 1-2 cm above the ground; DO NOT clip the crowns of perennial graminoids, as this will damage or kill the plant (Figure 3)
LFB	Leguminous forbs; includes all herbaceous annual and perennial members of the Fabaceae family	Clip tissue produced in the current year; DO NOT clip any aboveground perennial parts
FRB	Non-leguminous herbaceous annual and perennial forbs	Clip tissue produced in the current year; DO NOT clip any aboveground perennial parts
WST	Woody-stemmed plants with ddh < 1 cm	Clip only leaves and twigs produced in the current year, and only portions of individuals that fall within the clip-strip. Not necessary for rooting point to lie within the clip-strip.
OSD	Old standing dead material produced in a previous growing season	Make sure standing dead material produced in the <i>current</i> growing season is sorted into the correct group above



9. If required to facilitate clipping, remove any cactus biomass lying within the clip-strip. If the individual is rooted within the clip-strip, clip off the cactus plant at the soil level. **Only clip cactus plants that prevent access to herbaceous biomass that must be clipped.**
- Dispose of the cactus biomass outside of the plot.

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10. Clip and sort all herbaceous aboveground biomass rooted within the clip-strip into the biomass groups in Table 10. See Table 11 at end of this SOP for common points of confusion and guidelines for problem plants.

- Do **NOT** clip herbaceous vegetation that passes through/leans over the clip-strip but is not rooted in the strip (WST group is an exception, see below).
- **DO** clip all herbaceous biomass of plants rooted within the strip. That is, include leaves in the harvest that leave the strip but originate from stems rooted within the strip.
- **DO** clip leaves and twigs of woody stemmed plants with ddh < 1cm that fall within the clip strip. It is not necessary that the individuals are rooted in the clip strip.
- Working in pairs, technicians may split the clipping labor one of two ways:
 - Divide the 2 m clip-strip into 1 m sections, label two bags for each biomass code so that each technician has a set of bags, and then combine the biomass for each biomass code when clipping is finished.
 - Divide the clipping labor among the biomass codes. For example, one technician clips cool- and warm-season graminoids while second technician clips leguminous and non-leguminous forbs.

BEST PRACTICE TIPS



- Target one biomass code at a time.
 - Clip slowly, and immediately sort clipped vegetation into labeled bags.
 - Place full bags immediately into a cooler with cold packs.
-

11. When clipping is finished, group all bags from the current clipID into a larger 25# bag and return to the cooler.
12. Record the total number of bags from the clip-strip in the “bagCount” field of the “Field Datasheet”.

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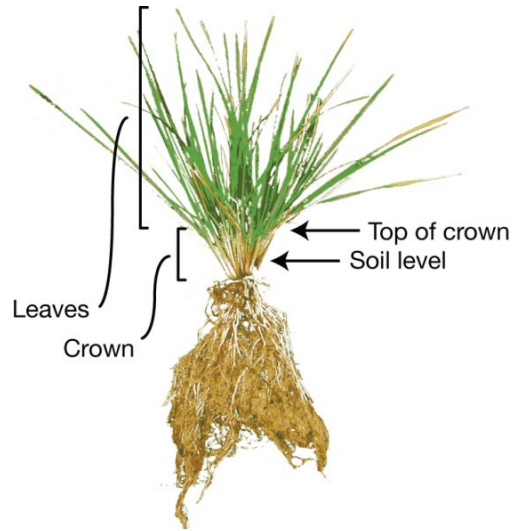


Figure 3. A perennial graminoid, showing the location of crown material relative to leaves and the soil surface

B.2 Points of confusion

Table 11. Additional guidelines for plant growth forms that require special handling or consideration.

Growth Form	Guidelines
Barrel- and saguaro-type cactus species	DO NOT CLIP; clip around these plants.
Cholla- and pad-type cactus species	DO NOT CLIP; these plants are counted and measured with the Vegetation Structure protocol
<i>Toxicodendron spp.</i>	Handle according to procedure in 8Appendix F
Agave, Yucca, and related species	DO NOT CLIP; these plants are counted and measured with the Vegetation Structure protocol
Ferns	DO NOT CLIP; these plants are counted and measured with the Vegetation Structure protocol
Clumped plants (caespitose graminoids, large rosette forbs, etc.)	Clip only the part of the clump that is rooted within the clip-strip.

B.3 Sample Preservation

- Keep paper bags with clipped vegetation in a cooler with cold packs to minimize wilting and to preserve diagnostic features for the post-harvest sort-check.
- Change cold packs for fresh ones every 12 h or transfer to a 4 °C refrigerator if a drying oven is not immediately available.
- Transfer bags to the drying oven as soon as possible. Monitor drying progress with the “Lab Drying QC Datasheet”.



IMPORTANT: Record the number of hours bags were stored cold in the “storageHours” column of the “Field Datasheet”.

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SOP C Field Sampling with Grazing Management

At “grazed” sites, which are defined here to mean those that are actively managed for grazing, two clip-strips per plot or subplot per bout are harvested. One clip-strip per plot or subplot is protected from grazing with a small, portable enclosure, and the second clip-strip per plot or subplot is exposed to the managed level of grazing pressure. Enclosures should be placed at a suitable random grid-cell location prior to grazing onset in the current season. After each clip-harvest sampling event, the enclosure is moved to the next suitable random location.

At grazed sites, sorting clipped biomass to functional group is only required for one harvest per year, and the harvest that is sorted to functional group should be selected to coincide with peak biomass. If there are multiple biomass peaks, choose the time point with the highest peak biomass for functional group sorting. All other harvests are not sorted to functional group.

C.1 Sample Collection in the Field

1. Navigate to the plotID to be sampled (using the GPS if necessary).
2. **You will sample two clip-strips per plot.** The first clip-strip is the one underneath the grazing enclosure that was previously put in place. The second clip-strip location will be identified using the clip-strip coordinate list: [plotID]_clip_coords.csv , that is provided by Science Operations.
 - The clip-strip coordinate list provides the randomized list of potential clip-harvest strips per plot or subplot.
 - The clip-strip coordinate list also indicates which clip-strips have already been harvested or rejected.

For each clip-strip in the plot:

3. Locate the relative X,Y-coordinates of the clip-strip SW corner within the plot or subplot. For the new clipID taken from the list, the procedure used to locate the X-coordinate depends on the value of the relative Y-coordinate:

If the Y-coordinate is < 10:

- a. Run a tape East/West along the south edge of the plot or subplot between the (0,0) → (20,0) plot markers (Figure 1), and stretch the tape taut.*
- b. Place a pin flag at the desired relative X-coordinate.
- c. Standing directly over the pin flag that was just placed at the X-coordinate, use the TruPulse in **HD** mode with a reflective surface to locate the Y-coordinate.
 - Make sure the azimuth is 0° (True North) when shooting the TruPulse to find the Y-coordinate (see Appendix G).
 - Avoid trampling the potential clip-harvest strip as much as possible.
- d. Place a pin flag at the clip-strip (X,Y) location.

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If the Y-coordinate is > 10:

- a. Run a tape* East/West from the plot centroid (10,10) to either the (0,10) position or the (20,10) position (Figure 1):

X-coordinate	Tape Layout
1 < X < 10	From (10,10) to (0,10) ¹
10 < X < 20	From (10,10) to (20,10) ¹

¹Use the TruPulse in **AZ** mode to guide the tape along the correct azimuth

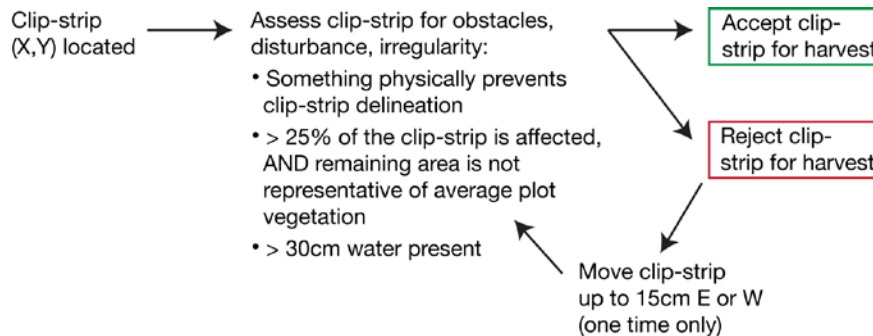
- b. Place a pin flag at the desired relative X-coordinate.
- c. Standing directly over the pin flag that was just placed at the X-coordinate, use the TruPulse in **HD** mode with a reflective surface to locate the Y-coordinate.
 - Make sure the azimuth is 0° (True North) when shooting the TruPulse to find the Y-coordinate (see Appendix G).
 - Avoid trampling the potential clip-harvest strip as much as possible.
- d. Place a pin flag at the clip-strip (X,Y) location.

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- If the plot slope is > 10 %, or there is significant brush or obstacles that prevent accurately stretching a tape, the TruPulse laser rangefinder can be used in **HD** mode to place the initial pin flags relative to the plot markers.
- Plot slope can be quickly estimated using the inclinometer in the TruPulse (**INC** mode).

4. Assess whether the clip-strip location is suitable, and accept or reject the location. Obstacles, disturbances, and/or irregularities may include trees, large rocks, ant nests, etc.). Strips should also be rejected if clipping an uncommon plant in the strip would influence plot-level diversity.



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5. If the clipID is rejected, record why in the “status” column on the clip-strip coordinate list, select the next clipID on the list, and return to step (3) above. Otherwise, update the “status” column and proceed to step (6). Update the “status” column in the clip-strip coordinates list using codes in Table 12:

Table 12. Codes to document acceptance/rejection of clip-harvest strips on the list of clip-strip coordinates

Code	Definition
0	Rejected; disturbance, obstacle, and/or irregularity encountered within the clipID cell
1	Accepted, no enclosure
2	Accepted, enclosure
3	Rejected temporarily, inundated
4	Rejected temporarily, uncommon plant

6. Delineate the accepted clip-strip for harvesting.
- Using one of the pre-marked string and stake sets, line up one of the marks with the pin flag, and push one stake into the ground.
 - Stretch the string and second stake from the South to the North end of the clip-strip, using the compass or the TruPulse to orient the string in a North/South direction.
 - Keep the compass or TruPulse at least 50 cm from non-aluminum metal plot markers, eyeglasses, wristwatches, etc.
 - Use a ruler to place the second string-and-stake set 10 cm to the right of the first set. Check that the distance between the two strings is exactly 10 cm at both ends of the clip-strip.
 - The two sets of marks on the two string-and-stake sets now clearly delineate a 0.1 m x 2 m area for clip-harvesting.
7. Record salient observations about the clip-strip in the “remarks” field of the “Field datasheet”.
- Example:* Record the estimated % area of the clip-strip disturbed by a gopher mound or the extent of minor ant nesting activity within the strip.
 - If there is no herbaceous biomass in a clip-strip, AND the clip-strip is deemed representative, record “No herbaceous biomass” in the “remarks” column of the Field Datasheet.
8. Using a sharpie, label 8# kraft paper bags (use larger bags if vegetation is large-stature) with the following information:
- boutID;** use *[siteID]XX* format, where siteID = 4-letter site code, and XX is the bout number assigned by Field Operations, e.g. CPER01. The bout number portion of the boutID starts over for every growing season.
 - date;** use *YYYY-MM-DD* format
 - clipID;** as provided in clip-strip coordinate list; use *[plotID]_XXX* format, e.g. CPER_001_126
 - enclosure;** use *Y/N* format
 - biomassCode;** use 3-letter codes (Table 13)
 - bagNumber;** use *X of Y* notation, where X is the bag # and Y is the total # of bags within a given biomassCode.

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Table 13. Herbaceous clip-harvest biomass codes, descriptions of herbaceous groups, and clipping guidelines.

Biomass Code	Description	Clipping Guidelines
BRY	Bryophytes; lichens are not part of this group, and are ignored	Clip only species that show distinct annual growth, and only clip current year growth
CSG	Cool-season graminoids; includes all grasses, sedges, rushes, etc. with the C3 photosynthetic pathway	Clip 1-2 cm above the ground; DO NOT clip the crowns of perennial graminoids, as this will damage or kill the plant (Figure 4)
WSG	Warm-season graminoids; includes all grasses, sedges, rushes, etc. with the C4 photosynthetic pathway	Clip 1-2 cm above the ground; DO NOT clip the crowns of perennial graminoids, as this will damage or kill the plant (Figure 4)
LFB	Leguminous forbs; includes all herbaceous annual and perennial members of the Fabaceae family	Clip tissue produced in the current year; DO NOT clip any aboveground perennial parts
FRB	Non-leguminous herbaceous annual and perennial forbs	Clip tissue produced in the current year; DO NOT clip any aboveground perennial parts
WST	Woody-stemmed plants with ddh < 1 cm	Clip only leaves and twigs produced in the current year, and only portions of individuals that fall within the clip-strip
OSD	Old standing dead material produced in a previous growing season	Make sure standing dead material produced in the <i>current</i> growing season is sorted into the correct group above



9. If required to facilitate clipping, remove any cactus biomass lying within the clip-strip. If the individual is rooted within the clip-strip, clip off the cactus plant at the soil level. **Only clip cactus plants that prevent access to herbaceous biomass that must be clipped.**

- Dispose of the cactus biomass outside of the plot.

10. Clip and sort all herbaceous aboveground biomass rooted within the clip-strip into the biomass groups in Table 13. See Table 14 at end of this SOP for common points of confusion and guidelines for problem plants.

- Do **NOT** clip vegetation that passes through the clip-strip but is not rooted in the strip (WST group is an exception).
- **DO** clip all herbaceous biomass of plants rooted within the strip. That is, include leaves in the harvest that leave the strip but originate from stems rooted within the strip.
- Working in pairs, technicians may split the clipping labor one of two ways:
 - Divide the 2 m clip-strip into 1 m sections, label two bags for each biomass code so that each technician has a set of bags, and then combine the biomass for each biomass code when clipping is finished.
 - Divide the clipping labor among the biomass codes. For example, one technician clips cool- and warm-season graminoids while second technician clips leguminous and non-leguminous forbs.

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- Target one biomass code at a time.
 - Clip slowly, and immediately sort clipped vegetation into labeled bags.
 - Place full bags immediately into a cooler with cold packs.
11. When clipping is finished, group all bags from the current clipID into a larger 25# bag and return to the cooler.
 12. Record the total number of bags from the clip-strip in the “bagCount” field of the “Field Datasheet”.
 13. If you have just finished clipping the strip that was protected by the grazing enclosure, move the enclosure to the next suitable clip-strip location on the clip-strip coordinates list.
 14. Stake the enclosure to the ground.

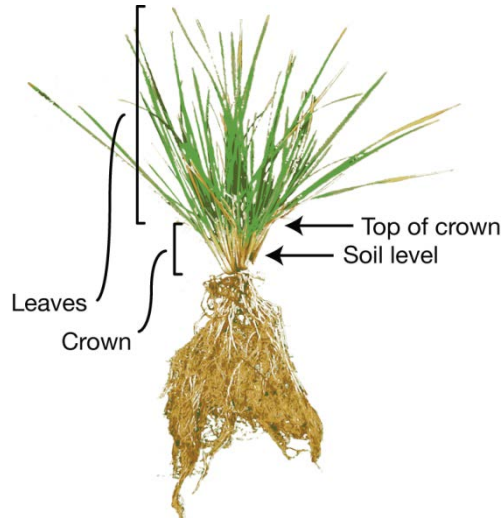


Figure 4. A perennial graminoid, showing the location of crown material relative to leaves and the soil surface.

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C.2 Points of Confusion

Table 14. Additional guidelines for plant growth forms that require special handling or consideration.

Growth Form	Guidelines
Barrel- and saguaro-type cactus species	DO NOT CLIP; clip around these plants.
Cholla- and pad-type cactus species	DO NOT CLIP; these plants are counted and measured with the Vegetation Structure protocol
<i>Toxicodendron spp.</i>	Handle according to procedure in Appendix F
Agave, Yucca, and related species	DO NOT CLIP; these plants are counted and measured with the Vegetation Structure protocol
Ferns	DO NOT CLIP; these plants are counted and measured with the Vegetation Structure protocol
Clumped plants (caespitose graminoids, large rosette forbs, etc.)	Clip only the part of the clump that is rooted within the clip-strip.

Sample Preservation

- Keep paper bags with clipped vegetation in a cooler with cold packs to minimize wilting and to preserve diagnostic features for the post-harvest sort-check.
- Change cold packs for fresh ones every 12 h or transfer to a 4 °C refrigerator if a drying oven is not immediately available.
- Transfer bags to the drying oven as soon as possible. Monitor drying progress with the “Lab Drying QC Datasheet”.



IMPORTANT: Record the number of hours bags were stored cold in the “storageHours” column of the “Field Datasheet”.

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SOP D Post-Field Sampling Tasks

D.1 Check Sorting of Clipped Biomass

The objectives for this task are:

- **Within 24-h of harvest**, check bags of field-sorted biomass for sorting accuracy.
- Remove any litter produced in a previous year from material produced in the current year. The most common error, particularly for clip-harvests performed late in the growing season, is to confuse material that was produced in the current year and has already died with material that was produced in a previous year.
- Remove any material produced in the current year that belongs in another biomass group. For example, leguminous forbs should not be mixed with non-leguminous forbs.
- It is not necessary to check sorting accuracy for bags with biomassCode = OSD. The OSD material is used as a reference during the laboratory sort-check, and is discarded after the sort-check is complete.

To sort-check clipped biomass:

1. Select a 25# kraft bag (or bags) containing all of the biomass from a given clipID.
2. Make sure that all bags from a given clipID are collated in the correct 25# kraft bag(s).
3. Choose a smaller bag containing clipped biomass from one biomassCode only, and carefully check the biomass that was quickly sorted in the field.
 - a. Set aside biomass that does not belong in the bag into separate piles (i.e. one pile for each biomass code).
 - b. Place any previous years' litter into the "OSD" bag.
 - c. Place sorted, checked biomass back into the original bag.
4. Clean the bench of any debris, and proceed to the next biomassCode from the same clipID, sorting again as in step (3).
5. Once all biomassCodes have been checked for sorting accuracy, place piles of resorted biomass into the appropriately labeled biomassCode bags, and place all smaller bags back into the 25# bag(s).
 - Exception: Discard the OSD bag.
6. Place the 25# bag(s) into the drying oven (SOP E), or return to the cooler for continued temporary storage.
7. Clean the workspace, and proceed to checking biomassCode bags from the next clipID.

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- The lead plant technician or botanist should spot-check 10% of the re-sorted biomass piles before they are re-bagged.
 - Spot-checks from a person skilled in plant species identification is particularly important early in the field season when seasonal field technicians may be less familiar with local flora.
-

D.2 Refreshing the Sampling Kit

- Make sure the following consumables are available in sufficient quantity for the next round of clip-harvests:
 - Paper bags, 8# and 25# kraft (or the necessary size given site vegetation stature)
 - Rite-in-the-Rain paper for printing field datasheets
- Return cold packs to the -20 °C freezer to refresh.

D.3 Equipment Maintenance and Cleaning

- Clean blades of hand clippers with either water or ethanol, whichever is most suitable.
- Recharge batteries for the GPS unit (if necessary).
- Recharge batteries for the TruPulse (if applicable).

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SOP E Laboratory Processing of Herbaceous Biomass Samples

E.1 Drying and Weighing Clipped Herbaceous Biomass

Clipped herbaceous biomass should be placed in the drying oven as soon as possible following harvest in the field.

1. Label each 25# bag (with smaller bags for each biomassCode inside) with the date and time it will be placed in the drying oven.



- **Critical step:** Labeling bags allows assessment of how long different batches of bags have been in the oven, especially when harvests from multiple days occupy the same oven.

2. Record the total number of hours each 25# bag was in cold storage prior to being placed in the drying oven in the “storageHours” column of the “Field Datasheet”.
3. Place 25# bags labeled with the date and time into a 60 °C drying oven for 48h – 120h (2d – 5d).
4. After placing in the oven, check the drying progress of clipped biomass using the “Lab Drying QC” datasheet.
 - a. Check the weight of the same subset of n=10 bags per eventDate after day 1, 2, 3, etc.
 - b. Calculate the difference in weight between the latest two time points for each bag.
 - c. Samples are dry when the average weight difference between the latest two timepoints = 0 (averaged across all n=10 bags, ± 0.05 g)
5. Remove bags of dried biomass from the drying oven, and place into a large plastic bag (e.g. a black plastic garbage bag or equivalent), seal the plastic bag, and let dried biomass come to room temperature.



- Placing samples in a bag is important because otherwise they will absorb water from the air as they cool, particularly in humid environments.
- **If mosses were harvested and dried (biomassCode = BRY), place this biomass into a desiccator.** Mosses are extremely hygroscopic and will readily absorb moisture from the air if not stored in a desiccator.

Once dried, samples may be stored for up to 30 days prior to weighing.

6. Weigh biomass from each biomassCode from each clipID using a mass balance (0.01g accuracy) and a large weigh boat.
 - a. Record the mass to the nearest 0.01g on the “Lab Weighing” datasheet.
 - b. For large volumes of biomass that do not readily fit into a large weighboat, use the following strategies:
 - Use a paper bag or a large cardboard box lid (or equivalent) instead of a weigh boat.
 - Crush or chop the biomass to reduce volume so it will fit into a weigh boat.
 - Avoid splitting the biomass into subgroups for weighing, as uncertainty values must be added each time a subgroup is created.

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- Once all weights have been recorded, return biomass to the large plastic bag, seal, and place in temporary storage. Samples in temporary storage can then be prepared as time permits for bioarchive and chemical analysis.

E.2 Data Verification and QA

To quantify uncertainty associated with weighing dried biomass, a portion of dried samples are re-weighed by a different technician than the person who originally weighed the biomass.

- Per unique boutID, select 10% of dried, previously weighed samples for re-weighing.
- Record QA weight data in the “qaHerbaceousMass” column of the “Lab Weighing” datasheet.
- Return plant material to temporary storage.

E.3 Grinding Dried Biomass for Archive and Chemical Analysis

- Coarsely grind each biomassCode per clipID with a Wiley Mill (0.85mm, 20 mesh size). If large amounts of biomass per biomassCode are present, a representative subsample may be ground instead of the entire sample (i.e. grind 1 of 10 bags). However, **the subsample must be representative.**
- Use an appropriately sized splitter/microsplitter to generate two representative sub-samples of approximately 20 mL volume:
 - Sample 1: Bioarchive sample
 - Sample 2: Chemical analysis sample
 - If there is insufficient sample to generate 20 mL per sub-sample, evenly split the sample.

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- If the split sub-sample is too large to fit into the vial in its entirety, continue splitting until a sub-sample of the desired size is generated.
 - DO NOT create sub-samples with a scoopula or spatula. These tools should only be used to transfer an ENTIRE sub-sample into a vial.
-

3. Place the split sub-samples into 20 mL polypropylene scint vials, and label the vials with:
 - boutID
 - clipID
 - biomassCode
4. Discard excess ground biomass from each biomassCode per clipID.
5. Clean grinding mill thoroughly with compressed air between samples.

E.4 Equipment Maintenance

- Balances should be calibrated with a standard calibration weight set:
 - After initial installation.
 - Any time the balance is moved.
 - Every 6 months.
- If you suspect readings are inaccurate for any reason.
 - Clean the grinding mill after grinding each biomass group, and after grinding is complete:
 - Clean with air after grinding each biomass group.
 - Clean with air and ethanol after grinding is complete on a given day.

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SOP F Data Entry and Verification

As a best practice, field data collected on paper datasheets should be digitally transcribed within 7 days of collection or the end of a sampling bout (where applicable). However, given logistical constraints, the maximum timeline for entering data is within 14 days of collection or the end of a sampling bout (where applicable). See RD[04] for complete instructions regarding manual data transcription.

Data recorded manually into field and lab datasheets must be transcribed into a spreadsheet for ingest into NEON's Cyberinfrastructure (CYI). There should be one Excel file containing Herbaceous Biomass ingest sheets (RD[09]). The data ingest document contains at least the following spreadsheets:

- **hbpTableSummary_in**: Table describing each ingest table, and corresponding measurement resolution of ingested data.
- **hbpFieldSummary_in**: Spreadsheet summarizing each data ingest table, and defining table field names and ingest rules.
- **hbp_perBout_in**: Metadata describing individual sampling events on a per clipID per plotID per boutID basis.
- **hbp_massData_in**: Oven-dried biomass data for each biomassCode per clipID per eventID, as well as weighing QA data.

F.1 Field Datasheets

Data from Field Datasheets may be transcribed within 14 days of collection.

1. Transcribe data from the Herbaceous Biomass Field Datasheets to the "hbp_perBout_in" ingest table.
 - Consult the "hbpFieldSummary" table in the Herbaceous Biomass ingest Excel file to determine appropriate values and formats for each field in the ingest table.
2. If a representative clipID contained no herbaceous biomass (noted in the "remarks" column of the Field Datasheet), enter the following values in the "hbp_massData_in" ingest table:
 - biomassCode = ALL
 - herbaceousMass = 0.00
3. Once all data from the most recent boutID have been collected and transcribed, save only the data from the most recent boutID to a .csv file for ingest by NEON CYI.
4. Update permanent digital versions of the "clip-strip coordinate" lists with "date" and "status" data recorded in the field.

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F.2 Lab Datasheets

Data from the “Lab Weighing” datasheet should be transcribed into the data ingest within 14 days after biomass values and biomass QA values are recorded for all plots from a given boutID. Lab Drying QC data are not transcribed for ingest into the NEON CYI.

1. Transcribe data from the “Lab Weighing” datasheet to the “hbp_massData_in” ingest table.
 - Consult the “hbpFieldSummary” table in the Herbaceous Biomass ingest Excel file to determine the appropriate values and formats for each field.

Once all data from the most recent boutID have been collected and transcribed, save only the data from the most recent boutID to a .csv file for ingest by NEON CYI.

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SOP G Sample Shipment

Information included in this SOP conveys science-based packaging, shipping, and handling requirements, not lab-specific or logistical demands. For that information, reference the [CLA shipping document](#) on [CLA’s NEON intranet site](#).

G.1 Handling Hazardous Material

None.

G.2 Supplies/Containers

Samples should be contained in 20 mL plastic scint vials, and should be accompanied by a copy of the relevant “hbp_processing_in” datasheet.

G.3 Timelines

Dried, ground samples may be stored indefinitely before shipping.

G.4 Conditions

Dried, ground samples sealed in plastic vials may be shipped at ambient temperature without preservatives.

G.5 Grouping/Splitting Samples

Samples originating from the same clipID should be grouped together. If there is more than one clipID per plotID, samples originating from the same plotID should be grouped together if possible.

G.6 Return of Materials or Containers

Not applicable.

G.7 Shipping Inventory

Not applicable

G.8 Laboratory Contact Information and Shipping/Receipt Days

See the [CLA shipping document](#) on [CLA’s NEON intranet site](#).

G.9 Additional Directions/Notes

Not applicable.

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8 REFERENCES

The Nutrient Network Experimental Protocol page (http://www.nutnet.umn.edu/exp_protocol).
Accessed 2013-09-19.

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APPENDIX A DATASHEETS

The following datasheets are associated with this protocol:

Table 15. Datasheets associated with this protocol

NEON Doc. #	Title
NEON.DOC.001574	Datasheets for TOS Protocol and Procedure: Measurement of Herbaceous Biomass

These datasheets can be found in Agile or the NEON Document Warehouse.

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APPENDIX B QUICK REFERENCES

B.1 Delineating the Clip Harvest Strip

LOCATE AND ASSESS POTENTIAL CLIP AREA

STEP 1 – Locate southwest corner of sample plot - plot coordinate (0,0)

STEP 2 – Select first available clip-strip location from Work Order list.

STEP 3 – Locate X-coordinate, anchor and stretch east-west tape, place pin flag.

Y-Coordinate	East-West Tape Location
1, 4, or 7	(0,0) →(20,0)
10, 13, or 16	(0,10) →(20,10)

STEP 4 – Locate Y-coordinate with TruPulse in HD mode (azimuth 0°), place pin flag.

STEP 5 – Assess suitability of clip-strip. Relocate 15 cm west or east OR reject if not suitable.

STEP 6 – Record (X,Y) coordinates of southwest corner of actual clip-strip area if initial location not acceptable.

STEP 7 – Remove and relocate enclosure to next suitable random location, if applicable.

DELINEATE 0.1 M X 2 M CLIP-STRIP

STEP 1 – Place north-south oriented string-and-stake set on west side of clip-strip. Use TruPulse to orient string.

STEP 2 – Place second string-and-stake set EXACTLY 10 cm to the east of first set.

STEP 3 – Check distance between strings at both ends with ruler.

CHARACTERIZATION

STEP 1 – Record in “remarks” (Field Data Sheet) any site features that might impact plant biomass.

STEP 2 – If cactus are present, remove and discard only those cactus pads that would physically prevent clipping herbaceous biomass.

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B.2 Clipping and Sorting

STEP 1 – Label 8# kraft paper bags (lunchbag size), use multiple bags as needed.

STEP 2 – Clip biomass ROOTED in clip-strip area, sorting vegetation into coded bags as you go.

STEP 3 – Record on Field Datasheet the total number of bags harvested per clip strip.

STEP 4 – Place all 8# bags from single clip-strip into one 25# bag.

STEP 5 – Store bag in cooler with ice packs (or sealed ice) for transport back to lab.

STEP 6 – Transfer clip bags to 4° C refrigerator in domain lab (if possible).

STEP 7 – Check sorting at end of day or next morning in lab.

STEP 8 – Confer with lead plant technician to check that all biomass is correctly sorted.

STEP 9 – Place clipped biomass in a drying oven as soon as possible after clipping and sorting.

STEP 10 – Record the number of hours biomass was stored before drying in the “storageHours” column on the Field Datasheet.

QUALITY DEPENDS ON PROPER:

- Sorting into groups.
- Separation of previous and current years’ growth.
- Labeling of all samples.

CLIPPING GUIDELINES

- Only clip biomass ROOTED in the clip-strip area.
- Sort clipped vegetation into appropriate bags as you go.
- Clip as close to the ground as possible (i.e., 1-2 cm above ground).
- DO NOT CLIP crowns of perennial grasses, as this will kill or damage the plant.
- Do not clip barrel cactus
- Clip *Toxicodendron spp.* according to Appendix F.

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APPENDIX C REMINDERS

Collecting quality biomass data with the clip harvest technique

At the plot: Be sure to...

- Avoid walking on targeted clip area and plant diversity subplots.
- Assess suitability of potential clip-strip and accurately delineate.
- Relocate enclosure to next suitable location, if applicable.
- Record (X,Y) coordinates of clip area on data sheet.

Label Information

- boutID
- date
- clipID
- enclosure (Y or N)
- biomassCode (3 letter code)
- bagNumber (e.g. 2 of 3)

Functional Group Name	Biomass Code
Bryophytes (not lichens)	BRY
Cool-season graminoids (C3)	CSG
Warm-season graminoids (C4)	WSG
Leguminous forbs	LFB
Non-leguminous forbs	FRB
Woody-stemmed plants, ddh < 1.0 cm	WST
Previous years' old standing dead	OSD

- Cross out sampled or rejected clip-harvest locations from Work Order list

Clip harvesting: Be sure to...

- Count pad-forming cactus; clip and discard only if necessary.
- Fill in Field Datasheet and check that all bags are accounted for.
- Store bags in cooler.
- Check sorting in lab at end of field day or next morning.
- Confer with lead plant technician to check biomass sorting.

Using the TruPulse: Pay close attention to...

- Declination – Is it set for your current location?
- Selection choices in drop-down menu.
- Battery charge (replace when low-charge indicated).
- Transcription of measurements onto data sheet.
- Metal objects – Keep them at least 2 feet away from instrument when using internal compass.

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APPENDIX D ESTIMATED DATES FOR ONSET AND CESSATION OF SAMPLING

The dates in the table below are based on historic records and are estimates for the start and stop dates of sampling. It is essential that domain staff monitor real-time conditions to determine when to start (and stop) sampling, as described in Section 4 of this protocol.

At sites managed for grazing, Distributed plots should be clip-harvested at approximately the same peak biomass date that functional groups are sorted in the Tower plots (see “Additional Sampling Information” field in Table 16 below). All dates are estimated from MODIS-EVI phenology data averaged from 2001-2009. See Table 17 for “day-of-year → Gregorian date” conversion data.

Table 16. Site-specific grazing status and per bout sampling start dates for herbaceous clip-harvest. All dates are provided in day-of-year format. Site ID values that are bold/italic are sites in Operations in 2014.

Domain Number	Site ID	Grazing Mgmt	Bouts per Growing Season	Start Date (Day-of-Year)	Additional Sampling Information
01	BART	No	1	220	
	BURL	No	1	220	
	HARV	No	1	220	
02	BLAN	No	1	210	
	SCBI	No	1	220	
	SERC	No	1	220	
03	DSNY	No	1	190	Tower plot parcel may be managed for grazing. Candidate site for continuous growth/decomp SOP.
	JERC	No	1	220	
	OSBS	No	1	190	
04	GUAN				Candidate site for continuous growth/decomp SOP.
	LAJA				Candidate site for continuous growth/decomp SOP.
	MAME				Candidate site for continuous growth/decomp SOP.
05	STEI	No	1	215	
	TREE	No	1	215	
	UNDE	No	1	215	
06	KONA				
	KONZ	Yes	Every 4 wks	90	End sampling by day 300, or when livestock removed. Sort clip to functional group on first bout occurring after day 210.
	KUFS				
07	GRSM	No	1	215	
	MLBS	No	1	220	

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Domain Number	Site ID	Grazing Mgmt	Bouts per Growing Season	Start Date (Day-of-Year)	Additional Sampling Information
	ORNL	No	1	210	
08	CHOC	No	1	200	
	DELA	No	1	205	
	TALL	No	1	195	
	DCFS	Yes	Every 4 wks	120	End sampling by day 290, or when livestock removed. Sort clip to functional group on first bout occurring after day 205.
09	NOGP	Yes	Every 4 wks	115	End sampling by day 290, or when livestock removed. Sort clip to functional group on first bout occurring after day 200.
	WOOD	No	1	210	Intermittent, low-level grazing. Domain Mgr must communicate with site host to determine whether Tower plots are grazed in a given year.
	CPER	Yes	Every 4 wks	90	End sampling by day 350, or when livestock removed. Sort clip to functional group on first bout occurring after day 210.
10	RMNP	No	1	180	
	STER	No	1 bout per crop, per growing season	2-4 wks before crop harvest	Domain Mgr communicates with site host to determine crop harvest dates.
	CLBJ				
11	KLEM	Yes	Every 4 wks	75	End sampling by day 310, or when livestock removed. Sort clip to functional group on first bout occurring after day 165.
	<i>TBD</i>				
	BOZE				
12	PARA				
	YELL	No	1	190	
	MOAB	Yes	Every 4 wks	85	End sampling by day 300, or when livestock removed. Sort clip to functional group on first bout occurring after day 225.
13	NIWO	No	1	220	
	<i>TBD</i>				

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Domain Number	Site ID	Grazing Mgmt	Bouts per Growing Season	Start Date (Day-of-Year)	Additional Sampling Information
14	JORN	No	1	245	Grazing not anticipated in Tower plots, but may occur. Domain Mgr communicates with site host to determine if Tower plots grazed.
	SRER	No	1	240	Grazing not anticipated in Tower plots, but may occur. Domain Mgr communicates with site host to determine if Tower plots grazed.
	TBD				
15	ONAQ	Yes	Every 4 wks	75	End sampling by day 280, or when livestock removed. Sort clip to functional group on first bout occurring after day 170.
	RBUT				
	TBD				
16	ABBY				
	THAY				
	WREF	No	1	210	
17	SJER	Yes	Every 4 wks	270	End sampling by day 155 of next calendar year (Mediterranean growing season). Sort clip to functional group on first bout occurring after day 95.
	SOAP	No	1	185	
	TEAK	No	1	205	
18	BARO	No	1	210	
	TOOL	No	1	205	
	TBD				
19	DEJU	No	1	210	
	HEAL	No	1	210	
	POKE	No	1	205	
20	OLAA				Candidate site for continuous growth/decomp SOP.
	PUFO				Candidate site for continuous growth/decomp SOP.
	PUGR				Candidate site for continuous growth/decomp SOP.

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Table 17. Day-of-year calendar for non-leap years

Day	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Day
1	001	032	060	091	121	152	182	213	244	274	305	335	1
2	002	033	061	092	122	153	183	214	245	275	306	336	2
3	003	034	062	093	123	154	184	215	246	276	307	337	3
4	004	035	063	094	124	155	185	216	247	277	308	338	4
5	005	036	064	095	125	156	186	217	248	278	309	339	5
6	006	037	065	096	126	157	187	218	249	279	310	340	6
7	007	038	066	097	127	158	188	219	250	280	311	341	7
8	008	039	067	098	128	159	189	220	251	281	312	342	8
9	009	040	068	099	129	160	190	221	252	282	313	343	9
10	010	041	069	100	130	161	191	222	253	283	314	344	10
11	011	042	070	101	131	162	192	223	254	284	315	345	11
12	012	043	071	102	132	163	193	224	255	285	316	346	12
13	013	044	072	103	133	164	194	225	256	286	317	347	13
14	014	045	073	104	134	165	195	226	257	287	318	348	14
15	015	046	074	105	135	166	196	227	258	288	319	349	15
16	016	047	075	106	136	167	197	228	259	289	320	350	16
17	017	048	076	107	137	168	198	229	260	290	321	351	17
18	018	049	077	108	138	169	199	230	261	291	322	352	18
19	019	050	078	109	139	170	200	231	262	292	323	353	19
20	020	051	079	110	140	171	201	232	263	293	324	354	20
21	021	052	080	111	141	172	202	233	264	294	325	355	21
22	022	053	081	112	142	173	203	234	265	295	326	356	22
23	023	054	082	113	143	174	204	235	266	296	327	357	23
24	024	055	083	114	144	175	205	236	267	297	328	358	24
25	025	056	084	115	145	176	206	237	268	298	329	359	25
26	026	057	085	116	146	177	207	238	269	299	330	360	26
27	027	058	086	117	147	178	208	239	270	300	331	361	27
28	028	059	087	118	148	179	209	240	271	301	332	362	28
29	029		088	119	149	180	210	241	272	302	333	363	29
30	030		089	120	150	181	211	242	273	303	334	364	30
31	031		090		151		212	243		304		365	31

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APPENDIX E SITE-SPECIFIC INFORMATION

N/A

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APPENDIX F CLIP-HARVESTING *TOXICODENDRON* SPECIES

The following are best-practice techniques for minimizing exposure to toxic oil during clip-harvest of *Toxicodendron* species.

1. Prior to field work:
 - a. Count out bags for storing and drying ONLY *Toxicodendron* biomass. Don't mix *Toxicodendron* biomass with any other biomass.
 - b. Pre-weigh (to nearest 0.01 g) and label each paper bag that will be used for storing and drying clip-harvested *Toxicodendron* biomass. Once the weight of each empty bag is included on the bag label, the biomass inside the bag will never have to be touched after it is initially placed in the bag.
2. To handle and clip *Toxicodendron* biomass in the field:
 - Wear cotton gloves and dispose after single use. Toxic oils can pass through nitrile or latex gloves.
 - Use a pair of clippers dedicated solely to clipping *Toxicodendron spp*, and clean with Tecnu (or equivalent) after each use. Store separately from other clippers to prevent accidental contact.
 - Bring a clean, new plastic bag to the field for storing and transporting contaminated gloves and clippers after use.
 - Wear a thin outer layer of disposable PPE over clothes and shoes.
 - After field work is complete, wash clothing according to these guidelines or similar: <http://laundry.about.com/od/removeoutdoorstains/a/poisonivylaundry.htm>
3. To process *Toxicodendron* biomass in the laboratory:
 - a. Minimize potential spread of toxic oil by putting *Toxicodendron* biomass bags into the same drying oven every time.
 - b. When drying is complete, clean drying oven shelves used for drying *Toxicodendron* biomass bags with hot water and Tecnu. Wear appropriate PPE when cleaning.
 - c. Record weight of bag + dried biomass to nearest 0.01 g, and also record weight of individual empty bag (to 0.01 g) on data sheets. Dried *Toxicodendron* biomass should never leave the bag.
4. After weighing, dispose of all *Toxicodendron* biomass bags. At this point in time, *Toxicodendron* tissue will not be specimen mounted or archived.

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APPENDIX G USING AND CALIBRATING THE TRUPULSE 360R LASER RANGEFINDER

G.1 Setting the Declination Offset

1. Press the “Power/Fire” button to turn on the unit. The viewfinder will display the main “Measurement Mode” screen.
2. Press and hold ▼ for 4 s to enter “System Setup Mode”.
3. Press ▼ until **H_Ang** is displayed in the viewfinder, then press “Power/Fire”.
4. **dECLn** will be displayed in the viewfinder, press “Power/Fire”.
5. **no** and **dECLn** will blink. Press ▼ until **YES** and **dECLn** blink, then press “Power/Fire” again. The current declination is shown in the viewfinder.
6. If this is the correct value, press and hold ▲ to return to the main “Measurement Mode” screen.
7. If the displayed value is incorrect for your current location:
 - a. Press either ▲ or ▼ to change the tenths value, press “Power/fire”.
 - b. Press either ▲ or ▼ to change first integer value, press “Power/fire”.
 - c. Press either ▲ or ▼ to change second integer value, press “Power/fire”.
 - d. The value just entered will blink. Press “Power/fire” to confirm and return to the “Measurement Mode” screen.

G.2 Tilt Sensor Calibration

1. Press the “Power/Fire” button to turn on the unit. The viewfinder will display the main “Measurement Mode” screen.
2. Press and Press and hold ▼ for 4 s to enter “System Setup Mode”.
3. Press ▼ until **inC** is displayed in the viewfinder, then press “Power/Fire”..
4. **no** and **CAL** will blink. Press ▼ until **yes** and **CAL** blink, then press “Power/Fire” again.
 - a. Calibration can be aborted by pressing “Power/Fire” when **no** and **CAL** are alternately displayed.
5. **C1_Fd** will be displayed in the view finder.
6. Place the TruPulse on a flat, relatively flat surface (within 15deg of level). Follow the sequence outlined in Figure 5.
 - a. At each step wait approximately 1 second before pressing “Power/fire”, then wait another second before moving to the next position. It is important that the unit is held steady when you press “Power/fire”.
 - b. To abort and return to previous calibration at any point hold ▲ or ▼ for 4 sec.

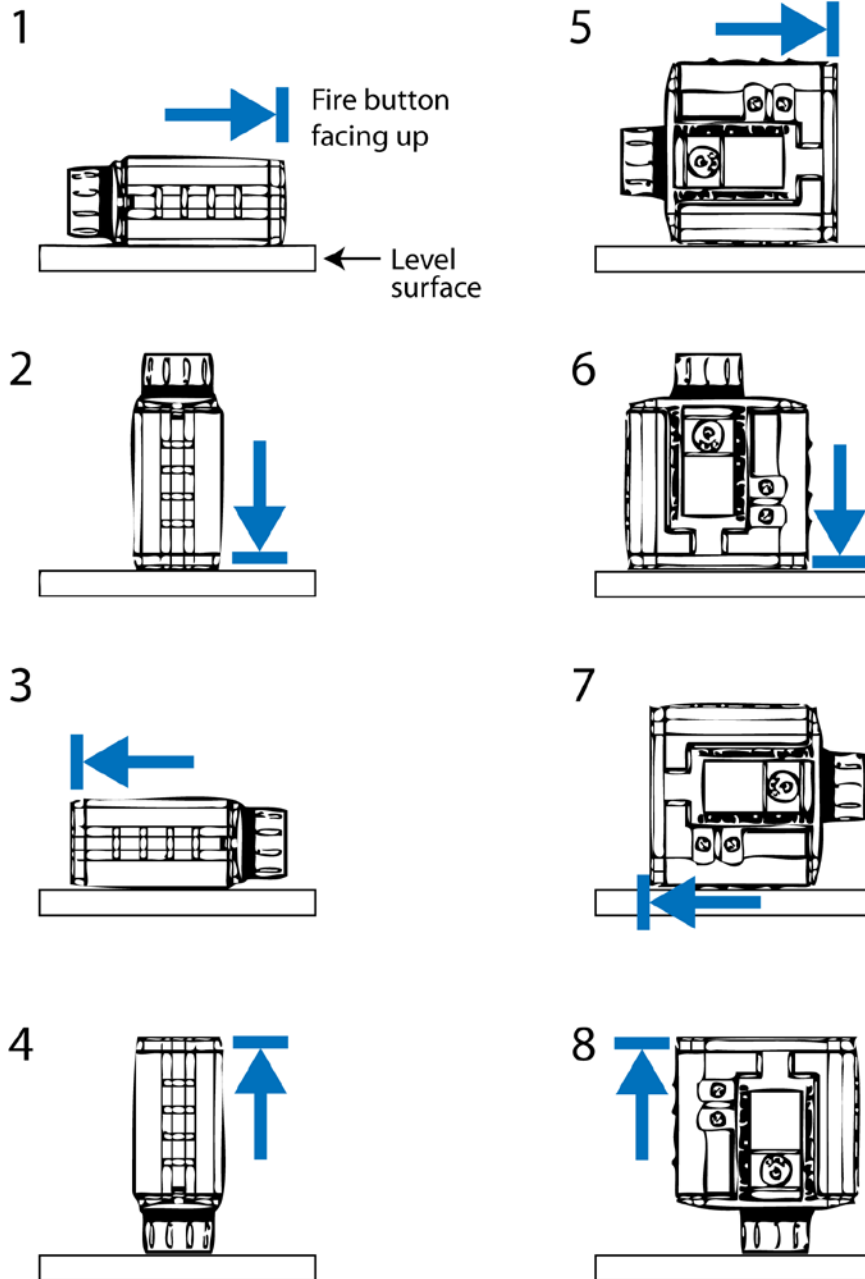


Figure 5. The tilt-sensor calibration routine for the TruPulse 360R laser rangefinder. The blue arrow and line indicate the direction of the lens at each calibration step.

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7. After all 8 positions have been run through, look through the eyepiece. Either a **PASS** or **FAiL** message appears in the view finder.
 - **PASS:** Press the “Power/Fire” Button to return to the measurement mode.
 - **FAiL1:** Excessive motion during calibration. Unit was not held steady.
 - **FAiL2:** Magnetic saturation error. Local magnetic field too strong.
 - **FAiL3:** Mathematical fit error.
 - **FAiL4:** Calibration convergence error.
 - **FAiL6:** Orientations were wrong during the calibrations.
8. If **FAiL** appears, press the “Power/Fire” button. **No** and **CAL** will alternately blink allowing you to do a new calibration. IF the calibration fails, the unit reverts to the previous calibration.

G.3 Using and Calibrating the TruPulse 360R Compass

Like any compass, the internal compass of the TruPulse is susceptible to error and to interference from common metallic objects. The following objects may affect the compass performance, and should be kept at least 50 cm (20 in) away from the TruPulse during operation:

Batteries	Nails
Data collectors or computers	Pin flags w/ metal stakes
Portable electronics	Steel-rimmed eyeglasses
Metal watch bands	Eyeglass spring-hinges
Non-aluminum tripods	

When using the TruPulse compass, it is good practice to check the compass performance against a standard mirror-site compass or a previously established plot-line at the beginning of each day, or when beginning a new plot. In addition, **ALWAYS CHECK AND RECALIBRATE THE COMPASS AFTER CHANGING THE BATTERIES**. It is common for the compass calibration to be inaccurate when the low battery indicator is displayed in the viewfinder, and you should always replace the batteries when this indicator appears.

If the compass requires calibrating, you must first determine that you are in an area free from local magnetic interference. Either of the following simple tests can be used in the field to test for local magnetic interference:

1. Choose a target at least 100 m away, and shoot to it. Note the azimuth. Then step backward or forward 1 m along the sight-line to the target and shoot again. Note the second azimuth.
 - The second azimuth should be within 1/10 to 5/10 of a degree of the first azimuth. If it is, you are likely in an anomaly-free area.
 - For increased confidence, repeat the test with a second target at 90° to the azimuth of the first target.

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2. Select a target at least 10 m away, shoot to it, and note the azimuth. Move to the target that was just shot, and shoot back toward the spot that was just occupied. Note the second azimuth.
 - The two azimuths should be 180° different, plus or minus no more than a few tenths of a degree.

Once you have ascertained that the current location is free from local magnetic interference, complete the following steps to calibrate the TruPulse 360R compass:

1. Press the “Power/Fire” button to turn on the unit. The viewfinder will display the main “Measurement Mode” screen.
2. Press and hold ▼ for 4 s to enter “System Setup Mode”.
3. Press ▼ until **H_Ang** is displayed in the viewfinder, then press “Power/Fire”..
4. **dECLn** is displayed. Press ▼ to display the **HACAL** option, then press “Power/Fire” again.
5. **No** and **HACAL** will alternately blink. Press ▲ or ▼ to display **YES** and **CAL**, then press “Power/Fire” to begin calibration.
 - a. Calibration can be aborted by pressing “Power/Fire” when **no** and **CAL** are alternately displayed.
6. **C1_Fd** will be displayed in the view finder.
7. Use a standard mirror-site compass to determine the direction of **magnetic** North. Holding the TruPulse 360R and facing close to **magnetic** North ($\pm 15^\circ$), the lenses should be facing as shown in Figure 6. To complete the calibration routine, follow the sequence outlined in Figure 6.
 - At each step wait approximately 1 second before pressing “Power/fire”, then wait another second before moving to the next position. It is important that the unit is held steady when you press “Power/fire”.
 - To abort and return to previous calibration at any point hold ▲ or ▼ for 4 sec.
8. After all 8 positions have been run through in sequence, look through the eyepiece. Either a **PASS** or **FAiL** message appears in the view finder.
 - **PASS**: Press the “Power/Fire” Button to return to the measurement mode.
 - **FAiL1**: Excessive motion during calibration. Unit was not held steady.
 - **FAiL2**: Magnetic saturation error. Local magnetic field too strong.
 - **FAiL3**: Mathematical fit error.
 - **FAiL4**: Calibration convergence error.
 - **FAiL6**: Orientations were wrong during the calibrations.

If **FAiL** appears, press the “Power/Fire” button. **No** and **CAL** will alternately blink allowing you to do a new calibration. If the calibration fails, the unit reverts to the previous calibration.

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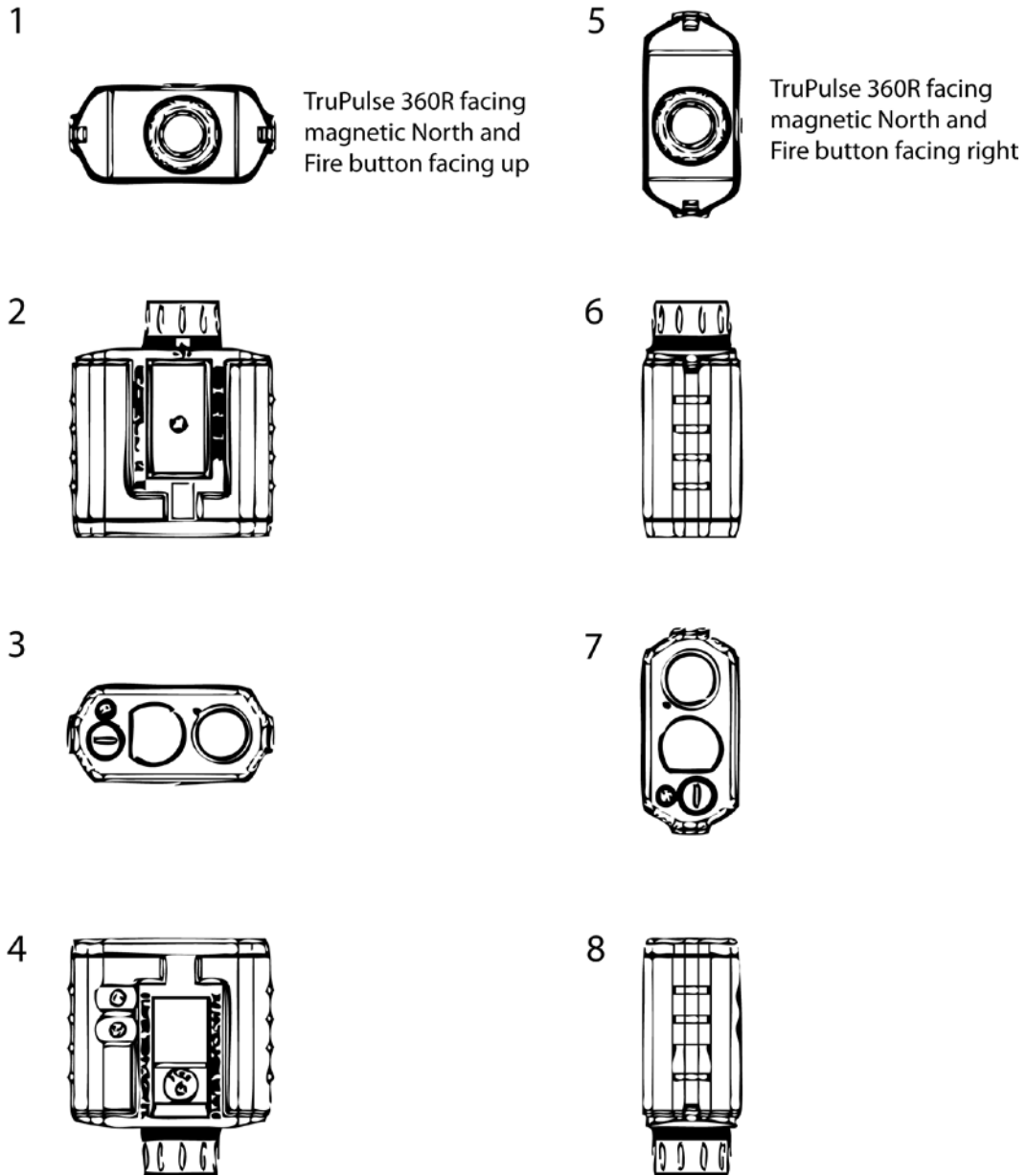


Figure 6. The internal compass calibration routine for the TruPulse 360R laser rangefinder.

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G.4 Measuring Distance from a Known Point

1. Press “Power/Fire” to turn on the TruPulse.
2. Set the unit to Target Mode = Filter
 - a. Press ▲ for 4 seconds. The active Target Mode appears in the viewfinder. Press ▲ or ▼ to cycle through available Target Modes. Available Target Modes are listed in Table 18 below.
 - b. Choose “Flt” and press “Power/Fire” to make the chosen Target Mode active.

Table 18. Laser Target Modes available for the TruPulse 360 laser rangefinder/clinometer models.

Target Mode	Definition	When to Use
Std	Standard, single-shot	Clear shot to unobstructed target
Con	Continuous; pressing and holding “Power /Fire” will continuously acquire targets for up to 10 s	Useful for scanning trees in order to find the highest point
CLO	Closest; pressing and holding “Power /Fire” will acquire multiple targets, the viewfinder displays the closest target	Narrow targets in the foreground
FAR	Farthest; identical to CLO, but the viewfinder displays the farthest target	<ul style="list-style-type: none"> • Target partially obscured by brush • Finding highest point of a tree
Flt	Filter; the sensor sensitivity is reduced to only detect laser pulses returned from a reflective surface; ‘F’ appears in the viewfinder	<ul style="list-style-type: none"> • Measuring targets through thick brush • In very heavy brush, the optional foliage filter can be used in this mode (but is not required)

3. Press either the ▲ or ▼ button until **HD** (i.e. Horizontal Distance) appears in the viewfinder.
4. Person 1: Hold the reflective surface at the base of the stem so that it is visible to Person 2.
5. Person 2: Look through the TruPulse viewfinder, aim the crosshairs at the reflective surface held by Person 1, and press and hold “Power/Fire” until the distance is displayed in the viewfinder; record this distance.

G.5 Measuring Azimuth from a Known Point

1. After recording the **HD** to the stem above, press ▲ three times until **AZ** (i.e. azimuth from True North) appears in the viewfinder and the angle in degrees is displayed; record this angle.

The angle should be preceded by a “**d**” indicating that declination has been set for the TruPulse at your current location (as described previously).