TOS PROTOCOL AND PROCEDURE: BRYOPHYTE PRODUCTIVITY

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Change Record

REVISION	DATE	ECO #	DESCRIPTION OF CHANGE
A	04/12/2016	ECO-03183	Initial release
B	01/23/2017	ECO-04321	) Updated logo and funding statement to match new template) Clarified qualifying vegetation) Removed targeted net placement strategy) Removed per plot sampling threshold, if the site qualifies for sampling, all Tower plots within the site are considered) Generalized data recording instructions to be applicable to either datasheets or tablet data entry) Appendix D: Changed DOY format to MM/DD

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1 OVERVIEW

1.1 Background

Bryophytes, when in abundance, have been shown to significantly affect both the balance between above and below ground carbon pools as well as the overall estimate of ecosystem productivity and carbon use efficiency (Binkley and Graham 1981, Shaver and Chapin 1991, Street et al. 2012, Bona et al. 2013). How these important community members will respond to various global change drivers, however, is still widely debated. For example, it is unknown whether there will be greater accumulation or decomposition of carbon in bryophyte-rich peatland ecosystems under conditions predicted by global change models (Loisel et al. 2012). Quantifying bryophyte productivity in sites where they are abundant is therefore an essential element of NEON's broader above-ground vegetation sampling strategy.

The group of non-vascular species that includes mosses, liverworts and hornworts are often referred to, collectively, as bryophytes. For the purpose of this protocol, bryophyte is more narrowly defined and refers to all mosses, including *Sphagnum* species, and liverworts. Such bryophytes can be the most abundant vegetation at arctic sites (Walker et al. 2002, Pouliot et al. 2010), and may also comprise significant portions of boreal, alpine, or temperate site biomass (Glime 2007).

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.

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 Ms. Aissa L. Feldmann of the New York Natural Heritage Program 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	EHSS Policy, Program and Management Plan
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.050005	Field Operations Job Instruction Training Plan
AD[05]	NEON.DOC.000914	NEON Science Design for Plant Biomass and Productivity
AD[06]	NEON.DOC.004104	NEON Science Performance QA/QC 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.002652	NEON Level 1, Level 2, and Level 3 Data Products Catalog
RD[04]	NEON.DOC.001271	NEON Protocol and Procedure: Manual Data Transcription
RD[05]	NEON.DOC.002136	Datasheets for TOS Protocol and Procedure: Bryophyte Productivity
RD[06]	NEON.DOC.001927	NEON Raw Data Ingest Workbook for TOS Bryophyte Productivity
RD[07]	NEON.DOC.001717	TOS Standard Operating Procedure: TruPulse Rangefinder Use and Calibration
RD[08]	NEON.DOC.014037	TOS Protocol and Procedure: Measurement of Herbaceous Biomass
RD[09]	NEON.DOC.001710	TOS Protocol and Procedure: Litterfall and Fine Woody Debris
RD[10]	NEON.DOC.014042	TOS Protocol and Procedure: Plant Diversity Sampling

2.3 Acronyms

Acronym	Definition
ANPP	Annual net primary productivity

2.4 Definitions

N/A

3 METHOD

Bryophyte annual net primary productivity (ANPP) will be measured at all Tower plots for sites with 10-100% bryophyte cover, based on plant diversity surveys conducted in the site characterization phase of Construction at each site. Distributed plots will not be sampled.

Bryophyte ANPP will be determined using a standard harvest technique: clipping annual growth above nets. The methods described here are best suited for acrocarpous mosses – species with a largely unbranched, vertical growth form. Pleurocarpous mosses, which produce lateral branches, will be harvested according to this protocol but it is acknowledged productivity of this type of bryophyte will be underestimated using this method; new growth below the growing tips of the moss shoots will not be captured (Rieley et al. 1979), and the contributions of new biomass production and stem elongation to "growth" above the net cannot be determined.

The location of clip strips within the plot moves on an annual basis to avoid clipping of the same area more than once over the life of the observatory. This is accomplished by: a) the establishment of new clip strips (within a given plot) following bryophyte sampling in a given year, with these new clip strips to be sampled in the following year; b) the removal of clip strips that were sampled in that year; c) careful recording of the location of previously sampled clip strips, so that no location is ever sampled more than once.

This sampling protocol is initiated in Tower plots at sites where mean bryophyte cover is 10% or more. Per plot mean bryophyte cover is assessed by averaging moss cover from the eight 1-m^2 sub-plots set aside for plant diversity sampling (RD[10]) in 20-30 plots per site. At sites with 40 m x 40 m Tower plots, a maximum of 20 plots (2 clip strips per plot) will be sampled. At sites with 20 m x 20 m Tower plots a maximum of 30 plots (1 strip per plot) will be sampled. NEON Science Operations will recommend sites with the minimum cover of mosses.

3.1 Plots and Subplots

For all sites with 20-100% bryophyte cover, annual harvest of bryophyte growth occurs within 400 m^2 plots ("stand-alone" 20 m x 20 m plots) or subplots (20 m x 20 m subsections of a larger 40 m x 40 m plot; **Figure 1**) following the "clip harvest from net height" method (Clymo 1970, Russell 1988). Within 40 x 40 m plots; two randomly selected subplots will be sampled for bryophyte productivity.

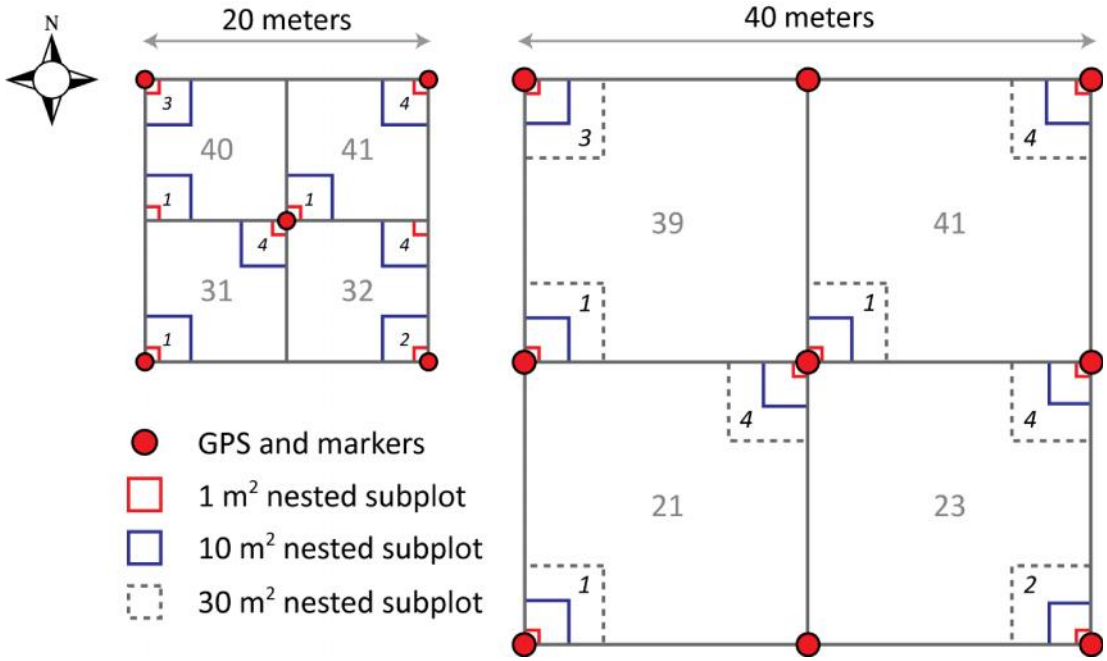

Figure 1. Illustration of NEON plot and subplot sizes. Bryophyte sampling takes place in 20 m x 20 m plots or subplots. Each plot (20 m x 20 m, or 40 m x 40 m here) is divided into four subplots and within each subplot are located nested subplots, the identities of which are indicated in the legend. Grey numbers indicate subplotIDs, and italic numbers indicate nested subplotIDs. Destructive clip-harvesting does not occur in $1m^2$ and $10m^2$ nested subplots used to estimate % cover by species. The $30m^2$ nested subplots are not used for % cover measurements and may contain clip-harvest nets.

3.2 Clip Strips and Sampling Areas within Plots

Bryophyte sampling will utilize the clip cell grid system outlined in the herbaceous clip harvest protocol (RD[08]). Each 20 m x 20 m plot or subplot is divided into a series of 0.5 m x 3 m numbered "clip cells" that cover the available sampling area within the plot or subplot; cells overlapping nested subplots used for Plant Diversity sampling are automatically omitted from consideration for clip harvest. Clip cells are shown as dashed blue lines in Figure 2.

Each clip cell contains a single "clip strip," as shown in Figure 2. The clip strips are north/south-oriented strips with dimensions of 0.1 m x 2 m. Relative x,y coordinates are assigned to the southwest corner of each clip strip (marked with a red "X" in Figure 2). The coordinates are in meters and are relative to the southwest corner of the plot or subplot as a whole; the southwest corner of each plot or subplot is defined as (0,0), and the northeast corner of the plot or subplot is (20,20). A clip strip with coordinates of (3, 17), then, is located 3 meters east and 17 meters north of the southwest corner of the plot.

During clip strip establishment, up to five 10 cm x 10 cm bryophyte clip-harvest nets are placed within the clip strip, to be sampled the following year; these 10 cm x 10 cm squares are the sampling areas for this protocol. Only one clip strip (and its associated 10 cm x 10 cm sampling areas) per 20 m x 20 m plot is sampled each year. Clip cells used for collecting herbaceous biomass, litterfall, or soil sampling, at any

time prior to the current bout are excluded from consideration from bryophyte clip-harvest sampling. Clip cells selected for bryophyte sampling will be excluded from consideration for future sampling for all other protocols.

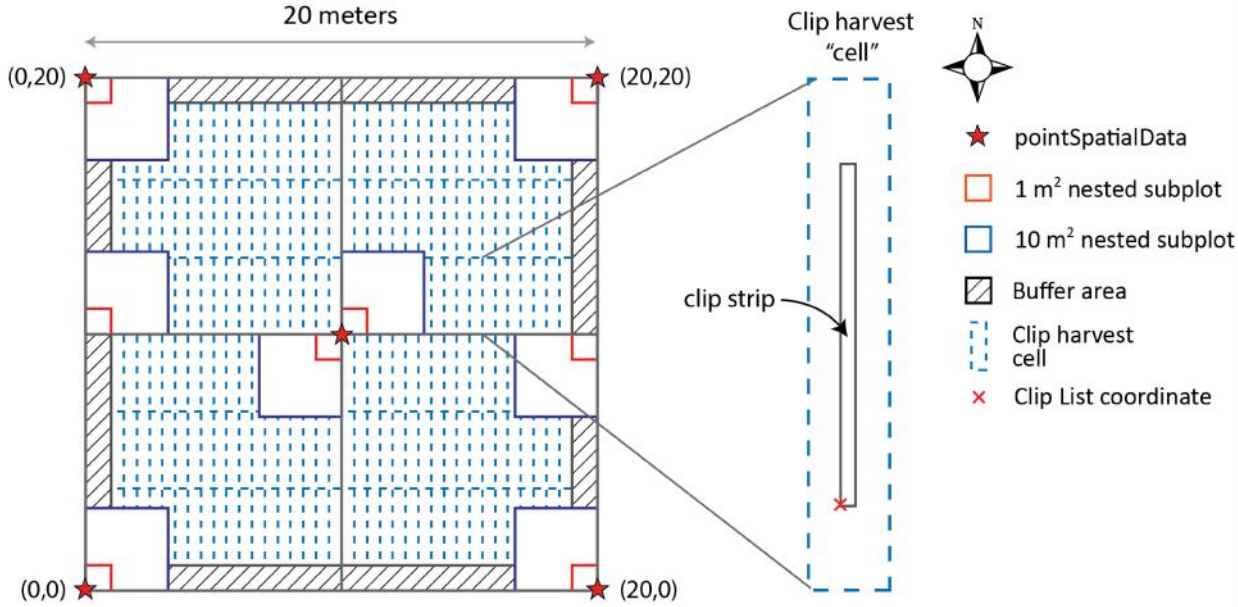

Figure 2. A Tower plot showing the locations of 0.5 m x 3 m "clip cells" (dashed blue lines) that contain potential 0.1 m x 2 m "clip-harvest net strips" or "clip strips." Larger plots will have different nested subplots, but the coordinate numbering system for the 20 m subplot within these plots will follow the same conventions as shown above. Coordinates corresponding to the SW corner of the clip-strip (red 'X') are provided to technicians in plot-specific Clip Lists.

3.3 Overview of Sampling

Technicians will utilize the random list of potential clip strips and coordinates to be sampled in each 20 m x 20 m plot or subplot (referred to as "Clip Lists" hereafter). This list is generated randomly and a priori by NEON Science Operations, and it contains more clip strips than will be suitable for clip-harvest net sampling over the lifetime of the observatory. The inclusion of more clip strips than will be sampled is due to unforeseen obstacles (such as rocks, trees, ant nests, etc.) at any given location that will prevent the sampling of some clip strips on the list. Technicians should work down this Clip 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 a given clip strip over the lifetime of the Observatory is minimized or eliminated.

Clip-harvest nets are fabricated from nylon netting with appropriately sized mesh constructed of material that is fine enough to preclude a significant shading of the plants beneath (Russell 1988). For ease of cutting and marking, netting with a mesh size of approximately 1.25 cm is recommended. Netting is cut into 20 cm x 20 cm squares and a permanent marker is used to delineate a 10 cm x 10 cm sampling area in the center to indicate the boundaries of the clip-harvest area. If permanent marker

fades too much on nets at very wet sites, brightly colored darning thread or yarn may be woven through the netting along the 10 cm x 10 cm sampling area boundary.

Upon arriving at a plot or subplot to place sampling nets, it is the field technicians' responsibility to navigate to the first proposed random clip strip, assess its representativeness and suitability for sampling (described in SOP B) (rejecting and moving on to the next location if necessary), and anchor up to five evenly spaced (from south to north) nets within the strip.

Clip-harvest nets are anchored to the bryophyte mat and the location of the clip strip is marked with a pin flag at the SW corner of the strip to aid in locating the net for sampling the next year. Litter and woody debris are cleared from the bryophyte surface to accommodate nets and expose the bryophytes prior to securing nets in place. Nets are placed flush against the bryophyte canopy so that new growth will occur through the mesh; minor manipulation of the bryophytes may be necessary to ensure that nets do not affect their growth pattern. In order to place the nets effectively, vascular plants may need to be clipped to the level of the bryophyte surface, or otherwise manipulated to ensure that they do not affect moss growth (see SOP B for more details). Nets are anchored using standard garden staples, if possible, but chaining pins or longer landscape stakes (with looped tops to secure the nets' mesh) may be used if required by the depth of the vegetation. These anchors are placed just outside of the 10 cm x 10 cm sampling area, so that they do not interfere with bryophyte growth inside of the clip harvest area.

At the end of the growing season (approximately one year after nets were placed), technicians return to the nets to carry out clip sampling and to place nets in different clip strips for the next sampling year. Within the central 10 cm x 10 cm clip-harvest area, bryophytes are clipped to the level of the nets and placed in labeled collection bags. A thin, metal ruler placed flat on the nets can be used as a guide when clipping new growth.

Once clip harvest of bryophytes is complete at the plot, samples must be kept cold until the material can be dried and weighed. Clipped specimens must be stored in a cooler containing cold packs immediately after clipping; keeping clipped moss cold is critical to prevent extension growth (Clymo 1970).

3.4 Best Practices and SOPs

Key requirements for successful implementation of this protocol and collection of quality data are:

- Careful placement of the clip-harvest nets against the vegetation
- Precise clip-harvest of bryophyte growth within the collection area
- Immediate placement of specimens in cold storage

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 communicate with NEON Science Operations

to resolve any field issues associated with implementing this protocol. The SOPs are briefly summarized here:

1. **SOP A**: Preparing for Sampling. This SOP lists the tasks needed to prepare for establishing clip-harvest nets in the field, and to prepare for the actual clip harvests.
2. **SOP B**: Bryophyte clip harvest net deployment. This SOP provides steps for locating assessing and delineating a clip strip, and placement of clip-harvest nets.
3. **SOP C**: Field Sampling. This SOP describes in detail how to harvest the bryophyte annual growth and store the samples.
4. **SOP D**: Post-Field Sampling Tasks. This SOP lists the tasks needed to prepare for the next round of sampling – refreshing sampling supplies and cleaning and maintaining sampling equipment.
5. **SOP E**: Laboratory Processing of Bryophyte Samples. This SOP describes drying and weighing procedures for bryophyte samples in the lab, and describes steps for re-weighing a subset of samples for quality control.
6. **SOP F**: Data Entry and Verification. This SOP describes data entry for field and lab data.

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]). Other quality control checks may be used to ensure that equipment is used properly in the field, bryophytes are stored properly before drying, samples are dried properly in the lab, and dried samples are processed according to the protocol.

4 SAMPLING SCHEDULE

4.1 Sampling Frequency and Timing

At sites selected for bryophyte sampling, clip-harvests will be performed on an annual basis within all Tower plots. Before the first sampling year, clip-harvest nets will be placed in the field at a seasonally appropriate time, determined on a site-by-site basis (Appendix D). After one year and in all subsequent years, clip-harvest sampling will occur and clip-harvest nets will be placed for the next year's sampling. As explained above, the clip-harvest nets are placed in a plot in one sampling bout in one sampling year, then clipped the next sampling year (12 months later); nets are then moved to a new random clip cell for clipping the following (3rd) year.

To ensure that the bryophyte community does not change appreciably before all target plots are sampled, a given clip sampling bout should be concluded within 14 days of initiation (**Table 1**). This

guideline ensures that data collected across all plots within a given bout are representative of the same sampling period. The number of field technicians assigned to the clip-harvesting task should be optimized so that this goal is feasible.

After bryophytes are clipped from a given clip cell, the following points are critical with respect to timing:

- Place labeled bags of clipped bryophytes immediately into a cooler with cold packs, and keep stored cool until they can be placed in a drying oven.
- Record the date and time samples were placed into cold storage after being clipped in the field, and the date and time samples were placed in the drying oven. These data will enable automatic calculation of the number of hours that samples were kept in cold storage.

Table 1. Frequency and timing guidelines for bryophyte clip-harvest net placement and clip sampling.

Plot Type	Events	Yearly Interval	Event Start	Event Stop
Tower	Net deployment Clip harvest	Annual	Lull in bryophyte growing season or late summer (Appendix D provides site-specific dates)	Within 14d of start

4.2 Criteria for Determining Onset and Cessation of Sampling

To calculate ANPP, annual bryophyte clip harvest bouts occur as close to one calendar year as possible after the nets are placed in the field, to ensure that all samples reflect one full year's worth of growth. Therefore, the timing of sampling is dependent on the timing of net placement. Nets should be initially established during periods of slow growth for bryophytes.

In temperate regions with seasonality, bryophytes will experience two periods of growth, during the cooler, wetter seasons (Rincon and Grime 1989). Typically, there is a flush of growth in the spring, when conditions tend to be moist and cool, and the tree or shrub canopy (if present) has not yet leafed out. During summer when conditions are warmer and often drier, and the canopy shades undergrowth, bryophyte growth is generally slower. A second period of growth is common in the fall, when conditions are again cooler and moister, and the canopy is losing leaves. Bryophytes can grow while under snow cover if there are periods of daylight, so nets must be placed when the ground is free of snow or ice. Therefore, in temperate regions, late summer may be the best time to place nets for the next year's sampling.

In regions lacking seasonality, bryophytes grow year-round and should simply be sampled at the same time every year, preferably late summer. In warm desert or near-tropical regions, nets should be placed

during drier, hotter periods when bryophyte growth is more likely to be slow; precipitation patterns for these regions will dictate sample timing.

In arctic and boreal sites, the logistical variables of snow/ice cover and available daylight will largely determine the timing of net placement. This will likely be in late summer, approximately late July or early August, when days are shorter and temperatures are still warm. By this time of the year, snow or ice should have melted and the relative warmth and dryness will slow the bryophyte growth. Establishing the nets prior to snowfall in the fall is essential, since snow will prevent evaluation of bryophyte presence and hence the representativeness of the clip strip. In addition, snow will prevent placement of the nets flush with the bryophyte surface; nets should still be placed if the surface is accessible through light snow cover and anchors can be installed.

Site-specific event start windows are provided in **Appendix D**, and it is incumbent upon Field Operations to select onset dates within these windows.

4.3 Timing for Laboratory Processing and Analysis

Because bryophyte 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. Samples should be processed (i.e. Sphagnum capitula removed, vascular species removed) prior to being placed in a drying oven within 24 h of clipping in the field. Samples 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 weighed immediately after removing from the oven, or placed in a desiccator for temporary storage prior to weighing. There are no scientific limits on the time oven-dried samples may be placed in temporary storage prior to weighing and processing. However, samples placed in temporary storage must be re-dried in the oven for 24 h prior to weighing. Because bryophytes can rapidly absorb moisture from the air, it is best to remove small batches of samples from the drying oven, and weigh immediately.

4.4 Sampling Timing Contingencies

When unexpected field conditions require deviations from this protocol, the following field implementation guidance must be followed to ensure quality standards are met:

Table 2. Decision tree associated with bryophyte clip-harvest sampling

Delay/ Situation	Action	Outcome for Data Products
Hours	If delay prevents completion of clip-harvest nets: 1. Ensure all bags of bryophytes are labeled, 2. Resume harvest of same clip-harvest net ASAP	No adverse outcome.
	If delay occurs between plots, resume harvest of next clip-harvest net ASAP.	
1-7 days	If delay prevents completion of clip-harvest nets: 1. Ensure all bags of bryophytes are labeled, 2. Store already clipped bryophytes in a cooler with fresh cold packs (okay), or oven-dry as per protocol (best), 3. Resume harvest of same clip-harvest net ASAP with new labeled bags, and 4. Combine dried bryophytes from clip strip for weighing when all samples are dry.	Difficult to complete clip-harvest of all plots in 10-14 day window if delay approaches 7 days. This creates potential change in observed ANPP.
	If delay occurs between clip-harvest nets, resume harvest of next net ASAP.	
8-13 days or longer	If delay prevents completion of clip-harvest nets: 1. Ensure all bags of bryophytes are labeled, 2. Store already clipped bryophytes in a cooler with fresh cold packs or a refrigerator (okay), or oven-dry as per protocol (best), 3. Resume harvest of same clip-harvest net ASAP with new labeled bags, and 4. Combine dried bryophytes from clip strip for weighing when all samples are dry.	Aboveground biomass per unit area may change in the field over this length of time. This introduces more uncertainty into ANPP estimates.
	If delay occurs between clip-harvest nets, resume harvest of next strip ASAP	

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 EHSS Policy, Program and Management Plan (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.

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 – SOP B, placing bryophyte clip-harvest nets

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
Durable items						
MX100703	R	GPS unit, pre-loaded with plot or subplot locations	Navigate to plot or subplot	All	1	N
MX100320	R	Compass, mirror-sight	Navigate to clip-harvest strips; used in combination with GPS unit	All	1	N
MX100322	R	TruPulse 360R laser rangefinder and clinometer with current declination entered	Navigate to clip-harvest strips	All	1	N
MX103218	R	Foliage filter	Allow laser rangefinder use in dense vegetation	Densely vegetated plots	1	N
MX104359	R	White reflector or reflective tape	Reflective target for laser rangefinder; aids in measuring distance to target accurately	All	1	N

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
MX100722	R	Fiberglass meter tape, at least 30 m long	Navigate to clip-harvest strips	All	2	N
	R	Field map	Field reference	All	1	N
	R	Per plot or subplot Clip List	Navigate to clip-harvest strips, document sampling status	All	1	N
MX100543	R	Thin, steel ruler, 30cm length, with cm demarcations	Delineation of accepted clip-harvest strip	All	1	N
Consumable Items						
MX111228	R	0.5 inch mesh for clip harvest nets.	Delineation of clip-harvest sample area	All	5-10 per plot	N
	R	Pre-marked string and stake sets	Delineation of accepted clip-harvest strip	All	2 sets	N
	R	Standard garden staples, 6” and 10”, preferably made from non-oxidizable materials (e.g. aluminum, galvanized steel, plastic, or equivalent)	Anchor clip-harvest nets to substrate	Most shallow mats	8-10 per net	N
MX104361	S	Chaining pins, steel, 14” long	Anchor clip-harvest nets to substrate	Mat too deep for adequate anchoring with staples	8-10	N

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
	S	Longer landscape stakes with looped top (e.g. Garden Plus 36” metal landscape stakes)	Anchor clip-harvest nets to substrate	Mat too deep for adequate anchoring with chaining pins	8-10 per net	N
	R	4” x 5” pin flags with PVC stakes	Aids in accurate location of clip-harvest nets; PVC useful to stabilize flag	All	1	N
RD[05]	R	Bryophyte Productivity Field Datasheets	Collect locational data	All	1	N

R/S=Required/Suggested

Table 4. Equipment list – SOP C, clip-harvesting bryophyte field samples

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
Durable items						
MX100703	R	GPS unit, pre-loaded with plot locations	Navigate back to actual sampling location.	All	1	No

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
MX100320	R	Compass, mirror-sight	Navigate back to actual sampling location	All, in combination with GPS unit	1	
	R	Field map	Field reference.	All	1	No
MX100543	R	Thin, steel ruler, 30cm length	Held horizontally at net height to provide a reference point when clipping new growth.	All	1	No
MX103532	R	Hand clippers, fine tip	Clipping plants	All	2	No
	S	Work gloves	Hand protection (if necessary)	Coarse, sharp vegetation	2	No
	R	Permanent marker	Label paper bags	All	2	No
	R	Large chest-style cooler, with frozen cold packs (at –20 ˚C)	Keep clipped biomass cool after harvest, preserve species' diagnostic features, reduce unintended mass loss.	All	1	No
Consumable Items						
MX103232	R	Paper bags, 25# kraft[1, 2]	Storage of clipped bryophyte biomass	All	10	No
MX105089	R	Paper bags, 8# kraft[1, 2]	Storage of clipped bryophyte biomass	All	10	No

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
	R	Pencils	Record sampling metadata	All	2	No
RD[05]	R	Bryophyte Productivity Field Datasheets	Record sampling metadata	All	Varies	No

R/S=Required/Suggested

[1] Bag size may be adjusted as necessary based on size/bulk of plants being clipped.

[2] Quantity may be adjusted as necessary based on field experience at a given site.

Table 5. Equipment list – Post-field sampling

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
Durable items						
	R	Large chest-style cooler, with frozen cold packs (at –20 ˚C)	Keep clipped bryophytes cool	All	1	N
	R	Permanent marker	Label paper bags	All	2	N
Consumable Items						
RD[05]	R	Bryophyte Productivity Field Datasheets	Record sampling data	All	Varies	N
	R	Pencils	Record sampling data	All	2	N
MX105089	S	Paper bags, 8# kraft[1, 2]	Storage of clipped bryophyte biomass	All	10	N
MX103232	S	Paper bags, 25# kraft[1, 2]	Storage of clipped bryophyte biomass	All	10	N

R/S=Required/Suggested

[1] Bag size may be adjusted as necessary based on size/bulk of plants being clipped.

[2] Quantity may be adjusted as necessary based on field experience at a given site.

Table 6. Equipment list – Processing bryophyte clip-harvest samples in the lab

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable Items					
MX100230	R	Drying oven	Dry field-clipped biomass for weighing	2 (typically)	N
	R	Desiccator	Storing dry bryophytes before weighing. Prevents hygroscopic bryophytes from absorbing water from the air after being dried	1	N
MX111227	R	Jar of dessicant	For maintaining dry conditions in dessicator	1 (more as needed)	
MX100265	R	Mass balance (0.01g accuracy)	Weigh oven-dried samples	1	N
Consumable Items					
	R	Pencils	Recording dry weight of bryophyte biomass	2	N
RD[05]	R	Datasheets:) Lab Drying QC Datasheet) Lab Weighing Datasheet	Recording dry weight of bryophyte biomass	As needed	N
MX100689	R	Weigh boats	Hold dried sample during weighing	Varies	N

R/S=Required/Suggested

6.2 Training Requirements

All technicians must complete required safety training. 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 distinguish target bryophytes (those that form continuous or large-patch carpets) at the sites within the region of employment.

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

- Distinguish bryophytes from vascular plants, lichens, and pteridophytes (specifically club mosses).
- Distinguish bryophyte types (Acrocarps, Pleurocarps, *Sphagnum,* liverworts)

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 to complete the first plots of the season). Use this estimate as a 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 1-2 hours to harvest bryophytes within a given clip-strip. This time range includes, clipping qualifying biomass from previously set nets, and deployment of nets in a new clip-strip location – this latter task involves identifying an acceptable sampling location for next year's nets, delineating the clip-harvest strip, and installing the mesh nets. Harvesting may take less time if vascular plant cover is sparse. Time required will be slightly less in the first year of bryophyte sampling because no harvesting will take place.

7 STANDARD OPERATING PROCEDURES

SOP A Preparing for Sampling

A.1 Preparing for Data Capture

Mobile applications are the preferred mechanism for data entry. Mobile devices should be fully charged at the beginning of each field day, whenever possible.

However, given the potential for mobile devices to fail under field conditions, it is imperative that paper datasheets are always available to record data. Paper datasheets should be carried along with the mobile devices to sampling locations at all times.

A.2 Preparing equipment

Table 7 contains actions required to prepare materials for location and placement of clip-harvest nets (SOP B) and clip-harvest net sampling (SOP C). Note that the items in this table are only those that require preparation; complete equipment lists can be found in section 6.1.

Table 7. Actions necessary to prepare equipment for two technicians placing clip harvest nets (SOP B) and performing bryophyte sampling (SOP C).

Item Description	Qty	SOP	Action(s)
GPS unit	1	B, C	⎫ Charge ⎫ Load target plot or subplot locations ⎫ Set to correct coordinate system (lat-long or UTM zone)
Compass, mirror-sight	1	B, C	Check/set correct declination[1]
TruPulse 360R laser rangefinder and clinometer[1]	1	B, C	⎫ Check battery, charge (if possible) ⎫ Clean lenses with lens cloth or lens tissue (if necessary) ⎫ Check/set correct declination[1]. See RD[07] for details ⎫ Calibrate TruPulse tilt-sensor (only necessary after severe drop-shock; see RD[07] for details)
Pre-marked string and stake sets[2]	2 sets	B	Fabricate if necessary: 1. Cut 2.5 m of ⅛" diameter nylon cord 2. Mark cord at each end with a permanent marker so that the center section of the cord measures exactly 2 m between the markings. 3. Tie each end to an 8" or longer tent stake.

Item Description	Qty	SOP	Action(s)
Pre-marked bryophyte clip-harvest nets[3]	5	B	Fabricate if necessary: 1. Cut a 20 cm x 20 cm square from thin nylon netting with mesh size of about 1.25 cm (0.5") 2. Mark a 10 cm x 10 cm square in the center using a permanent marker. There will be a 5 cm frame around the center square. 3. If permanent marker fades too much on nets at very wet sites, brightly colored darning thread or yarn may be woven through the netting along the 10 cm by 10 cm sampling area boundary.
Standard garden staples, longer stakes, or chaining pins to anchor clip-harvest net (non-oxidizable metals or plastic materials are acceptable)	As needed	B	Clean and straighten (if necessary)
Field map	1	B	Print on all-weather paper
Per plot or subplot Clip Lists[4]	1	B	Print on all-weather paper
Bryophyte Productivity Field Datasheets (RD[05])	As needed	B, C	Print on all-weather paper
List of clip strips with netting	As needed	C	Export **clipIDs that have netting established** from bryophyte productivity database. Print on all-weather paper
Hand clippers, fine tip	1	B, C	Clean and sharpen blades (if necessary)
Re-usable cold packs	As needed	C	Place in –20 ˚C freezer
Field map of actual sampling location labeled with clipID	1	C	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-harvest net strip boundaries, and require fabrication prior to field work. Each set consists of two tent stakes connected by nylon cord.
[3] Pre-marked bryophyte clip-harvest nets are used to delineate clip-harvest sampling locations and must be fabricated prior to fieldwork. Each net consists of one 20 cm x 20 cm piece of nylon netting with a 10 cm x 10 cm center square delineated with permanent marker.

[4] Provided separately by Science Operations once plot establishment has been completed.

SOP B Bryophyte clip-harvest net deployment

This sampling protocol will be initiated in Tower plots at sites where mean bryophyte cover is 10% or more. Plot level bryophyte cover is assessed by Science Operations, and is calculated by averaging moss cover from the eight 1-m^2 sub-plots set aside for the plant diversity protocol (RD[10]) which is then averaged across all plots surveyed for site characterization to arrive at a site level estimate of bryophyte cover.

- A maximum of 20 plots will be sampled at sites with 40 m x 40 m Tower plots; at these plots, two subplots will be selected for sampling based on random subplot lists provided by NEON Science Operations.
- A maximum of 30 plots will be sampled at sites with 20 m x 20 m Tower plots; each plot will contain a single clip strip per year.
- Each clip strip, containing 5 clip-harvest nets, will be sampled following one year's worth of growth.
- NEON Science Operations will provide a list of sites at which bryophyte cover meets the 10% threshold, based on site characterization data.

Data generated in this SOP are recorded on the Bryophyte Productivity: Clip Strip Establishment Field Datasheet or as part of the set activity if data are recorded on a mobile digital device.

B.1 Locating randomly selected clip strips and placing clip-harvest nets

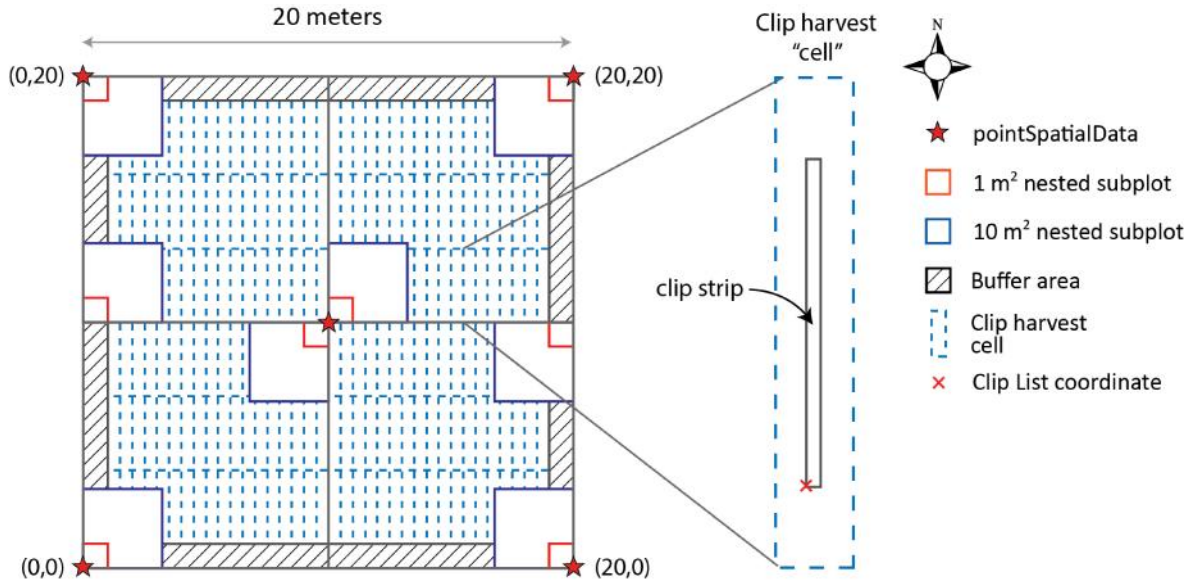

Figure 3. (Same as Figure 2) A Tower plot showing the locations of 0.5 m x 3 m "clip harvest cells" or "grid cells" (dashed blue lines) that contain potential 0.1 m x 2 m "clip-harvest net strips" or "clip strips." Larger plots will have different nested subplots, but the coordinate numbering system for the 20 m subplot within these plots will follow the same conventions as shown above. Coordinates corresponding to the SW corner of the clip-strip (red 'X') are provided to technicians in plot-specific Clip Lists.

1. Navigate to the **plotID** to be sampled using GPS, map, and compass, as needed.
2. Use the plot- or subplot-specific Clip List ([**plotID**] clipList.csv) to identify the first potential clip strip (with "**clipID**") that has not already been sampled or rejected. The clip strips are the 0.1 m x 2 m strips nested within the "clip cells," as shown in **Figure 3** above. Where relevant, subplot number is included in the file name and is also provided as a field in the Clip List spreadsheet.
    - The Clip List provides a randomized list of potential clip strips per plot or subplot, and the coordinates associated with the SW corner of each clip strip.
    - The Clip List also indicates which clip strips have already been sampled or rejected.
3. Locate and flag the southwest corner of the clip strip (the red "X" in Figure 3 above), using the relative (X,Y) coordinates of its 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:

- Run a tape East/West along the south edge of the plot or subplot between the (0,0) and the (20,0) plot markers (**Figure 3**) and stretch the tape taut.
- Place a pin flag at the desired relative X-coordinate.
- 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 RD[07]).
    - Avoid trampling vegetation as much as possible.
- Place a pin flag at the (X,Y) location.

If the Y-coordinate is > 10:

- Run a tape (see Tips, below) East/West from the plot centroid (10,10) to either the (0,10) position (if the X-coordinate is between 1 and 10) or the (20,10) position (if the X-coordinate is between 10 and 20; see **Figure 3** and Table 8):

Table 8 Tape layout instructions for a clip-harvest cell.

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

- Place a pin flag at the desired relative X-coordinate.
- 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 RD[07]).
- Avoid trampling the vegetation as much as possible. Avoid walking across plot to extent possible. Walk along edges of plot, and along edges of clip strip as it is being delineated. While following edges to extent possible, also minimize the distance walked inside the plot. Do not walk inside clip strip being delineated.

- Place a pin flag at the (X,Y) location.

TIPS

- If the plot slope is > 20%, 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 bryophyte vegetation in the clip strip is representative of the site as a whole and whether the clip strip is suitable for sampling, and accept or reject the location (**Figure 4**).

- Bryophyte vegetation within the clip strip should be representative of the target bryophyte vegetation in the 20 m x 20 m plot/subplot.
  - In practice, 'representative' is quantified as having similar patterns of continuous or large-patch carpets of bryophytes as are found in the plot.
  - If the bryophytes occur discontinuously in the plot or across other plots in the site, then areas with no bryophyte cover in the clip strip may still be considered representative.
- In some plots, absence of target bryophytes or partial cover of target bryophytes (the edge of a larger carpet) may be considered representative, and the location acceptable.
- If the site has less than 50% cover of bryophytes, and the plot has no bryophytes, it may still be considered representative, since some areas of the site might have no bryophyte cover.
- Obstacles, disturbances, and/or irregularities in the strip that make it unsuitable for sampling may include trees, large rocks, ant nests, standing water deeper than 30 cm, etc. If something physically prevents the delineation of the clip strip, >25% of the strip is affected by obstacles, disturbances, and/or irregularities, or >30 cm of water is present, there are two options:

  1) The strip may not be moved, but individual nets can be shifted if suitable habitat is present (step 8, below);

  2) otherwise the location is rejected and the next clip strip for that plot is evaluated.

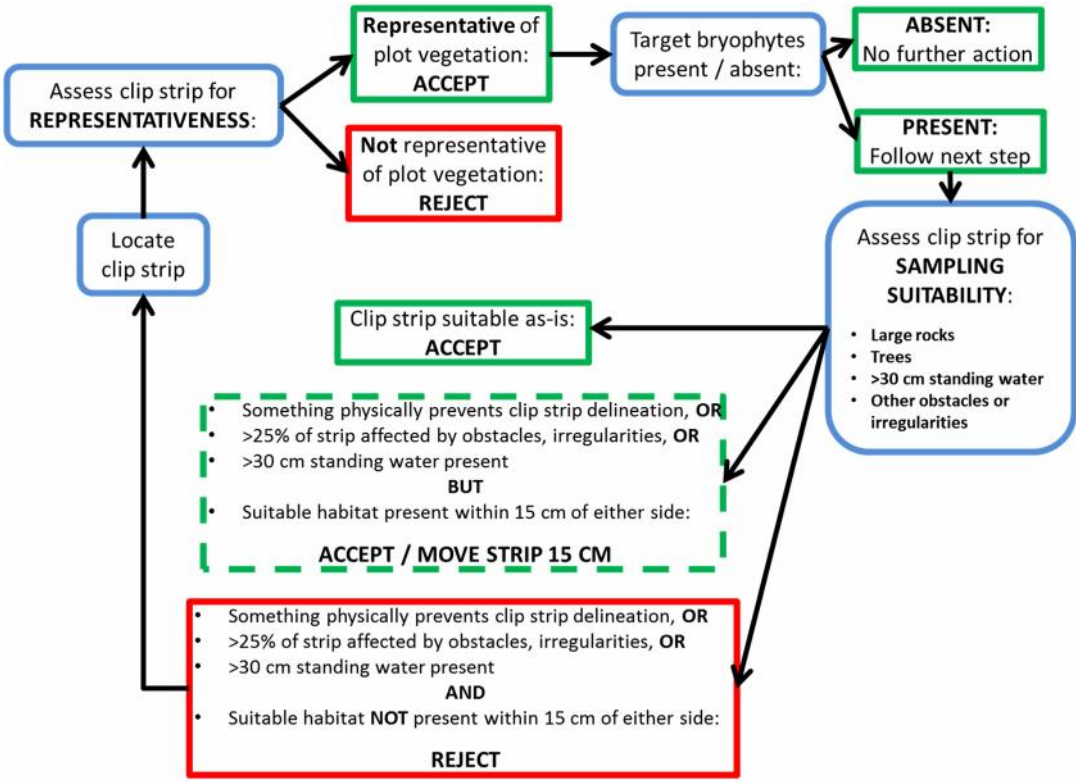

Figure 4. Flow chart to guide assessment of clip strip suitability for sampling.

5. If the clipID (in the Clip List) is rejected, record why in the "status" column on the Clip List (codes in **Table 9**), select the next clipID on the list, and return to step (3) above. Otherwise, update the "status" column using codes in Table 9 and proceed to step (6).
   - If 3 consecutive clipIDs are rejected based on being non-representative of the plot, before moving to the next location, take a moment to assess the plot to re-calibrate what is considered representative.

UNCOMMON SPECIES

Randomly located clip strips should also be rejected if placement of nets and subsequent clipping is likely to impact a particular plant specimen that would ultimately influence plot-level diversity, that is, a plant present in the clip strip exists nowhere else in the plot or subplot and cannot be avoided by shifting net placement.

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

Code	Definition
0	Rejected; non-representative or disturbance, obstacle, and/or irregularity encountered within the clipID cell
1	Accepted, no exclosure
2	Accepted, exclosure (not applicable to bryophyte sampling)
3	Rejected temporarily, inundated
4	Rejected temporarily, uncommon plant
5	Co-located belowground biomass core sampling

6. Record:
   - **clipID** – identifier for the unique clip cell
   - **targetTaxaPresent** (Yes/No) – indicates whether bryophytes are present
     - If target bryophytes are present, **targetTaxaPresent** = "Yes" move to step (8).
     - If target bryophytes are absent from the clip strip, AND the strip is deemed representative **targetTaxaPresent** = "No" and proceed to the next plot/subplot
7. Delineate the accepted clip strip.
   - Using one of the pre-marked string and stake sets, line up one of the marks with the pin flag at the southwest corner of the clip strip, 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 bounding the clip strip.
8. Establish the five clip-harvest nets in a row within the 0.1 m x 2 m area of the clip strip, 20 cm apart.
   - Place the 10 cm x 10 cm sampling area in the center of each net **flush** with the bryophyte canopy,
   - Nets must be **taut**, and secured **firmly**, to ensure that next year's clip harvest reflects the *entire* growth for the year. Nets placed, even slightly above the bryophyte surface, or placed such that they are pushed up by new vascular plant growth, new bryophyte growth will be unaccounted for.
   - Net placement within the 0.1 m x 2 m area may be shifted up to 10 cm in any direction to avoid vascular vegetation that may interfere with net placement or to accommodate microtopographical features.

- Before securing the first net, look at the strip to approximate a layout for all five nets that will allow them to be at least 20 cm apart while avoiding vegetation that may interfere with the net.
- The total north/south distance of all nets may not exceed 2 m in length (the length of a clip strip)

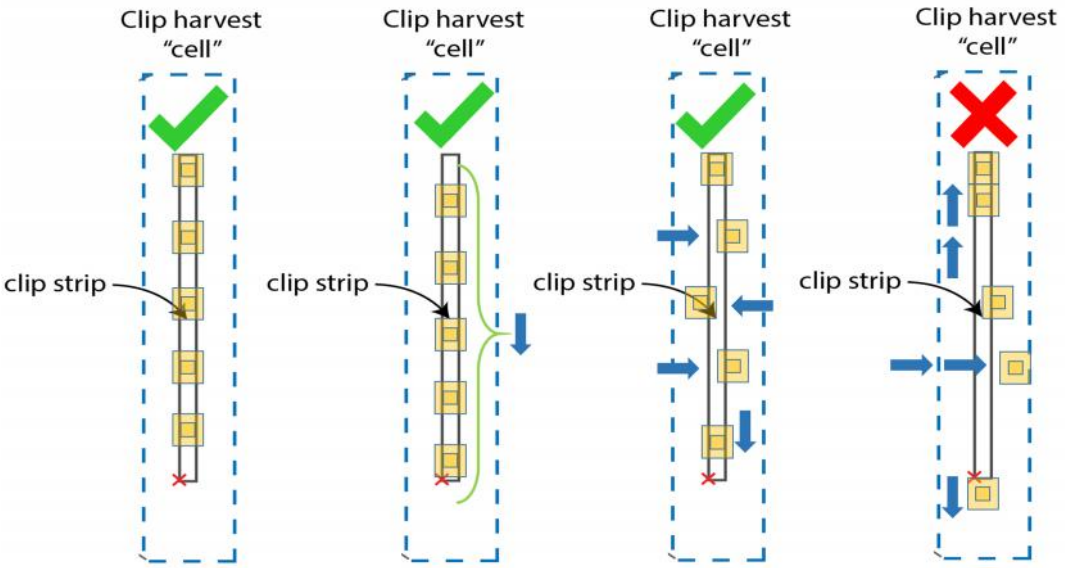

Figure 5. Nets may be shifted up to 10 cm in any direction to avoide obstacles provided nets are a minimum of 20 cm apart and do not span > 2 m across all nets. Blue arrows indicate a 10 cm shift in the specified direction.. In the unacceptable example here, the two northern-most nets are too close together, the third trap down is shifted too far to the east, and the southern most trap has been shifted too far to the south such that the total distance from north to south is > 2 m.

- Within the selected clip net locations, clear litter and small woody debris to expose the bryophytes and accommodate the net placement, if necessary.
  - Vascular plants may need to be clipped and eased through the mesh, or otherwise manipulated, to ensure that they do not affect secure placement of the net and will not affect net position with annual growth.
  - Herbaceous plants such as sedges or small forbs may be clipped to the bryophyte surface, or pulled through the mesh.
  - Where there are shrubs or trees present (e.g., leatherleaf *Chamaedaphne calyculata*), net placement may need to be adjusted to avoid placing nets over shrub/tree stems.
  - If a suitable location cannot be identified due to the presence of woody stems, minor manipulation is acceptable. If fine stems (<1 cm diameter) must be cut, leave enough stem that the remaining portion may be threaded through the mesh so that new growth from the cut tips occurs above the level of the net and does not push the net up as they grow back. Vascular plants *must not be pulled out of the ground* to accommodate nets.
  - Dispose of clipped vascular plant biomass outside the plot.

- Align the first clip-harvest net with the southern edge of the clip strip. Place the net *flush* against the bryophyte canopy so that all new growth will occur through the mesh; minor manipulation of the bryophytes may be necessary to ensure that the net does not affect their growth pattern.
  - For *Sphagnum* species, carefully thread capitulum through the 10 cm x 10 cm portion of the net so that all new growth within the delineated measurement area occurs above the level of the net.
- Affix the clip-harvest net firmly to the bryophyte mat using anchors of appropriate length, either garden staples, chaining pins, or landscape stakes.
  - If possible, when visiting each plot throughout the year for other sampling activities, check that net is still firmly anchored in place. Re-set anchors if they have loosened.
- Move string-and-stake set as nets are secured so strings can be removed when net deployment is complete.
- Measure 20 cm north from the first clip-harvest net and place the next net. Repeat until all five nets have been established. If target bryophytes are absent from any of the five net locations, those nets should not be placed.
- Record:
  - **setDate** – the date on which nets were set
  - **setNetCount** – the number of nets that were placed; values range from 1 to 5.

9. Remove the string-and-stake set after all nets have been placed. **Leave only one PVC pin flag in the plot/subplot, at the southwest corner of the clip strip**.

SOP C Field Sampling

Data generated in this SOP are recorded on the Measurement of Bryophyte Productivity: Clip-Harvest Field Datasheet or as part of the collect activity if data are recorded on a mobile digital device.

C.1 Procedure

1. Navigate back to the **plotID** to be sampled using GPS, map, and compass, as needed.
   - Field sampling should be delayed if snow cover would prevent accurate location and collection of nets.
2. Navigate back to the clipID where nets were set the previous year (should be marked with a pin flag). Avoid trampling vegetation as much as is possible.
   - Locate the pin flag marker and the clip-harvest net.
3. Record required field data for the clip strip, including:
   - **missingNetCount** – number of nets missing, damaged, or disturbed such that clip harvest on annual growth is not possible (biomass will be calculated based on the average biomass per net, which will account for any loss of nets).
   - **sampledNetCount** – number of undisturbed nets from which biomass could be collected. This number includes nets that are present, but have no biomass to be clipped. (setNetCount – missingNetCount = sampledNetCount)
   - **bagCount** - number of bags of clipped biomass collected. Biomass from multiple nets may be pooled in a single bag.
   - **bryType** – dominant (>50%) type of bryophyte present in the clipped sample. Select either:
     - Pleurocarps (ple)– Mosses with lateral growth habit
     - Acrocarps (acr)– Mosses with upright growth habit
     - Sphagnum (sph)– Mosses from the genus *Sphagnum* sp.
     - Liverworts (liv)– Members of the division Marchantiophyta
     - Mixed (mix)- > 1 type of bryophyte in sample, and no single type represents > 50%
     - Other (oth) - specify in remarks
4. Using a permanent marker, pre-label 8# kraft paper bags with the following information (use larger bags if it appears that they will be needed):
   - **plotID**
   - **subplotID**
   - **date**; use *YYYYMMDD* format
   - **clipID**; as provided in Clip List; use "*plotID_XXX"* format, e.g. CPER_001_126
   - **bagNumber**; use *X of Y* notation, where X is the bag # and Y is the total # of bags within a given **clipID**.

5. For all nets in a strip, clip and bag all bryophyte biomass that is growing above the height of the mesh in the marked 10 cm x 10 cm center square.
    - Use a thin metal, or light weight plastic ruler placed horizontally on the net as a height guide, if needed.
    - Pull out the anchors and remove each clip-harvest net after it has been clipped.
    - Be careful not to clip the net itself while clipping bryophyte growth.

BEST PRACTICES

- Clip slowly and **immediately** put clipped vegetation into labeled bags.
- Place full bags **immediately** into a cooler with cold packs.
- Be sure to record the time placed in the cooler on the field datasheet.

6. *Optional field task:* If bryophytes are in the genus *Sphagnum*, remove capitulum according to guidelines in E.1.
    - Clipping of capitulum is likely easier to complete in the lab but may occur in the field. There is no scientific requirement, other than prior to drying, dictating when this step is completed.
    - Discard clipped capitulum outside plot boundaries
7. When clipping is finished, if multiple bags were used, group all bags from the current clip strip into a larger, labeled, 25# bag, record **time** (the time placed in the cooler).
8. When all nets have been sampled, remove the pin flag.
9. Place nets in a new random clip strip according to steps in SOP B.
    - If stems threaded through the net are likely to be damaged to a great degree when net is removed, cut the net away and deploy a new net in the new location.

C.2 Sample Preservation

- Keep paper bags with clipped vegetation in a cooler with fresh cold packs to prevent further shoot elongation.
- Change cold packs for fresh ones every 12 h, or transfer to a 4 ˚C refrigerator if a drying oven is not immediately available.
- Process clipped bryophytes and transfer bags to the drying oven as soon as possible.

SOP D Post-Field Sampling Tasks

D.1 Refreshing the sampling kit

- Review Table 4 for a listing of the equipment and materials needed to conduct field sampling; make sure the following consumables are available in sufficient quantity for the next round of clip-harvests:
  - Paper bags, 8# and 25# kraft
  - Field datasheets
  - Permanent markers and pencils
- Return cold packs to the –20 ˚C freezer to refresh.

D.2 Equipment maintenance and cleaning

Review Table 4 for a listing of the equipment and materials needed to conduct field sampling, and clean and prepare equipment as needed:

- Clean blades of hand clippers with ethanol.
- Clean and straighten net anchors (garden staples, etc.) and pin flags, if necessary.
- Clean used clip-harvest net squares for reuse, if possible.
- Recharge batteries for the GPS unit, if necessary.

SOP E Laboratory Processing of Bryophyte Samples

Data generated in this SOP are recorded on the Measurement of Bryophyte Productivity: Lab Weighing Datasheet or as part of the weigh activity if data are recorded on a mobile digital device or webUI.

E.1 Removing *Sphagnum* spp. capitula

Mosses in the genus *Sphagnum* have a unique tuft-like structure, called the capitulum, located near the stem apex. New branches with young leaves will form in groups (called "fascicles") below the stem apex as the stem elongates over time. The capitulum remains relatively constant through time as a stem continues to grow, making it difficult to determine when the capitulum was actually formed, and thus it should not be included in net primary production estimates (Vitt 2007). The capitulum of harvested *Sphagnum* biomass must therefore be identified and removed prior to drying (see **Figure 6**).

1. Remove harvested biomass from field collection bags.
2. Identify the capitula of harvested bryophyte biomass as you prepare for drying.
    a. The shape, color, and arrangement of branches on the capitulum can vary widely by species, but the capitulum length generally ranges between 1 – 10 mm of the terminal section of the stem.
3. Separate the capitulum (including any developing branches or leaves on the capitulum) from each stem by clipping with scissors directly below where the capitulum and stem meet.
4. Discard the removed capitula and return the remaining harvested biomass to the field collection bags before proceeding to drying and weighing (SOP E.2).

5.

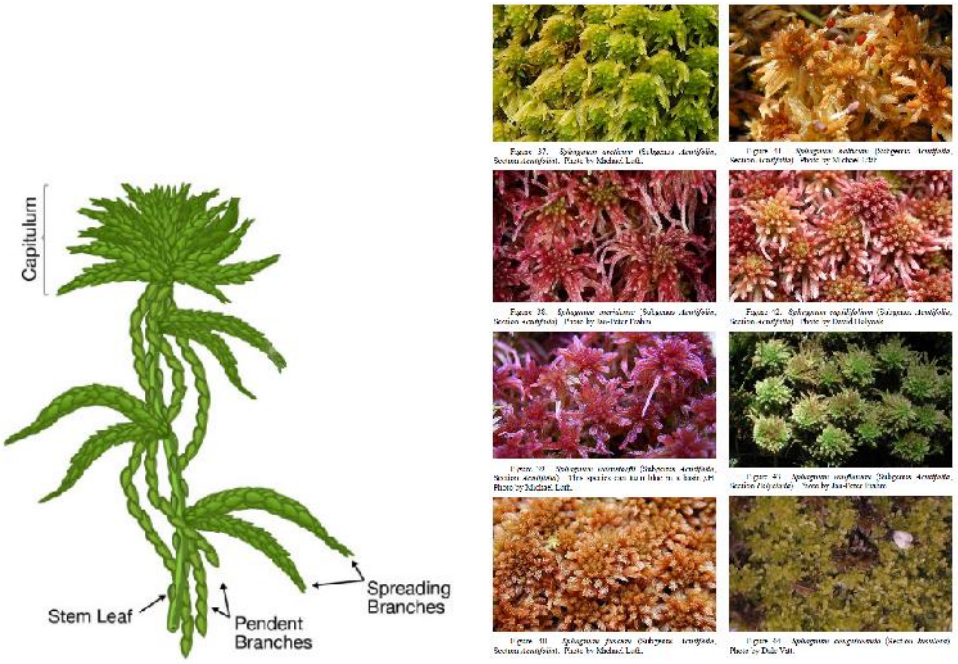

Figure 6. (Left) Schematic of a *Sphagnum* plant consisting of stem, leaves, and capitulum; adapted from Weston et al. (2015). (Right) Close up photographs showing various colors and morphologies of sphagnum capitula; taken from Glime (2013).

E.2 Drying and weighing clipped bryophyte biomass

Clipped bryophytes should be placed in the drying oven as soon as possible following harvest in the field. Note that once the samples are ready to be weighed, the biomass harvested from all of the nets within a given clip strip will be combined and weighed as a single sample for that clip strip.

1. Label each 8# bag or 25# bag (with smaller bags inside) with the date and time it will be placed in the drying oven. Record **ovenStartDate** and **startTime** on the "Lab Drying QC" datasheet.

- ***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. It also allows for the calculation of how long the samples were in cold storage prior to being placed in the drying oven.

2. Place bags labeled with the date and time into a 65 ˚C drying oven for 48h – 120h (2d – 5d).
3. After placing in the oven, check the drying progress of clipped biomass every 24 hours using the "Lab Drying QC" datasheet. Record **dryMass**, as needed, on the "Lab Drying QC" datasheet. If samples are sufficiently dry before the 120h check, not all of those fields may need to be recorded.
   - Check the weight of the same subset of n=10 bags per **ovenStartDate** at days 1, 2, 3, etc.
   - Calculate the difference in weight between the latest two time points for each bag.

- Samples are dry when the average weight difference between the latest two time points = 0 (averaged across all n=10 bags, ± 0.05 g)

4. Remove bags of dried biomass from the drying oven, and place into a desiccator to let dried biomass come to room temperature. Record **ovenEndDate** and **endTime**
    - Placing bryophytes in a desiccator is critical because bryophytes are extremely hygroscopic and will readily absorb moisture from the air.
    - Work in small batches, to avoid overloading the desiccator.
    - Once dried, samples may be stored for up to 30 days prior to weighing. Samples treated in this manner must be returned to the drying oven for 24 h prior to weighing.

5. Weigh biomass from each **clipID** (clip strip) using a mass balance (0.01g accuracy) and a weigh boat.
    - As you prepare to weigh the biomass for a given clip strip (**clipID**), you may need to combine the biomass from multiple bags into a single sample to be weighed.
    - Record the mass to the nearest 0.01g on the "Lab Weighing" datasheet under the **dryMass** field, and record **weighDate**. Be sure to tare the scale with the weigh boat on it, prior to weighing the plant material. If material weighs <0.01g, record actual value from balance; if balance does not register material, record value as 0.005g.
    - Record **plotID**, **subplotID**, **boutNumber**, and **clipID**, copying from those IDs as recorded on the paper bags containing the sample.
    - For large volumes of biomass that do not readily fit into a large weigh boat, use the following strategies:
        - Use a paper bag, large cardboard box lid, plastic tray (or equivalent) instead of a weigh boat. Be sure to tare the scale with whatever item will be used to contain the plant material during weighing.
        - 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.*

6. Once all weights have been recorded, place biomass in temporary storage at ambient temperatures.
    - QA may also be performed on a subset of samples at this time, but due to the hygroscopic nature of mosses, QA must be completed immediately or samples must be returned to drying oven prior to re-measurement (SOP E.3).

E.3 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.

1. Per unique **boutNumber**, select 10% of dried, previously weighed samples for re-weighing.

- If QA weighing does not occur immediately after the initial weighing, return the selected samples to the drying oven for 24 h prior to QA weighing. Dry bryophyte samples will pick up moisture from the atmosphere very quickly.

2. Record QA weight data:
   - Record the QA weight in the **qaDryMass** field to the nearest 0.01 g. Record the mass of plant material only: do NOT include the weight of the paper bag.
3. Return plant material to temporary storage until all data have been successfully entered in the NEON database. Only after data have been successfully entered and the domain office has received notification that QC checking has been completed at NEON headquarters may samples be discarded.

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.

SOP F Data Entry and Verification

The importance of thorough, accurate data transcription cannot be overstated; the value of the efforts in the field is only manifested once the data are properly entered for delivery to NEON's end user community.

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).

Before entering data, all personnel ***MUST*** read RD[04] for complete instructions regarding manual data transcription. Prior to entering data via a web user interface (webUI), each technician shall enter a plot (or subplot) of data from one bout into the protocol-specific webUI housed on the Training portal, as described in RD[04].

Data recorded manually into field and lab datasheets must be transcribed into NEON's digital data entry system. Be sure to enter data for all plots within a bout. If an entire bout was missed, no data need be entered. There are three separate data entry apps that are combined to a single ingest table:

- **Set activity**: Metadata describing individual net setting events on a per clipID per plotID, date basis
- **Collect activity:** Metadata describing individual clip harvest events on a per clipID per plotID, date basis
- **Massdata**: Oven-dried biomass data for each sampleID per clipID per date, as well as weighing QA data.

Detailed steps for interacting with NEON's data entry applications are provided in the application user guide.

F.1 Set activity datasheets

Data from field datasheets are transcribed within 14 days of setting nets.

4. For data collected on paper datasheets: transcribe field data into the appropriate data entry application in accordance with data entry and data QA/QC protocols
5. If a representative **clipID** contained no bryophytes (i.e., **targetTaxaPresent** = 'No'), data must also be entered for collect and massdata and the record finalized.
   - Collect app
     - collectDate = setDate
     - sampledNetCount = 0
   - Massdata app

- ovenStartDate = NA
- ovenEndDate = NA
- drymass = 0

6. Data entry fields mirror the datasheet but may be organized differently
7. Update permanent digital versions of the clip strip lists (the Clip Lists) with "date" and "status" data recorded in the field.

F.2 Collect activity datasheets

Data from field datasheets are transcribed within 14 days of collection.

1. For data collected on paper datasheets: transcribe field data into the appropriate data entry application in accordance with data entry and data QA/QC protocols
2. If all set nets were destroyed or missing, **sampledNetCount** = 0, data must also be entered for massdata and the record finalized.
   - Massdata app
     - ovenStartDate = NA
     - ovenEndDate = NA
     - drymass = 0
3. Data entry fields mirror the datasheet but may be organized differently

F.3 Lab datasheets

Data from the "Lab Weighing" datasheets should be transcribed within 14 days after biomass values and biomass QA values are recorded for all clipIDs.

1. For data collected on paper datasheets: transcribe lab data into the appropriate data entry application in accordance with data entry and data QA/QC protocols
2. Lab Drying QC datasheets are for internal tracking only and are not transcribed for ingest into the NEON CYI.
3. Once all data from all activities conducted during the most recent sampling bout (set, collect, weigh) have been collected and entered, data will be ingested by CYI according to the guidelines provided in RD[04].

SOP G Sample Shipment

There are no sample shipments associated with this protocol, SOP not applicable.

8 REFERENCES

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APPENDIX A DATASHEETS

The following datasheets are associated with this protocol:

Table 10. Datasheets associated with this protocol

NEON Doc. #	Title
NEON.DOC.002136	Datasheets for TOS Protocol and Procedure: Bryophyte Productivity

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

APPENDIX B QUICK REFERENCES

B.1 Placing the clip-harvest nets

Locate and assess potential clip-harvest net strips

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

STEP 2 – Select first available clip-harvest net strip location (**clipID**) from Clip List.

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

Y-Coordinate	East-West Tape Location
1-9	(0,0) →(20,0)
10-19	(0,10) →(20,10)

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

STEP 5 – Assess suitability of strip for clip-net sampling. Relocate 15 cm west or east OR reject and locate next **clipID** if not suitable.

STEP 6 – Record (X,Y) coordinates of SW corner of actual clip-net strip if moved.

Delineate 0.1 m x 2 m clip-harvest 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.

Establish the clip-harvest nets

STEP 1 – Align the first 20 cm x 20 cm clip-harvest net with the southern edge of the clip-harvest strip flush with bryophyte surface. If necessary, clip vascular vegetation that might impede manipulation and placement of mesh netting over bryophytes.

STEP 2 – Anchor net in the 5 cm frame around the marked center square using garden staples, chaining pins, or landscape stakes.

STEP 3 – Measure 20 cm north from the first net and place the next net, repeating until all 5 nets have been placed. If target bryophytes are absent from a net location, do not establish a net.

STEP 4 – Place PVC pin flag at SW corner of clip-harvest net strip.

B.2 Sampling clip-harvest nets

STEP 1 – Return to location of nets previously placed in clip strip.

STEP 2 – Label 8# kraft paper bags (lunchbag size); use multiple bags as needed.

STEP 3 – Clip bryophytes above the height of each net in the strip, using a flat ruler as a gauge if necessary.

STEP 4 – Record the total number of bags harvested on the Field Datasheet

STEP 5 – If there are multiple 8# bags, place them into one labeled 25# bag.

STEP 6 – Store bag in cooler with frozen cold packs for transport back to lab and record time placed in cooler on the field datasheet.

STEP 7 – Transfer clip bags to freezer in domain lab (if immediate placement into drying oven is not possible).

STEP 8 – Place clipped biomass in a drying oven as soon as possible after clipping.

QUALITY DEPENDS ON PROPER:

- Placement of the harvest net against the vegetation
- Clip-harvest of bryophyte growth within the collection square

Clipping Guidelines

Place a thin metal ruler flat against the net surface to use as a height gauge while clipping.

APPENDIX C REMINDERS

Label Information
boutNumber
date
clipID
bagNumber (e.g. 2 of 3)

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-harvest location and securely anchor nets in place.
- ☑ Relocate harvest nets to next suitable location, if applicable.
- ☑ Record clipID on data sheet.
- ☑ Cross out sampled or rejected clip-net locations from Clip List.

Clip harvesting: Be sure to…

- ☑ Fill in Field Datasheet and check that all bags are accounted for.
- ☑ Store bags in cooler immediately.
- ☑ Record time that bags were placed in cooler.

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.

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.

All dates are derived from MODIS-EVI phenology data averaged from 2001-2009. See Section 4.1 Sampling Frequency and Timing for tips on how to determine per bout net placement and sampling dates for bryophyte clip-harvest.

Table 11. Site-specific per bout sampling start dates for bryophyte clip-harvest.

Domain Number	Site ID	Start Date (Day-of-Year)	Mean bryophyte cover across all surveyed plots	Additional Sampling Information
05	STEI	NA	3%	< 10% bryophyte cover, protocol not implemented
	TREE	NA	5%	< 10% bryophyte cover, protocol not implemented
	UNDE	08/03	24%	
18	BARR	07/29	57%	
	TOOL	07/24	55%	
19	DEJU	07/29	41%	
	HEAL	07/29	51%	
	BONA	07/29	TBD	
20	OLAA	TBD	TBD	