

<i>Title:</i> TOS Protocol and Procedure: Litterfall and Fine Woody Debris		<i>Date:</i> 02/08/2017
<i>NEON Doc. #:</i> NEON.DOC.001710	<i>Author:</i> K. Jones	<i>Revision:</i> E

## TOS PROTOCOL AND PROCEDURE: LITTERFALL AND FINE WOODY DEBRIS

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## Change Record

REVISION	DATE	ECO #	DESCRIPTION OF CHANGE
A	09/09/2014	ECO-02136	Initial release
B	10/15/2014	ECO-02357	Migration to new protocol template
C	04/16/2015	ECO-02771	Minor updates for clarification and to maintain consistency with other productivity protocols Revised steps for delineating clip cells Revised specifications for chemical analysis
D	01/02/2016	ECO-03416	New fields added : setDate (definition changed), addDate (replaces setDate on pertrap datasheet) Added Appendix G: clip cell coordinate maps Added Appendix H: Safe handling of Toxicodendron Added Appendix I: Troubleshooting Added Appendix J: Alternative materials Clarified relative position calculations in SOP B Updated text in SOP G: shipping to match instructions in herbaceous clip harvest protocol. Added dryMass QC instructions Modified lab drying QC datasheet to accommodate multiple drying ovens Added instruction for mass <0.01g Added supplementalDryingTime
E	02/08/2017	ECO-04373	Migrated to new protocol template. Clarified use cases for mixed vs. other functional groups, added mixed option for samples require >1 hr. to sort Removed supplementalDryingTime – no longer being used in litter data product. Added mixing step for creating plot level chemical analysis samples. Added mass guidelines for chemistry subsampling. General clarification throughout.

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## 1 OVERVIEW

### 1.1 Background

Quantifying production of litterfall and fine woody debris is required to estimate annual Aboveground Net Primary Productivity (ANPP) at plot, site and continental scales, and will provide essential data for understanding vegetative C fluxes over time. Litterfall and fine woody debris production will be estimated within Tower plots on an annual basis, based on litter accumulation in elevated and ground traps. Sampling point selection within a plot or subplot will be random; sampling points will be selected from the same randomized list generated to guide clip strip locations for herbaceous clip harvest. In ecosystems where the overstory is non-continuous (i.e. patchy) litterfall and fine woody debris sampling will be targeted rather than random across the plot. The selected sampling strategy will be used at all plots within a site. This protocol will not be implemented at sites with overstory vegetation < 2 meters tall.

Estimates of deciduous litterfall will be calculated on a per annum basis, with all of the litter produced in a given year contributing toward the yearly estimate. Evergreen litterfall estimates within a given calendar year do not necessarily reflect annual production due to the multi-year and somewhat variable lifespan of needles; however, the long-term average (n = at least 3 years) will be used to estimate per annum needle production.

This design calls for sorting fresh litter into specified functional groups prior to drying if time permits. If it is logistically not feasible to sort fresh material before drying, litter may be sorted after drying as time allows. However, sorting freshly collected litter is preferable because dry litter is easily fragmented and identifying small or desiccated litter fragments to functional group will introduce uncertainty in sorting accuracy.

Elevated litter trap size has been selected to be consistent with existing standards and are the same dimensions (70.7 cm x 70.7 cm x 80 cm) as traps used by Smithsonian Tropical Research Institute Center for Tropical Forest Studies (CTFS, **Figure 4**). To minimize the number of clip strips dedicated to fine woody debris sampling, which are therefore unavailable for herbaceous biomass sampling, ground traps will have the same dimensions as a single clip strip cell, 3 m x 0.5 m. If it is apparent that the volume of litterfall biomass collected from elevated and ground litter traps is too great to efficiently dry and process given limitations on drying oven space in the NEON laboratory, trap size or number may be reduced by NEON Science based on sample optimization analysis.

This protocol is divided into six Standard Operating Procedures (SOPs). Each SOP addresses one discrete task and may be utilized as a standalone document as needed for specific field or lab tasks.

- ) **SOP A: Preparing for Sampling:** Includes gathering the necessary equipment and preloading the GPS with the necessary waypoints.

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- ) **SOP B: Initial Deployment of Traps:** Describes the steps for locating sampling points and establishing litter trap pairs.
- ) **SOP C: Field Sampling:** Describes field collection of litterfall and fine woody debris from traps.
- ) **SOP D: Laboratory Processing for Dry Mass Measurement:** Covers laboratory processing including drying and weighing of samples.
- ) **SOP E: Data Entry and Verification:** Provides guidance for manual data transcription from paper data sheets if a mobile data recorder (MDR) is not available.
- ) **SOP F: Processing Litter Samples for Bioarchive and Leaf Chemistry:** Describes the steps for sub-sampling and grinding dried leaf and needle material.
- ) **SOP G: Sample Shipment for Bioarchive and Leaf Chemistry:** Provides science rationale for timelines and restrictions on sample handling and shipping to external facilities.

## 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

This protocol is modeled closely after the litter monitoring protocol written by Helene C. Muller-Landau and S. Joseph Wright (2010) for the CTFS Global Forest Carbon Research Initiative.



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## 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	TOS Science Design for Plant Biomass, Productivity, and Leaf Area Index
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.002132	Datasheets for TOS Protocol and Procedure: Litterfall and Fine Woody Debris
RD[06]	NEON.DOC.014037	TOS Protocol and Procedure: Measurement of Herbaceous Biomass
RD[07]	NEON.DOC.001025	TOS Protocol and Procedure: Plot Establishment
RD[08]	NEON.DOC.001711	TOS Protocol and Procedure: Coarse Downed Wood
RD[09]	NEON.DOC.001924	NEON Raw Data Ingest Workbook for TOS Litterfall and Fine Woody Debris
RD[10]	NEON.DOC.001813	TOS Elevated Litter Trap Assembly Instruction
RD[11]	NEON.DOC.001717	TOS Standard Operating Procedure: TruPulse Rangefinder Use and Calibration
RD[12]	NEON.DOC.001716	TOS Standard Operating Procedure: Toxicodendron Biomass and Handling
RD[13]	NEON.DOC.000987	TOS Protocol and Procedure: Measurement of Vegetation Structure
RD[14]	NEON.DOC.001024	TOS Protocol and Procedure: Canopy Foliage Chemistry and Leaf Mass per Area Measurements
RD[15]	NEON.DOC.014048	TOS Protocol and Procedure: Soil Biogeochemical and Microbial Sampling

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### 2.3 Acronyms

Acronym	Definition
ANPP	Aboveground Net Primary Productivity
CTFS	Center for Tropical Forest Studies
NLCD	National Land Cover Dataset
MODIS	Moderate Resolution Imaging Spectroradiometer (NASA Satellite)
SOP	Standard Operating Procedure

### 2.4 Definitions

**Litterfall:** Shed leaves and needles, reproductive parts (i.e. flowers, fruits, cones, seeds, etc.), and fine woody debris with butt-end diameter < 2 cm (modified from Clark et al. 2001, Bernier et al. 2008). Woody pieces with diameter ≥ 2 cm are considered coarse downed wood, and will be sampled according to the NEON Field and Lab Protocol for Coarse Downed Wood (RD[08]).

## 3 METHOD

To measure litterfall and fine woody debris, NEON will employ two types of sampling units: 1) square, elevated, mesh litter traps; and 2) rectangular, ground “traps” (Figure 4, SOP B). Elevated litter traps are designed to be large enough that the average size of abundant foliage and fine woody debris elements are easily intercepted by the trap. Ground traps are intended to intercept particularly large foliage elements that will not fit in elevated traps (e.g. palm fronds), and fine woody debris pieces that are too long to be sampled in elevated traps including small diameter branches.

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.

Quality assurance will be performed on data collected via these procedures according to the NEON Science Performance QA/QC Plan (AD[06]).

### 3.1 Sampling Methods

For both elevated and ground traps, only the portion of material that meets both the length and diameter criteria will be sampled (Muller-Landau and Wright 2010). Litter sampled from elevated traps will be sorted into functional groups following collection, using the groupings outlined in Table 1. Note that these functional groups differ from those used in NEON’s herbaceous clip harvest protocol (RD[06]); litter material larger than described in Table 1 will be sampled according to the Coarse Downed Wood protocol (RD[08]).

**Table 1.** Size limits for functional groups collected in Elevated and Ground litter traps

Functional Group	Elevated Traps	Ground Traps
Leaves	< 50 cm length	> 50 cm length
Needles	< 50 cm length	N/A
Twigs/branches	< 2 cm diameter AND < 50 cm length	< 2 cm diameter AND > 50 cm length
Woody material (e.g. seed cones, bark, other lignified structures)	< 50 cm length	> 50 cm length
Seeds (including fruits and other attached structures)	All	N/A
Flowers (includes pollen cones and attached structures e.g., pedicels, peduncles...)	All	N/A
Other (lichen, mosses, frass, unidentifiable material, etc.)	All	N/A
Mixed (unsorted litter material)	< 2 cm diameter AND < 50 cm length	N/A

To ensure the accuracy of annual litter production estimates, ground traps will be cleared of all qualifying litter material following the annual sampling bout.

Leaf and needle litter from elevated traps from a single sampling bout will be shipped to external laboratories to be analyzed for C, N,  $\delta^{13}C$  and,  $\delta^{15}N$  once every five years (refer to section 4 for more details about timing).

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### 3.2 Laboratory processing

Following collection and sorting in the field, litter is transported back to the laboratory and dried at 65°C until water weight has been removed, to within the allowed variance indicated in SOP D (minimum 48 hrs). The woody portion of litter is cut to fit in the drying oven then dried at a higher temperature than litterfall, 105°C to release bound water (Williamson and Wiemann 2010). Additionally, lignified structures associated with functional groups other than ‘Woody material’ or ‘Twigs/branches’ (e.g. hickory husks, walnut shells) may also require higher temperatures and extended dry times to release bound water.

### 3.3 Equipment

Design of PVC elevated litter traps is adopted from the CTFS design. Non-oxidizable metal rods (e.g. fiberglass, aluminum, galvanized steel, or equivalent) are used to anchor elevated litter traps in place. Where permitted by the land use agreement, the corners of ground traps will be marked with non-oxidizable metal or plastic stakes to facilitate precise re-measurement of the selected sampling area.

### 3.4 Spatial Distribution of Sampling

Consistent with existing protocols, NEON will establish one elevated litter trap and one paired ground trap in two randomly selected 400 m<sup>2</sup> subplots in 1600 m<sup>2</sup> Tower plots or one litter trap pair per 400 m<sup>2</sup> Tower plot (see RD[07] for description of different Tower plot sizes). The selected subplots are the same ones used for all other plant productivity sampling in Tower plots (RD[06], RD[08], RD[13]).

Only plots with woody vegetation > 2 m tall will be selected for litter sampling using this protocol. Vegetation surveys conducted during site characterization will inform plot selection. Initially, all Tower Plots will automatically be considered for litter sampling and then accepted according to the following criteria:

- ) 1 or more individuals with stem diameter ≥ 10 cm or;
- ) 10 or more individuals with stem diameter ≥ 5 cm

Plots that do not meet these criteria are not utilized for litter sampling. Guidance on whether litter sampling is expected will be provided by NEON Science based on analyses of data from vegetation characterization. Litter traps may be added if vegetation within a plot graduates to the size classes listed above; data from vegetation structure will be used to inform this decision.

### 3.5 Elevated traps

An elevated mesh litterfall trap (70.7 cm x 70.7 cm x 80 cm; 0.5 m<sup>2</sup>, 0.8 m tall) will be placed at a random location within each accepted plot/subplot, with trap locations selected from the herbaceous clip harvest list. Once set, traps will remain in the same location within the plot for sampling in

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subsequent years unless traps are removed for optimization. These traps will reliably sample shed leaves, needles, reproductive parts, and fine woody debris with butt-end diameter < 2 cm *and* length < 50 cm. Traps will be sampled according to the guidelines outlined in section 7 of this document.

Deciduous forests will be sampled once in the spring, then once every two weeks during leaf senescence. Evergreen systems including coniferous, xeric and tropical forests will be sampled year-round; the ideal sampling interval is every 4 weeks but may be extended to 8 weeks if dictated by logistical constraints. Sites with both deciduous and evergreen vegetation will be sampled according to a hybrid approach, monthly sampling with increased frequency during senescence.

In mixed woodland and grassland ecosystems (e.g. Domain 15 Onaqui, Domain 17 San Joaquin), woody vegetation cover is frequently patchy. As such, randomly placed litter traps are unlikely to adequately capture litter dynamics from woody vegetation. In this case, NEON will target litter trap placement to randomly selected areas of the plot with woody cover, and then use remote sensing imagery from NEON’s Airborne Observation Platform (AOP) to estimate woody vegetation percent cover of the plot to scale litter production from the trap to the plot level. Scaling of this data will occur as part of the preparation of data products and is not expressly part of this protocol.

### 3.6 Ground traps

Ground traps for collecting large leaves, fronds, and fine woody debris with butt-end diameter < 2 cm and length > 50 cm, will be randomly located in plots at least 2 meters from elevated traps, consistent with Muller-Landau and Wright (2010). To avoid interfering with other sampling within the plot, the basic ground trap sampling unit will be one randomly selected 0.5 m x 3 m herbaceous clip harvest grid cell within the same plot or subplot as the elevated trap (Figure 3, SOP B). Ground traps are cleared of all litter > 50 cm in length and < 2 cm diameter, one year prior to the onset of sampling so that any litter within the selected area can be assumed to be the result of annual production. Only portions of large fronds or long sections of fine woody debris that lie inside the ground traps will be sampled; these sample locations will not move from year to year and will be excluded from consideration as locations for herbaceous clip harvest.

## 4 SAMPLING SCHEDULE

### 4.1 Sampling Frequency and Timing

The primary objective is to generate annual or per growing season estimates of litterfall and fine woody debris production within the dominant vegetation type (i.e. within Tower plots).

Material left uncollected in the field for longer than the specified sampling interval may be subject to granivory by small mammals, herbivory by insects, or increased decomposition and resulting loss of mass. In deciduous forests, elevated traps must be checked at least every two weeks during leaf senescence, as traps may fill in relatively short periods. Collection of litter during leaf senescence may

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occur at intervals less than two weeks if litter volume is high and sufficient resources exist to support additional sampling; this is left to the discretion of the Domain manager and will not be dictated by NEON Science.

#### 4.1.1 Elevated traps

In Tower Plots dominated by deciduous vegetation with pronounced annual senescence, elevated litter traps will be sampled in the spring to account for winter production of fine woody debris, followed by biweekly sampling during the period of autumn senescence (Bernier et al. 2008). In systems dominated by plants that bear multi-year leaves or needles (e.g. D17 San Joaquin and D04 Guanica), elevated traps will be sampled throughout the year. Mixed forests, forests with both evergreen and deciduous species present, will be sampled according to a hybrid approach; sampling should occur once a month with increased, bi-weekly sampling during senescence. If the hybrid approach is selected, monthly sampling may be extended to the maximum 8-week sampling interval as needed to account for additional sampling bouts during senescence.

Litterfall in coniferous forests (e.g. D10 Rocky Mountain Park and D16 Wind River) or in xeric shrub systems (e.g. D14 Santa Rita and Jornada LTER) may be sampled with less frequency than deciduous broadleaf forests, but since there is no clear ‘litterfall season’ sampling will occur year round. NEON will sample litterfall in arid desert systems on a monthly basis (Table 2).

Once a month sampling is preferred; however, sampling frequency at coniferous, xeric, tropical or mixed forest sites may be reduced to once every 8-weeks if dictated by logistical constraints.

**Table 2.** Sample timing and frequency by vegetation type

Climate / Ecosystem	When to sample elevated traps
Temperate Deciduous	<ul style="list-style-type: none"> <li>) Once in the spring, ± 2 weeks of the calendar date spring sampling occurred in the preceding year</li> <li>) Every two weeks during leaf senescence period</li> </ul>
Coniferous / Evergreen / Tropical	<ul style="list-style-type: none"> <li>) Once a month*, all year</li> </ul>
Arid shrub	<ul style="list-style-type: none"> <li>) Once a month*, all year</li> </ul>
Mixed Deciduous/Evergreen	<ul style="list-style-type: none"> <li>) Once a month*</li> <li>) Every two weeks during leaf senescence period</li> </ul>

\* A 4 week sampling interval is ideal for purposes of data quality but may be decreased to once every 8 weeks if dictated by logistical constraints.

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#### **4.1.2 Ground traps**

Ground traps will be cleared and established during the initial trap deployment phase and will be sampled once annually ( $\pm 2$  weeks). Ground traps will be placed in Tower Plots only, and will remain in the same location unless moved to a new location or removed for logistical reasons.

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

The Elevated trap sampling schedule will vary depending on the vegetation present at a site (Table 2). Ground litter trap sampling will occur once a year, preferably during the dormant season, and should occur within  $\pm 2$  weeks of the date on which sampling occurred the previous calendar year. Initiation of 2 week sampling intervals during leaf senescence may be determined by checking an elevated trap from a plot near the Tower (as convenient, in the course of other scheduled sampling); once litter material from senesced falling leaves begins to accumulate in the trap, begin late season sampling.

#### **4.3 Timing for Laboratory Processing and Analysis**

Samples should be sorted and placed in the drying oven as soon as logistically feasible upon return to the domain lab to minimize loss of mass.

In dry environments, once samples are oven dry, they may be placed in temporary storage prior to weighing. However, in humid environments there is a tendency for dried samples to reabsorb water, so samples should be weighed soon after removal from the drying oven; if immediate weighing is not possible and samples must be stored, return samples to the drying oven for an additional 24 hours prior to weighing. For samples from collection events selected for chemical analysis and bioarchive there are no scientific limits on the time oven-dried samples may be placed in temporary storage prior to grinding, and subsampling for chemical analysis and bioarchive, however, samples stored prior to grinding must be dried an additional 24 hrs in the drying oven before grinding.

##### **4.3.1 Processing Samples for Bioarchive and Leaf Chemistry**

Processing of litter material for bioarchive and leaf chemistry will be completed as part of a suite of synchronized TOS measurements aimed at characterizing plant and soil biogeochemical dynamics. This includes the ‘biogeochemistry’ components of TOS Protocols Canopy Foliage Sampling (RD[14]) and Soil Biogeochemical and Microbial Sampling, including N Transformations (RD[07]), as well as TOS Protocol: Core Sampling for Plant Belowground Biomass (RD[15]). Co-execution of these protocols at a given site in the same year is a high priority.

Dried samples of leaf and needle material from elevated traps collected during a single collection bout are processed and sent to an external lab for leaf chemistry isotope analysis and bioarchive once every five years. These samples are shipped according to the process outlined in SOP G.

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In coniferous evergreen or broadleaf evergreen dominated systems, a sample is collected for archive from the October collection event. In deciduous and mixed forest systems, a sample from the period of peak senescence will be sent for additional analyses, the collection date varies based on phenology and therefore differs from site to site and from year to year.



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#### 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 3.** Contingent decisions

Delay/Situation	Action	Outcome for Data Products
Hours	If delay prevents completion of litter collection from a single trap, resume collection as soon as possible.	No adverse outcome
	If delay occurs between plots, resume litter trap collection as soon as possible.	
1-7 days	If delay prevents completion of litter collection from a single trap: <ol style="list-style-type: none"> <li>1. Store already collected litter in a cooler/refrigerator (acceptable), or sort and oven-dry as per protocol (best),</li> <li>2. Resume collection of litter trap ASAP with new labeled bags</li> <li>3. Combine dried biomass per functional group for weighing when all biomass is dry.</li> </ol>	No adverse outcome
	If delay occurs between litter traps, resume collection of remaining litter traps as soon as possible.	
8-13 days or longer	If all traps are not collected in a single bout, prioritize collection of litter from missed traps at the subsequent bout	Some litter mass may be lost from traps, increasing uncertainty in biomass and ANPP estimates.

Within a given year or growing season, Metcalfe et al. (2008) point out that litterfall collection efforts often have high levels of uncertainty and require greater sample size to accurately estimate annual production than other biomass pools. Additional traps may be installed following NEON Science review of data from initial collection events (see AD[05] for details).

If it is apparent that the volume of biomass collected from elevated and ground litter traps is too great to efficiently dry and process given limited drying oven space in the NEON domain laboratories, trap size or number may be reduced if justified based on sample optimization analysis conducted by NEON Science.

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#### 4.5 Criteria for Permanent Reallocation of Sampling Within a Site

Litterfall and fine woody debris sampling will occur on the schedule described above at 20-30 plots (all Tower) per qualifying site. Ideally, sampling will occur at these sampling locations for the lifetime of the Observatory (core sites) or the duration of the site’s affiliation with the NEON project (relocatable sites). However, circumstances may arise requiring that sampling within a site be shifted from one particular location to another. In general, sampling is considered to be compromised when sampling at a location becomes so limited that data quality is significantly reduced. If sampling at a given plot becomes compromised, a problem ticket should be submitted by Field Operations to Science.

There are two main pathways by which sampling can be compromised. Sampling locations can become inappropriately suited to answer meaningful biological questions (e.g., a terrestrial sampling plot becomes permanently flooded, or wildlife routinely disturb sampling equipment such that samples cannot be collected). Alternatively, sampling locations may be located in areas that are logistically impossible to sample on a schedule that that is biologically meaningful.

A given plot must be sampled at least 50% of the bouts expected over a two-year period (i.e., a minimum of 6 bouts per year, covering multiple seasons). Plots that cannot be sampled on this schedule should be considered compromised.

### 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 the 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.

A laser rangefinder/hypsometer/compass instrument is used to locate randomly assigned trap locations. Safety considerations for this instrument include:

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

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Pipe glue used to attach PVC legs to the elevated trap is highly flammable and may cause skin and eye irritation. Vapors are also potentially dangerous if inhaled. Technicians using glue should familiarize themselves with the hazards associated with this product (refer to the SDS), and with proper handling techniques.

Personnel assigned the task of constructing elevated traps shall complete Hand and Power Tool Safety Training and Machine Shop Safety (available on the Safety page of the NEON intranet) if cutting of PVC for construction is necessary, or if wood traps will be used instead of PVC. Personnel shall be trained in the safe use, maintenance and cleaning of the Wiley® Mill or equivalent. *Toxicodendron* spp. (i.e. poison ivy, poison oak and poison sumac) are common and may cause skin rashes on susceptible individuals. The best defense is the use of clothing that covers the body with long pants and long-sleeved shirts and application of over-the-counter products for exposure to urushiol oils. Refer to NEON Operations Field Safety and Security Manual AD[02] Section 7.1 and to Appendix H and/or RD[12] for safe handling instructions.

Heavy work gloves are recommended when collecting litter from ground traps or any time when sorting through litter where unseen hazards (e.g. spines, *Toxicodendron*, snakes, spiders) may be present.

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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 4.** Equipment list – Initial trap deployment, SOP B

Item No.	R/S	Description	Purpose	Quantity*	Special Handling
<b>Durable Items</b>					
	R	Non-oxidizable metal rods (e.g. aluminum, galvanized stainless steel, or equivalent) ~1 m length	Anchor trap to sampling location	4 per trap	N
0343220000	S	Aluminum stake	Mark corners of ground traps	4 per trap	N
MX104361	R	Chaining pins or other suitable anchor	Anchor measuring tapes	2	N
	S	Coin	Randomize selection of patches at sites with targeted selection	1	N
MX100320	S	Compass with mirror and declination adjustment	Locate X, Y coordinates of within-plot trap location; alternative to high-accuracy laser rangefinder (with less precise rangefinder)	1	N

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Item No.	R/S	Description	Purpose	Quantity*	Special Handling
EG07670000	R	Elevated litter trap assembly	Collect litter sample	40-50	N
MX103218	R	Foliage filter	Allow laser rangefinder use in dense vegetation	2	N
MX100322	R	Laser Rangefinder, ½ foot accuracy	Locate X, Y coordinates of within-plot trap location	1	N
MX104742	S	Laser Rangefinder, 1 yard accuracy	Measure distances. May be used, in conjunction with handheld compass, as an alternative to TruPulse	1	N
MX100722	R	Measuring tape, minimum 30 m	Locate clip-harvest strips within plots/subplots. Plot slope < 10 deg; grassland, savannah	1	N
MX103491	R	PVC pipe cutter	Cut PVC to length	1	N
MX110540	R	Torpedo bubble level	Check the angle of the elevated trap	1	N
MX103238	S	White reflector or reflective tape	Reflective target for laser rangefinder; aids in measuring distance to target accurately	1	N
<b>Consumable items</b>					
MX104908	R	CR123A battery	Spare battery for laser rangefinder	2	N
	S	PVC pipe glue	Permanently attach PVC from the elevated trap kits	1 jar	N

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Item No.	R/S	Description	Purpose	Quantity*	Special Handling
MX105416 (Blue) MX105397 (orange)	R	Survey marking flag, PVC or fiberglass stake	Delineate sampling area	4 per trap	N
<b>Resources</b>					
RD[05]	R	Datasheets for Litterfall and Fine Woody Debris	Record required data and metadata	Variable	N
	R	Per plot or subplot Clip Lists	Identify random clip-strip locations		N
	S	Random number list	Randomize selection of patches at sites with targeted selection	1	N

\* Adjust quantities as needed to accommodate site specific conditions  
R/S=Required/Suggested

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**Table 5.** Equipment list – Field sampling elevated and ground litter traps, SOP C

Item No.	R/S	Description	Purpose	Quantity*	Special Handling
<b>Durable Items</b>					
MX103524	R	Nylon rope	Delineate ground trap	1, 8 m	N
0343220000	S	Aluminum stake	Replace stakes on damaged ground traps	4	N
MX110542	R	Cotton bags, uniquely numbered <sup>1</sup>	Carry fresh, potentially wet, litter samples	2 per trap pair	N
EG07670000	R	Elevated litter trap assembly	Replace damaged traps	2	N
033674001	R	80 cm long, 0.5 in diameter PVC pipe	Replace damaged elevated trap leg pieces	As needed	N
0336740002	R	69.5 cm long, 0.5 in diameter PVC pipe	Replace damaged elevated trap frame pieces	As needed	N
0336750000	R	PVC right angle out, 3-way elbow, 0.5 in	Replace damaged elevated trap corner pieces	As needed	N
0336760001	R	122 cm x 122 cm, 1mm polyester window screen	Replace or repair damaged elevated trap screen	As needed	N
0345630000	R	6 in long, UV resistant zip-ties	Replace damaged elevated trap zip ties	As needed	N

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Item No.	R/S	Description	Purpose	Quantity*	Special Handling
0339860000	R	PVC slip coupling	Replace damaged elevated trap leg spacers	As needed	N
MX103218	R	Foliage filter	Allow laser rangefinder use in dense vegetation	2	N
MX109491	R	Handheld caliper, 0.1 cm precision	Measure branch diameters	1	N
MX100322	R	Laser Rangefinder, ½ foot accuracy	Locate X, Y coordinates of trap if thick brush prevents visual trap location in Thick brush	1	N
MX100497	S	Measuring stick, 1 m <sup>2</sup>	Measure and identify/discard litter > 50 cm	1	N
MX110541	S	Pruning lopper, heavy duty	Cut branches up to 2 cm diameter	1	N
MX110540	R	Torpedo bubble level	Check the angle of the elevated trap	1	N
MX109755	S	Flush cut clippers	Cutting screen material or zip ties	1	N
	S	Screen patch kit (pieces of 1 mm screen, wire, repair tape, wirecutters)	Repair minor holes in screen material	1	N
<b>Consumable items</b>					
MX104908	S	CR123A battery	Spare battery for laser rangefinder	2	N



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Item No.	R/S	Description	Purpose	Quantity*	Special Handling
MX104502	R	General Purpose Tags, may use rite in-the-rain	Label collection bags	2 per trap pair	N
<b>Resources</b>					
RD[05]	R	Datasheets for Litterfall and Fine Woody Debris	Record required data and metadata	Variable	N

\* Adjust quantities as needed to accommodate site specific conditions

<sup>1</sup> recommended size ~ pillowcase dimensions

<sup>2</sup> May also mark 50cm on plot frame with permanent marker.

R/S=Required/Suggested

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**Table 6.** Equipment list – Laboratory processing and analysis SOPs D & F

Item No.	R/S	Description	Purpose	Quantity*	Special Handling
<b>Durable Items</b>					
MX104799 MX104751	S	Paintbrushes, various sizes	For use in sorting litter	4	N
MX103932	S	Timer	Track sorting time and limit to one hour per field sample	1	N
	S	Domain specific litter sorting guide	Assist with identification of litter functional groups	1	N
MX100690 MX100689	S	Weigh boats, various sizes	For weighing sorted material	4	N
MX103237	R	Hy back pan	Receive sub-samples generated by splitter	2 per splitter	N
MX103235	R	Sample microsplitter, small capacity	Subsample from small volumes of ground sample. Relatively little litter mass per litterCode per trap	1	N
MX107196	R	Sample splitter, large capacity	Subsample from relatively large volumes of ground sample. Useful with fibrous leaves. Relatively large litter mass per litterCode per trap	1	N

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Item No.	R/S	Description	Purpose	Quantity*	Special Handling
<b>Consumable items</b>					
MX104801	R	Paper coin envelopes	Contain very small masses of sorted litter for drying	20	N
MX106814 MX102977 MX105089 MX110628 MX110629 MX103232	R	Paper bags, assorted sizes	Contain litter, sorted to functional group	50	N
MX104523	S	12 x 18 blank newsprint paper	Clean, high contrast surface for sorting	As needed	
MX101278	R	Scintillation vials with caps, 20 mL	Contain ground split samples for shipment to archive or chemical analysis	As needed	N
MX105583 (15 gal)	S	Trash bag	Contain oven-dried samples before they are weighed	Box of 100	N
MX110716	R	Ethanol wipes	Quickly clean gloves, buckets, sample splitter, etc. between samples		
<b>Resources</b>					
RD[05]	R	Datasheet Lab Drying QC	Record data	As needed	N

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<b>Item No.</b>	<b>R/S</b>	<b>Description</b>	<b>Purpose</b>	<b>Quantity*</b>	<b>Special Handling</b>
RD[05]	R	Datasheet Lab Weighing	Record data	As needed	N

\* Adjust quantities as needed to accommodate site specific conditions

R/S=Required/Suggested

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## 6.2 Training Requirements

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

Technicians must be proficient in the use of handheld GPS units in order to successfully navigate to plots for sampling.

## 6.3 Specialized Skills

The lead plant technician must possess the demonstrated ability to identify collected plant structures to functional group via visual inspection. Preferably, the technicians sorting litter are the same technicians who harvested the litter in the field.

## 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. Please note that if sampling at particular locations requires significantly more time than expected, Science may propose to move these sampling locations.

Field collection time is expected to only take a couple of minutes for each trap. The majority of time in the field will be spent travelling between plots; travel time will vary by site.

Lab processing time will depend heavily on the volume of material collected and number of functional groups present in a given collection. Sorting material prior to drying will likely take less than an hour per trap. Weighing dry material is also dependent on the sample volume but should not take more than a couple minutes per functional group per trap. Grinding, subsampling, filling and labeling vials may take 10-15 minutes per function group per trap.

## 7 STANDARD OPERATING PROCEDURES

### SOP A Preparing for Sampling

1. Print clip strip lists for the plots that will be visited. Clip lists are available on the FOPs TOS page on the NEON intranet:
  - ) Litterfall sampling locations will be selected from the plot-specific randomized lists created for herbaceous clip harvest locations (RD[06]). These lists are therefore essential for the completion of the trap deployment procedure (SOP B), and must be updated to reflect the fact that two of the clipID locations are occupied by litter traps (elevated and ground). For the purpose of this protocol, trap location and clipID are used interchangeably.
  - ) Make sure that all fields in the clip strip lists are up to date, that clip strips that have been harvested or rejected are current and indicated on the lists.
  - ) These lists will be utilized in the field regardless of selected trap placement strategy (i.e., random vs. targeted).
2. Gather all field equipment
  - a. If *Toxicodendron* is likely to be encountered, include cotton gloves and pre-weighed paper bags.
3. Number cloth collection bags, with a permanent marker, so they may be uniquely identified. This is the **bagID**.
4. Prepare GPS:
  - a. Charge batteries
  - b. Load plot locations
    - ) Defining a route to each plot prior to going to the field will enable completion of the field collection bout in the least amount of time.
5. Prepare laser rangefinder (if using).
  - a. Check battery and charge
  - b. Clean lenses with lens cloth or lens tissue (if necessary)
  - c. Check/set correct declination. See RD[11] for details.
  - d. Calibrate tilt sensor; see RD[11] for details.
  - e. Calibrate internal compass.
6. Prepare compass (if using).
  - a. Check/set correct declination. Note that declination changes with time and should be looked up annually per site: <http://www.ngdc.noaa.gov/geomag-web/>
7. Generate randomized number lists for sites with targeted selection.
8. Print datasheets (RD[05]) on all-weather paper.

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**SOP B Initial Deployment of Traps**

**B.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.

**B.2 Selecting litter trap location strategy**

Litter traps will be deployed in pairs, one elevated and one ground trap per pair. One elevated trap and one ground trap is deployed in each of two randomly selected 400 m<sup>2</sup> subplots within a 1600 m<sup>2</sup> Tower plots. In smaller, 400 m<sup>2</sup> Tower plots, only one litter trap pair is deployed. Because litter sampling will primarily occur in forested sites where plot size is typically 1600 m<sup>2</sup> or more, most plots will have at least two pairs of traps. Trap placement will utilize the clip cell grid developed for the herbaceous clip harvest protocol (RD[06]), and the random subplot selection list provided by NEON Science. Refer to the TOS Protocol and Procedure: Plot Establishment (RD[07]) for details on handling measuring tapes and plot delineation tips. Review and print Elevated Trap Assembly Instructions (RD[10]) for use in the field.

In order to enable scaling of litter production across the site, the strategy for trap placement (i.e. Targeted or Random) is consistently applied across *all plots at a site* rather than based on plot specific conditions.

- ) **Targeted selection** is utilized for patchy vegetation, where overstory species > 2 m height is present throughout < 50% of the Tower airshed sampling area.
- ) **Random selection** is employed in forested sites with > 50% canopy coverage by individuals >2 m tall

Refer to Appendix D for the recommended strategy by site; these recommendations are based on a combination of NLCD vegetation classification, satellite imagery and site characterization data. If the selected strategy/recommendation seems inappropriate (based on the criteria listed above) for a particular site given the conditions on the ground, use NEON’s problem reporting system to iterate with Science Operations about the trap placement strategy.

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**targetTaxaPresent = No**

Any site dominated by herbaceous species, where woody vegetation is infrequent and too short to be reliably sampled by elevated litter traps (i.e., < 2 m), will be excluded from consideration for implementation of this protocol. At sites where litter sampling will occur, all Tower Plots must be considered for deployment of litter traps.

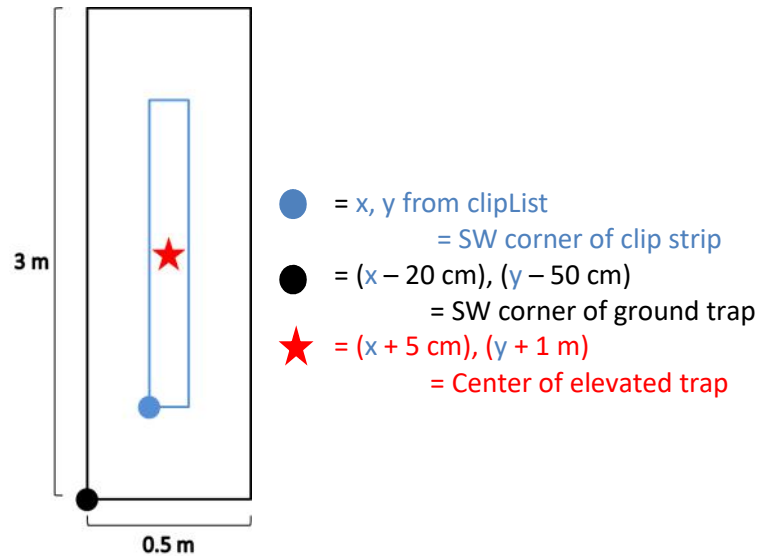
- ) If a random sampling strategy is employed and a given plot does not contain any vegetation > 2 m height
  - o Record **targetTaxaPresent = No**
  - o If **targetTaxaPresent = Yes** for the plot, but no qualifying vegetation is present in a given subplot, traps must still be deployed and sampled; **targetTaxaPresent** is a plot-level assessment.
- ) If a targeted sampling strategy is employed, a subplot or plot may be rejected if there is not sufficient woody vegetation >2 meters tall to allow for placement of both elevated and ground traps OR if all sampling locations beneath qualifying patches are within excluded sampling areas (i.e. 1 m buffer around plot edge and 1 m and 10 m diversity sampling areas).
  - o Excluded clipCells are NOT available on the provided clipLists
  - o Record **targetTaxaPresent = No** on datasheet or mobile app and continue to the next plot/subplot

**B.3 Litter trap coordinates**

Appendix G provides x, y-coordinates specific to litter trap placement but note that the clipLists posted on the NEON intranet only include coordinates for the SW corner of *clip strips* used to sample herbaceous biomass/productivity (RD[06]). If only the clipList is printed and taken to the field, technicians will have to navigate to the SW corner of the clipCell (ground traps) and the centroid of the clipCell (elevated traps) by calculating relative position based on clip strip coordinates (**Figure 1**). The step-by-step instructions provided in this SOP assume only the clipList is available.



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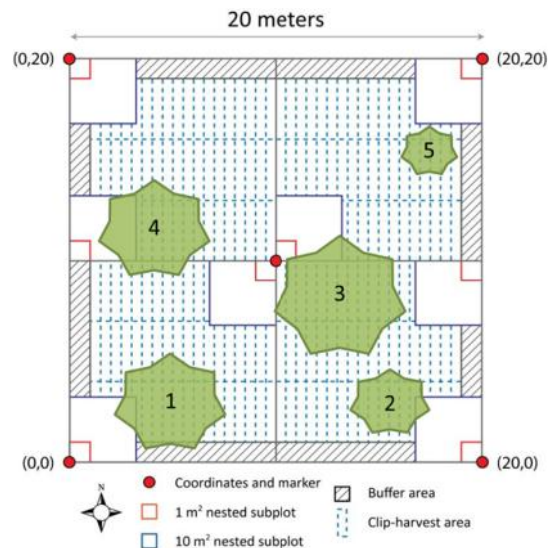
**Figure 1.**  $x, y$ -coordinates for litter trap placement (red star, black circle) differ from coordinates provided in the clipLists provided for use with the herbaceous clip protocol (blue circle) RD[06].

#### B.4 Locating targeted elevated trap location

1. Navigate to the desired plot and, if sampling in a 40 m x 40 m plot, the randomly selected subplot.
2. Assess location of patches of qualifying vegetation (>2 m tall, outside of 1 m and 10 m diversity plots) within the plot or subplot (depending on plot size). If no qualifying patches are present, record **targetTaxaPresent**=No for the plot or subplot.
3. Give each patch a numeric value. Assign values sequentially, left to right, bottom to top, beginning in the SW corner (Figure 2)
  - a. If only a single qualifying patch is available, elevated and ground traps may be placed on opposite sides of the patch (even if < 2 m apart), provided the elevated trap is not situated such that it could potentially affect large particle distribution to the ground trap.
4. Use either a random number list, a series of coin flips, or other unbiased method of selection to randomly select a patch to target for litterfall and fine woody debris sampling.
5. Once a patch is selected, select a location under the canopy, central to the patch to place an elevated litter trap.
  - a. Avoid the 1-meter buffer around the plot edge, and the 1 and 10 meter nested subplots used for diversity sampling. Clip cell coordinates are not generated for those locations.
  - b. If excluding 1 and 10 meter nested subplots removes all qualifying patches of vegetation from consideration, record **targetTaxaPresent** = 'No' and move to the next plot/subplot
6. Use the range finder to measure the distance to plot/subplot edges.
7. Determine where the nearest clip strip centroid is located.

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- a. From the selected location, measure distance to the nearest N-S plot boundary to determine the x-coordinate of this point
- b. Measure the distance to the nearest E-W plot boundary to determine the y-coordinate
- c. Use the clip cell map to identify the clip cell located closest to the selected point (Appendix G)
- d. Navigate to the centroid of that cell.
  - 1) clipStrip x-coordinate + 5 cm, clipStrip y-coordinate + 1 m
8. If practical, center trap over that point, this will minimize the number of clips that will be removed from consideration for herbaceous clip harvest.
  - ) In the example provided in Figure 2, the coordinates associated with the nearest clip strip centroid from the center of patch 4 are:  $x = 3.7$ ,  $y = 11.5$ .
  - ) Not centering the trap over a centroid is acceptable but not ideal as there will be more cells excluded from consideration for herbaceous clip harvest.
9. Place a pin flag at the selected trap location.

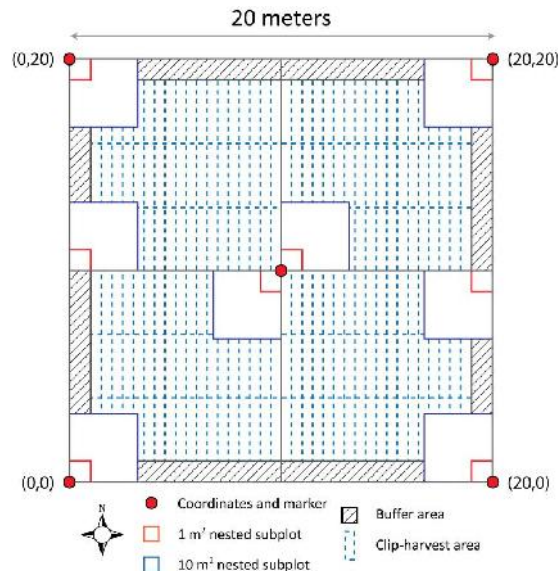


**Figure 2.** Example of numbering system for qualifying patches of vegetation within a plot

### B.5 Locating random elevated trap location

Use the plot- or subplot-specific Clip List ([plotID]\_[subplotID]\_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.

1. Navigate to the SW corner of the clip strip of the first available clipCell from the randomized list:



**Figure 3.** A 20 m x 20 m NEON plot showing the locations of 0.5 m x 3 m clip-harvest “cells” (dashed blue lines). 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. 40m x 40m plot schematic available in Appendix G

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 3), 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 laser rangefinder in **HD** mode with a reflective surface to locate the Y-coordinate.
  - ) Make sure the azimuth is 0° (True North) when shooting the laser rangefinder to find the Y-coordinate (see RD[11]).
  - ) Note: if laser rangefinder is not available, the same routine described here may be completed using a handheld compass to verify azimuth and a laser rangefinder or additional tape measure for distance.
- d. Place a pin flag at the clip-strip (X,Y) location.

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If the Y-coordinate is > 10:

- ) Run a tape East/West from the plot/subplot centroid (10,10) to either the (0,10) position or the (20,10) position (Figure 3) *Note: in 40 m x 40 m plots, subplot centroids may not permanently marked:*

X-Coordinate	Tape Layout <sup>1</sup>
1 < X < 10	From (10,10) to (0,10) <sup>1</sup>
10 < X < 20	From (10,10) to (20,10) <sup>1</sup>

<sup>1</sup> Use the laser rangefinder 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 laser rangefinder in **HD** mode with a reflective surface to locate the Y-coordinate.
- ) Make sure the azimuth is 0° (True North) when shooting the laser rangefinder to find the Y-coordinate (see RD[11]).

*Note: if laser rangefinder is not available, the same routine described here may be completed using a handheld compass to verify azimuth and a laser rangefinder or additional tape measure for distance.*

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

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- ) Use the laser rangefinder in **HD** mode to place the initial pin flags if the plot slope is > 20 %, or there is significant brush or obstacles that prevent accurately stretching a tape.
  - ) Plot slope can be quickly estimated using the inclinometer in the laser rangefinder (**INC** mode) or the inclinometer on the handheld compass.
- 

2. Assess the suitability of the clipCell for an elevated litter trap:
  - ) Accept the cell if no obstacles are present that prevent trap placement and anchoring (e.g. large shallow rock covering a majority of the clip cell, large boulders, impermeable vegetation, or low lying fallen trees that divert litter away from the trap location).
  - ) Reject trap location if the selected strip is within 2 meters of an LAI sampling point.
  - ) If the strip is not acceptable for placement of an elevated litter trap, move to the next strip on the list but do NOT record the strip status as rejected for herbaceous biomass sampling.
  
3. Navigate to center of the cell (clipStrip x-coordinate + 5 cm, clipStrip y-coordinate + 1 m, **Figure 1**), place a pin flag. Elevated traps will be centered over this point.
  - ) If the trap cannot be anchored over the clipCell centroid, the trap may be shifted up to 1 meter North or South.

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- ) Record a '1' in the **Status** column of Clip List sheet for clip-strip selected, Record the litter trap deployment date in the **Date** field, add a note that cell was used for litter collection.

## B.6 Locating ground trap clip strip

Ground traps will be placed across one entire clipCell and may not be placed such that more than one cell per 400 m<sup>2</sup> is occupied by a ground trap.

- ) **Targeted selection** – repeat the process described in B.5 for randomly selecting a patch in which to locate the ground trap. Do not exclude the patch selected for the elevated trap from consideration. If the same patch selected for elevated trap placement is chosen, according to randomized procedure, place ground trap on the opposite side of the selected patch or > 2 meters from the elevated trap such that the elevated trap cannot re-direct litter particles toward or away from the ground trap.
  - ) **Random selection** – continue using the randomized clip strip locations in sequential order as described in B.5; assess the suitability of the next potential clip-strip location that has not previously been sampled or rejected.
  - ) Reject the trap location if the selected strip is < 2 meters from the elevated trap or if conditions prevent placement of stakes in all four corners of the selected clip cell
1. Navigate to the SW corner of the selected cell (clipStrip x-coordinate – 0.2 m, clipStrip y-coordinate – 0.5 m, **Figure 1**), place a pin flag.
  2. Delineate the 3 m x 0.5 m clip cell that will be used for the ground trap using meter tape and compass or laser rangefinder to ensure that the trap is oriented to the cardinal directions.
  3. Hammer in brightly colored or aluminum stakes in each of the four corners leaving ~20cm visible above ground.
    - a. At sites/plots with shallow soil or high presence of rocks that preclude placement of stakes, mark the clipCell in an alternative appropriate method that is acceptable to the site host. Plots cannot be rejected from ground trap placement due to the presence of rocks.
  4. Remove all large leaves, large fronds, and all fine woody debris > 50 cm length and < 2 cm diameter from within the ground trap area.
    - ) It is not necessary to remove small leaves, fronds, etc. that are normally sampled with the elevated litter traps.

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## B.7 Elevated trap construction and installation

1. Center square trap frame over pin flag placed in the center of the selected clip strip cell
2. Mark plot corners with pin flags
  - ) The trap frame is 70.7 cm wide, since a clip strip cell is 50 cm wide, trap legs will be anchored 10 cm into the adjoining cells on either side of the selected cell
3. Hammer non-oxidizable metal stakes into ground at the pin flag locations to anchor trap legs, leaving 50 cm above ground
4. Attach trap legs to square frame
  - a. Legs may optionally be glued in place.
5. Cut the trap legs so that, once installed, the square frame is level (use bubble level to check), approximately 0.8 m above the ground.
  - a. Do not reject trap location if woody vegetation will be located beneath the trap, provided vegetation does not affect the shape/sag of the litter trap mesh.
  - b. If possible, do not manipulate existing vegetation, though some clipping of branches is allowed at sites with continuous mid-level vegetation where a suitable location would otherwise not be available.
6. Slide trap legs over stakes.
7. Use permanent marker and meter stick to draw 50 cm line along one side of the trap frame. This will be used during collection bouts to assess qualifying material.
8. Attach screen to square frame with the provided zip ties (Figure 4).
  - ) The pre-cut screen is larger than the trap area and should not be taut across the trap, a minimum of 20 cm difference between the plane of the trap frame to the lowest point in the mesh is ideal to prevent litterfall from blowing away. >20 cm sag may be employed as necessary to accommodate high litter production sites (e.g., deciduous forest).
9. Write **trapID** (clipID) and **subplotID** on one leg of elevated trap.
8. If trap is ready to begin collecting litter material, record **addDate** as the **setDate** for the first collection bout.

## B.8 Record data about trap deployment

When this protocol is implemented at a site, EVERY Tower Plot must be assessed for presence of qualifying vegetation.

1. For each plot record:
  - ) **addDate**: date of initial deployment
  - ) **plotID, subplotID, clipID**: location information
  - ) **targetTaxaPresent: Yes/No**. Does the plot contain vegetation that qualifies for inclusion in litter sampling?
  - ) **trapType**: elevated or ground

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- ) **trapSize:** 0.5 if trapType = elevated ; 1.5 if trapType = ground
- ) **trapPlacement:** random or targeted (this must be the same for all Tower Plots at a site and will be pre-populated on mobile app)
- ) **Remarks:** Free text entry about trap deployment



**Figure 4.** Fully constructed elevated litter trap.

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## SOP C Field Sampling

### C.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.

### C.2 Litter collection – Elevated traps

1. Navigate to plot.
2. Assess and record the **trapCondition** (Table 7).

**Table 7.** Prescribed trapCondition codes for paper datasheets

Code	Description
OK	Litter collected - Trap in good shape, no issues
TE	Litter not collected – Trap empty
HO	Litter not collected - Holes large enough for leaves to pass through. Holes near the base of the screen (the lowest hanging point) are worse than holes on the side of the screen.
TB	Litter not collected – trap blocked. Large branches or leaves (especially palm fronds) present in the trap which may have prevented trap from collecting litter or diverted falling litter away from the trap
TT	Litter not collected – trap tilted 10° (use clinometer on compass to measure)
RE	Litter not collected – trap broken
TS	Litter not collected, not discarded – trap skipped
PF	Litter collected – Trap previously flooded

3. If the trap is not in good condition, discard the litter within and around the trap footprint, then make necessary repairs. Broken traps should be replaced immediately if possible.
  - ) A damaged trap must be replaced or repaired within one week if repair/replacement is not possible at the time of collection. Record the date on which trap was repaired/replaced and reset as the **setDate** for the next collection bout.
  - ) *Note.* There is no defined threshold for when litter should be discarded from traps with holes (HO). As the size and location of holes in the mesh that may allow material to be lost will vary based on the dominant vegetation at a site, it is at the discretion of the technician collecting litter to determine if the sample should be discarded due to the presence of holes. If it is likely that < 5% of mass has been



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lost through the holes, material may still be collected with a trapCondition code = 'OK'. Holes should still be repaired.

4. If the trap is in good condition (OK) continue with collection procedure.
5. Discard litter > 50 cm in length, this material is not reliably collected in the elevated traps and will be sampled in ground traps
6. All woody material > 2 cm diameter will be measured according to the Coarse Downed Wood (CDW) protocol. Use calipers to measure diameter of woody branches
  - a. Discard branches > 2 cm at narrowest point
  - b. For branches that taper to 2 cm, cut off and discard the portion > 2 cm diameter; drop discarded portion of branches haphazardly (i.e. do not group or stack discarded material) beside the elevated litter trap
7. Transfer all other material, including parts hanging out of the trap, into the cloth bag designated for elevated trap litter
8. Create label with clipID, date, trap type, and technician name (Figure 5), and attach to bag or place inside bag prior to collecting material
  - a. If material from a single trap does not fit in a single cloth collection bag, create a duplicate tag for the second bag and add "1 of 2", "2 of 2" to each tag. Record additional **bagID** in the remarks column of the datasheet or mobile app and pool contents of each bag for sorting, drying and weighing.

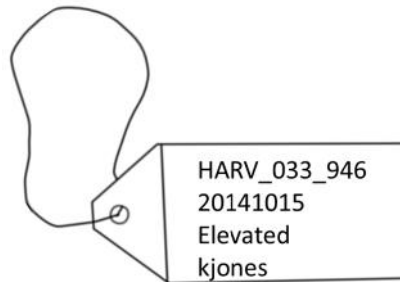


Figure 5. Example field collection label

9. Knot cloth bag to prevent material from falling out while in transport, do not use draw strings if present on bags
10. Using the 'SOP C: Field Sampling' datasheet, or tablet, record:
  - ) **measuredBy/recordedBy**
  - ) **setDate:** (pre-populated on PDA) the date the trap was set/reset, if trapCondition = OK then setDate=previous collectDate, else, date that damaged trap was replaced /repaired and reset.
  - ) **boutNumber:** optional field, used for internal work tracking.
  - ) **collectDate:** use YYYYMMDD format.
  - ) **collectTime:** 24hr hh:mm format.
  - ) **plotID:** xxxx\_### - assigned by NEON Science.

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- ) **subplotID**: see Appendix F for a plot map
  - ) **clipID** (aka **trapID**): unique identifier for trap location within the plot (format: plotID + ‘\_’ + clipCellNumber ex: OSBS\_033\_145)
  - ) **trapType**: Elevated
  - ) **trapCondition**: Table 7
  - ) **bagID**: transcribe from cloth bag, this is a unique number, written on the individual bag. In the event that a tag is lost, metadata can be recovered based on this value.
  - ) **trapMoved**: Yes, No (if ‘yes’, enter new **clipID** on ‘trap deployment’ datasheet or pertrap form on mobile app)
  - ) **trapReset**: Yes, No
11. Record remarks if necessary

### C.3 Litter collection – Ground traps

1. Locate stakes marking ground trap location
2. Assess and record **trapCondition** (Table 8)

**Table 8.** Modified trapCondition codes for ground traps

Code	Description
OK	Litter collected –Trap in good shape, no issues
TB	Litter not collected – trap blocked. Large branches or tree > 10 cm diameter have fallen over trap which may have diverted falling litter away from the trap
PF	Litter collected – Trap previously flooded

- ) If trap condition is blocked (code = TB), do not collect. If obstruction cannot be cleared, move ground trap to a new location from the clip strip list using either the random or targeted approach described in SOP B.
  - ) Record new **clipID** on the SOP B: initial deployment
  - ) Clear all litter from the new clip cell.
  - ) Do not collect
3. Wrap nylon cord around the four staked corners of the ground trap, delineating the trap edges.
  4. Identify qualifying litter, including all litter, (e.g. leaves, rachi, leaves, twigs) which is:
    - ) > 50 cm length and
    - ) < 2 cm diameter (averaged between major and minor axes if elliptical) and
    - ) < 2 m from soil surface (suspended litter, caught in overhanging vegetation, if within the 0.5 m x 3 m clip cell, qualifies)
  5. Cut off and discard portions of qualifying litter which extend beyond trap edges, retaining only the portion which lies within trap perimeter, even if the retained portion is < 50 cm in length.

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6. Cut off and discard portions of woody branches > 2 cm diameter
7. Collect all remaining qualifying litter from within the ground trap, transfer material to a uniquely numbered cloth bag
  - ) Pieces may be cut to smaller lengths if they are too long to fit in the cloth collection bags
8. Move remaining litter that is too large to qualify for collection outside of the ground trap boundaries to avoid re-assessing in future collection bouts
9. Create a label with clipID, collectDate, trapType, technician name (Figure 5), and attach to bag.
10. Knot cloth bag to prevent material from falling out while in transport, do not use draw strings if present on bags
11. Using the ‘SOP C: Field Sampling’ datasheet or tablet (PDA) record:
  - ) **measuredBy/recordedBy**
  - ) **collectDate:** use YYYYMMDD format
  - ) **plotID:** xxxx\_### - assigned by NEON Science
  - ) **subplotID:** see Appendix F for a plot map
  - ) **clipID (aka trapID):** unique identifier for trap location within the plot
  - ) **trapType:** Ground
  - ) **trapSize:** 0.5 if trapType = elevated ; 1.5 if trapType = ground
  - ) **trapCondition:** Table 7
  - ) **bagID:** transcribe from cloth bag, this is a unique number, written on the individual bag. In the event that a tag is lost, metadata can be recovered based on this value.
  - ) **trapMoved:** Yes, No (if ‘yes’, enter new location on ‘trap deployment’ datasheet or pertrap mobile app)
12. Record **remarks** if necessary

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**SOP D Laboratory Processing for Dry Mass Measurement**

**D.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.

**D.2 Sorting, drying and weighing litter samples**

- ) If litter and bags are very wet (i.e. dripping), hang bags to air dry before further processing.
- ) If transfer of arthropods or gastropods between sites is a concern, freeze collection bags prior to sorting
- ) If sorting immediately following collection is not possible, store samples in refrigerator to slow decomposition

1. Sort litter from each Elevated and Ground bag per trap pair to litter functional group.
  - a. Clear adequate bench space in the laboratory.
  - b. Empty the cloth bag filled with litter onto the bench, and sort litter pieces to the functional groups in Table 9 (Elevated trap collection bags) or Table 10 (Ground trap collection bags).



*Note: it may be useful to create a domain specific litter sorting guide to help streamline identification of litter material and overall sorting time.*

- c. Clean off any dirt attached to litter from ground traps.
  - ) If the bout will be processed for bioarchive and leaf chemistry analyses, or there is a chance it may be used for bioarchive and leaf chemistry, wear nitrile or latex-free gloves while sorting. This will prevent contamination of leaves from sweat and oils. Gloves may be re-used between samples.
- d. Cut or break any large seeds (i.e. dime size or greater) into smaller section. The primary goal is to break the seed coat to allow water to escape in the drying process; if seeds cannot be cut all the way through, partial cuts are acceptable for this purpose.

**Table 9.** Elevated trap litter functional group codes (for use on paper data sheets, data entry application will have the full functional group name)

Code	functionalGroup - Description
ELVS	Leaves (including petioles, rachis and non-woody tendrils)
ENDL	Needles from coniferous species

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Code	functionalGroup - Description
ETWI	Twigs/branches < 2 cm diameter <i>and</i> < 50 cm length
EWDY	Woody material (e.g. bark, seed cones, etc.)
ESDS	Seeds (including fruit and attached structures)
EFLR	Flowers (including pedicels and pollen cones)
EOTR	Other (lichen, mosses, frass, unidentifiable material, etc.)
EMXT*	Mixed, unsorted, all litter functional groups included

\* Use only if 1 hour sorting limit is reached, or if only tiny fragments are left to sort and material < 1% of total mass, or if directed by Science.

**Table 10.** Ground trap litter functional group codes (for use on paper data sheets, data entry application will have the full functional group name)

Code	functionalGroup - Description
GLVS	Leaves and needles > 50 cm length (including petioles, rachis and non-woody tendrils)
GTWI	Twigs/branches < 2 cm diameter <i>and</i> > 50 cm length
GMXT*	Mixed, unsorted, all litter functional groups included

\* Use only if 1 hour sorting limit is reached, or if directed by Science.

e. Label paper bags to hold sorted litter functional groups from each trap. Include sampling information from tag on cloth bag, as well as the appropriate **litterCode**. Choose either 8# or 25# kraft bags, or smaller, depending on the quantity of litter.

f. Tips for efficient sorting:

- ) Do not spend more than 1 hour sorting material from a given elevated trap.
- ) Sort largest, most easily identifiable material (i.e. cones, bark, twigs) first - this should account for the majority of the biomass
- ) Work your way down to increasingly more difficult to identify material
- ) Do not spend extra time sorting material that represents <1% of total mass even if the hour threshold has not yet been reached.
- ) If you reach an hour, group all remaining material into 'mixed, unsorted' and move on.

2. Label the **ovenStartTime** (24 hr time, e.g. 1645 for 4:45 pm) and **ovenStartDate** (YYYYMMDD) that bags are placed in the drying oven on the back side of the tag.

a. Place all bags from a given clipID or collectDate in the drying oven at the same time.

b. **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. Additionally, organizing the oven by grouping samples from a given day in the same area will streamline the re-measurement process; samples may be located and removed for weighing without requiring a complete unloading of the contents of the oven.



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A custom stamp with blank fields for all required information may facilitate consistent labeling of bags and organization of samples in drying ovens.

3. Record the number of bags and the specific litterCodes present for each clipID on the “Sorting QC Datasheet”.
4. Place bags of litter (excluding ETWI, EWDY and GTWI) in a drying oven set to 65°C for 48h – 120h (2d – 5d), until constant mass is attained.
5. Check the drying progress of litter bags using the generalized “Drying Datasheet” available on the NEON intranet.
  - a. Check the weight of the same subset of n=10 bags per date 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,  $\pm 0.05$  g or 1%, whichever is greater)
  - d. Once constant mass is achieved, remove bags from oven, label bags with the **ovenOutDate** and **ovenOutTime**.
6. Place bags of ETWI, EWDY, lignified ESDS, and GTWI litter in a drying oven set to 105° C for 24-72 hours, until constant mass is attained. If multiple drying ovens are available, steps 5-6 and 8-9 may occur simultaneously, otherwise, complete drying of litter material at 65° C before increasing the temperature to dry lignified tissue. Woody material requires higher drying temperatures to release bound water.
7. Check the drying progress of litter bags using the “Lab Drying QC” datasheet.generalized “Drying Datasheet” available on the NEON intranet.
  - a. Check the weight of the same subset of n=10 bags per **collectDate** 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,  $\pm 0.05$ g or 1%, whichever is greater)
  - d. Once constant mass is achieved, remove bags from oven, label bags with the **ovenOutDate** and **ovenOutTime**.
8. Weigh material from each functional group (i.e. **litterCode**) with a mass balance (0.01 g minimum accuracy).
  - ) Weigh dried plant material immediately after removing from the drying oven, as it will absorb moisture from the air if left in ambient room conditions (particularly in humid environments). If practical to do so, remove bags from the oven and weigh one at a time.
  - ) If material cannot be weighed immediately, store sorted material in labeled paper bags (8# or 25# kraft bags, or similar), inside a larger, sealed, plastic bag (e.g. a black plastic garbage bag or equivalent).

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- ) If necessary, dried samples may also be stored for up to 30 days in ambient room conditions prior to weighing. Samples treated in this manner must be returned to the drying oven for 24 h prior to weighing.
    - ) The data entry application will provide a constrained list of field samples based on data uploaded from the mobile app. Only trap + date combinations from which samples were collected (i.e. **trapCondition** = OK or PF) will be available for **dryMass** data entry.
  - b. Record **dryMass** = '0' for all functional groups, except Mixed, not present in the sample. The mixed functional group only requires a value if this category was used.
    - ) If there is no parent sample (i.e. if a trap was not collected), no entry should occur for **dryMass** for any **functionalGroup**. Do not enter '0' for traps for which samples were not collected.
  - c. Record the **litterMass** to the nearest 0.01 g. For large volumes of biomass that do not readily fit into a large weigh boat, use any of the following strategies:
    - ) Crush or chop the biomass to reduce volume so it will fit into a weigh boat.
    - ) Use an HDPE tray, 'larval tray' plastic box lid (or equivalent) instead of a weigh boat.
    - ) Avoid splitting biomass into subgroups for weighing as this will increase the total amount of error introduced by the weighing process



*Note: paper bags or a large piece of cardboard may absorb atmospheric moisture resulting in skewed mass measurements, if using a paper or cardboard container as a weigh boat, balance must be zeroed out immediately prior to adding litter mass for each measured sample, or such containers should be avoided in humid environments.*

9. Re-weigh a subset of mass samples to assess uncertainty associated with the measurement process.
  - a. QA measurements must be completed by a different technician than the person who originally weighed the sample
  - b. Per bout, for each site, select 5% of dried, previously weighed samples for re-weighing.
    - 1) If QA weighing does not occur within one hour of the initial weighing, return the selected samples to the drying oven for 24 h prior to QA weighing. In humid environments, samples will pick up moisture from the air.
  - c. Record QA weight data to the nearest 0.01 g in the **qaDryMass** field of the "Litter weight measurements" datasheet.
  - d. Return litter samples to temporary storage until all data have been successfully entered to the NEON database.
10. If the collection event has been selected for bioarchive and leaf chemical analyses, return biomass from the leaves and needle functional groups to paper bags and store together in the

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large plastic bag, seal, and place in temporary storage. Samples in temporary storage can then be prepared as time permits for bioarchive and leaf chemical analysis (SOP F).

11. All other material may be discarded in a manner approved by the site host or domain office.
12. If the collection event has not been selected for bioarchive and leaf chemistry analyses, all litter material may be discarded after data have been recorded in digital format via the NEON data entry application.



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## SOP E Data Entry and Verification

The importance of thorough, accurate data entry cannot be overstated; the value field efforts are only manifested once the data are properly entered for delivery to NEON’s end users.

Mobile applications are the preferred mechanism for data entry. Data should be entered into the protocol-specific application as they are being collected, whenever possible, to minimize data transcription and improve data quality. Mobile devices should be synced at the end of each field day, where possible; alternatively, devices should be synced immediately upon return to the Domain Support Facility.

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. 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 are entered via the following Mobile or Desktop applications:

- ) **Litter: Trap Deployment:** Metadata describing trap placement
- ) **Litter: Field Sampling:** Metadata describing individual sampling events on a per trap per plotID per sampling date basis.
- ) **Litter: Lab Mass Data:** Oven-dried biomass data for each functional group per clipID per collectDate, as well as weighing QA data.
- ) **Litter: BGC Sub-Sampling:** Lab processing for chemistry analysis and bioarchive

### E.1 Entering and uploading field data

1. For data collected on paper datasheets: Transcribe data into appropriate data entry application in accordance with data entry and data QA/QC protocols (AD[06]).
2. Upload data collected on mobile app to the NEON server
3. If this is the first bout at a site or a trap had to be moved to a new location and data were recorded on paper data sheets, transcribe data from the ‘SOP B: Initial Deployment of Traps’ Datasheets to the “**Litter: Trap Deployment**” application.
4. If **trapMoved** = ‘Yes’ for a given field collection record, record data for new **clipID** in the trap deployment application.
5. Update permanent digital versions of the “clip-strip coordinate” lists with **status** and **date** grid cells were used.
6. Once all data from the most recent sampling bout have been collected and transcribed, submit data for ingest to the NEON database according to the guidelines provided in RD[04].

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## E.2 Equipment maintenance, cleaning, and storage

1. Charge/replace laser rangefinder batteries, if necessary.
2. Charge GPS unit.
3. Clean grinding mill and splitters.
4. Rinse field collection bags as needed
  - ) Do not use soap, water only

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## SOP F Processing Litter Samples for Bioarchive and Leaf Chemistry

Samples from all elevated traps per plot from one collection event every five years will be ground and submitted for bioarchive and chemical analysis. Two functional groups, leaves and needles, will be targeted, mass from all other functional groups and from ground traps, will not be processed for chemical analysis and bioarchive.

### Timing

Conifer dominated forest: collect a sample for archive and leaf chemistry from the October collection event

Deciduous broadleaf forest: Select a sample from the period of peak senescence, this date may vary from site to site and from year to year

Mixed forest: Select a collection bout from fall senescence. If needle litter production is limited, select the bout with the greatest needle mass to process for leaf chemistry

Refer to the site-specific Appendix D for suggested sampling windows, use assessment of local conditions to ultimately decide when sampling occurs.

Domains will generate a maximum of one leaf sample and one needle sample per subsample type (C:N, lignin, archive) per plot (**Figure 6**).

1. Wear a pair of Nitrile (Latex-free) gloves when handling and subsampling foliage to be used for chemical analyses. Gloves may be used for >1 sample but should be changed if they become visibly dirty or coated in sap or residue.
2. In a clean container, mix dried leaves from the all (one or two) elevated traps in a given plot. In another container, mix needles from all (one or two) elevated traps in the same plot. In a mixed forest system, this will generate two samples for each selected plotID, one from the 'leaves' functional group, and one from the 'needles' functional group.
  - a. If *Toxicodendron* was present in the sample (and processed according to Appendix H), discard all samples after weighing, do not include any mass samples from traps containing *Toxicodendron* sp. in the samples to be ground and sent for bioarchive and chemical analysis.
  - b. Do not save and process for chemical analysis if dry mass is < 0.2 g
  - c. If the sum of dry mass from the trap(s) is > 15 g, subsample material by hand:
    - 1) With a nitrile gloved hand coarsely crush material into a clean container (e.g. bucket for large amounts of material, bowl for less)
    - 2) mix crushed material by hand to create an even blend
    - 3) haphazardly select one handful of crushed leaves, ~15 g to grind and process for chemical analysis



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Note: Use a balance to achieve the target mass for the first couple of samples but after this initial ‘calibration,’ it is okay to estimate sample quantity by eye.

3. If the combined dry mass from the two trap(s) is >1.5 grams for a given functional group, coarsely grind material with a Wiley Mill (0.85mm, 20 mesh size).
  - a. Continue grinding until all material is fully ground.
  - b. If, after grinding, a significant amount of material is adhered to the glass cover or interior of the mill (common when grinding needles), carefully clean the mill and collect adhered material.
    - 1) To clean the mill, remove the glass face, loosen the screen, knock the screen into the collection jar 3-4 times, then wipe the inside of the grinding mechanism and glass plate with a paint brush to collect ground material.

**USE AN APPROPRIATELY SIZED SPLITTER/MICROSPLITTER TO GENERATE 1-3 REPRESENTATIVE SUB-SAMPLES ACCORDING TO BEST PRACTICES**

- ) 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.

4. **Table 11.**
5. Place the split sub-samples into 20 mL polypropylene scint vials, and label the vials with the **sampleID**.
  - a. SampleIDs may be generated in advance of grinding based on mass values from SOP D. SampleIDs are formatted as follows:
    - ) **massSampleID:** (from SOP D)
      - o “NEON.ltr.”clipID[no underscores].date.functional group code[just ‘lvs’ or ‘nd’]
      - o Example: ‘NEON.ltr.OSBS025175.20151115.lvs’
    - ) **massSampleMixtureID:** generated in step F.2
      - o Remove the clip cell component from the **massSampleID**
      - o Example: ‘NEON.ltr.OSBS025.20151115.lvs’
    - ) **cnSampleID:** massSampleMixtureID + “.cn”
      - o Example: ‘NEON.ltr.OSBS025.20151115.lvs.cn’
    - ) **ligninSampleID:** massSampleMixtureID + “.lig”
      - o Example: ‘NEON.ltr.OSBS025.20151115.lvs.lig’
    - ) **archiveSampleID:** massSampleMixtureID + “.ar”
      - o Example: ‘NEON.ltr.OSBS025.20151115.lvs.ar’

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- b. Use a printed label or laboratory tape wrapped around the entire vial so that it overlaps itself. **Do not write directly on the vial, it will rub off.**

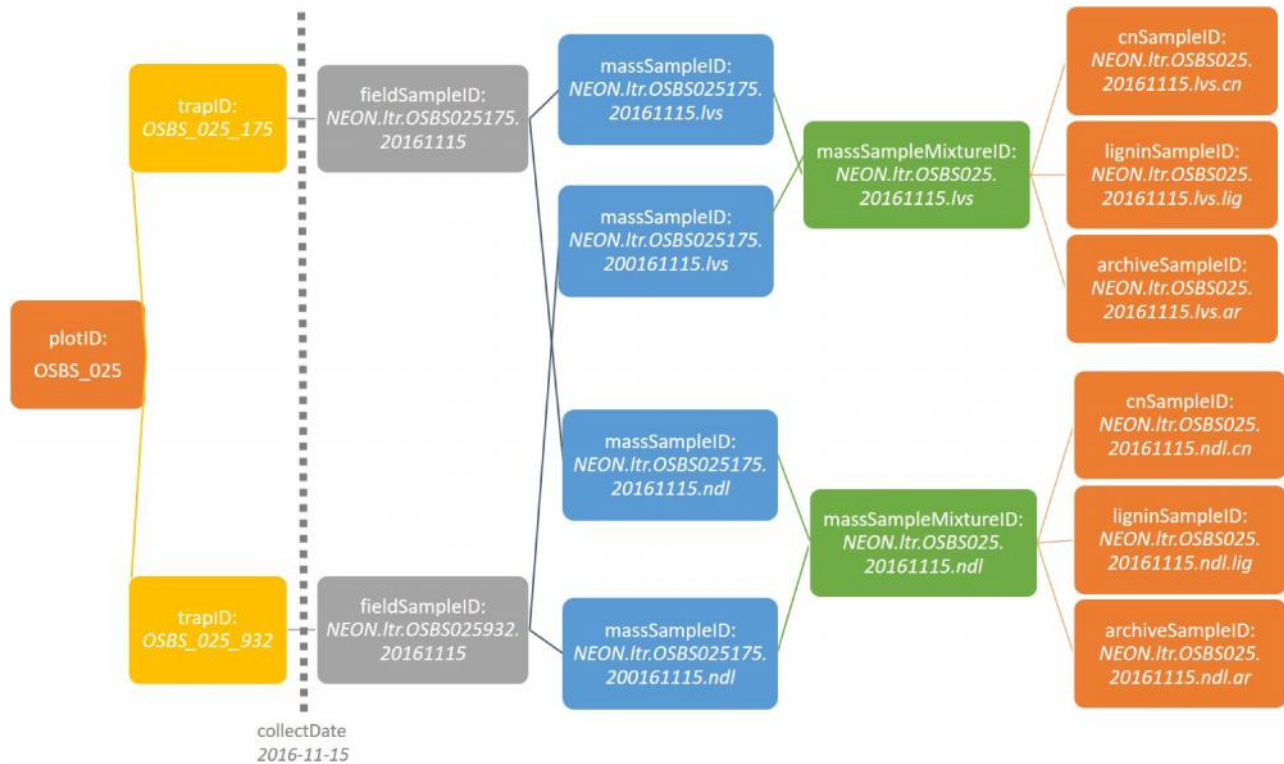
### BEST PRACTICES

- ) 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.

**Table 11.** Sub-sampling guidelines for C:N, Lignin and Archive subsamples.

dryMass	Samples to create			Processing guidelines
	C:N Sample <i>min 0.2 g</i>	Lignin Sample <i>min 1 g</i>	Archive sample <i>min 3 g</i>	
< 0.2 grams	-	-	-	Do not create subsample, discard all material
0.2 – 1.5 grams	X	-	-	Do not grind, place entire sample in scint vial. Use gloved hand to crush if necessary to fit.
1.5 - 5 grams	X	X	-	Grind sample, distribute 1/4 sample to C:N and 3/4 sample to lignin sample
5 - 15 grams	X	X	X	Grind sample, distribute 1/4 to C:N, 1/4 to lignin, 1/2 to archive.

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**Figure 6.** Anatomy of a sampleID. Note, if samples from only one trap are created, a massSampleMixtureID must still be created. Data generated will maintain traceability to original trap locations.

**SOP G Sample Shipment for Bioarchive and Leaf Chemistry**

**Only leaf and needle biomass samples from Litterfall collection bouts scheduled for archive and chemical analyses will be shipped to external facilities. Verify with Domain Manager and FOPS multi-year schedule on NEON intranet, that current year is scheduled for litter bioarchive and leaf chemistry analyses, before mailing samples.** Information included in this SOP conveys science-based packaging, shipping, and handling requirements, not lab-specific or logistical demands. For that information, reference the “Shipping Information for External Facilities” document on [CLA’s NEON intranet site](#).

**G.1 Handling Hazardous Material**

N/A

**G.2 Biogeochemistry: Supplies/Containers**

20 mL Scintillation vials with dried ground material in them do not require additional preservation. Vials will be shipped from the Domain lab to external labs for analysis:

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1. Take scintillation vial box containing processed samples out of the cabinet for shipment
2. Make sure each vial is labeled with all required information
3. Wrap the box in bubble wrap and tape securely, then place in a FedEx box for shipment.
4. Navigate to the “Shipping Information for External Facilities” document on [CLA’s NEON intranet site](#). Check whether there are other items, such as permits or cover letters, required for inclusion in the shipment. *Check this document frequently as instructions are subject to change.*
5. Print out required documents (if needed) and include in shipment box.
6. Prepare a shipping inventory detailing the contents of the shipment, using the protocol-specific template found on [CLA’s NEON intranet site](#). Include a printed copy in the shipment box.
7. Address and affix shipping label
8. Send Ground if alone – may affix ‘Up’ stickers

**G.3 Timelines**

There are no scientific limits on the time oven-dried, ground samples may be placed in temporary storage prior to shipping.

**G.4 Conditions**

Samples must be dry, ground, securely contained and clearly labeled.

**G.5 Grouping/Splitting Samples**

N/A

**G.6 Return of Materials or Containers**

N/A

**G.7 Shipping Inventory**

Prepare a shipping inventory using the protocol-specific template found on [CLA’s NEON intranet site](#). Include a printed copy of the inventory in the shipment box, and send an electronic copy to the email addresses listed in the “Shipping Information for External Facilities” document. Include the shipment tracking # in the email.

**G.8 Laboratory Contact Information and Shipping/Receipt Days**

See the “Shipping Information for External Facilities” and “External Facilities Closure Dates” documents on [CLA’s NEON intranet site](#).

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## 8 REFERENCES

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**APPENDIX A DATASHEETS**

The following datasheets are associated with this protocol:

**Table 12.** Datasheets associated with this protocol

<b>NEON Doc. #</b>	<b>Title</b>
NEON.DOC.002132	Datasheets for TOS Protocol and Procedure: Litterfall and Fine Woody Debris

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 used for litter trap placement

#### LOCATE AND ASSESS POTENTIAL CLIP CELL

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

**STEP 2** – If no woody vegetation is present in the plot, record targetTaxaPresent=No

**STEP 3** – Select first available clip-strip location from Work Order list.

**STEP 4** – Locate X-coordinate, anchor and stretch east-west tape, place pin flag.

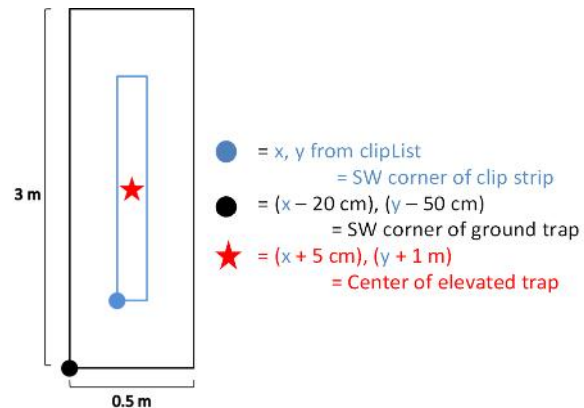
Y-Coordinate	East-West Tape Location
1, 4, or 7	(0,0) →(20,0)
10, 13, or 16	(0,10) →(20,10)

**STEP 5** – Locate Y-coordinate with laser rangefinder in HD mode (azimuth 0°), place pin flag.

**STEP 6** – Locate clip cell centroid (elevated trap)  
1 m North, 5 cm East

**STEP 6b** – Locate clip cell SW corner (ground trap) 0.5 m South, 20 cm West

**STEP 7** – Assess suitability of clip-strip. Reject if not suitable.



#### DELINEATE 0.5 M X 3 M CLIP-STRIP

**STEP 1** – Place one stake in SW corner of clip-cell.

**STEP 2** – Use laser range finder and tape measure to locate remaining three corners.

**STEP 3** – Check distance between all four corner with ruler or tape measure. Use handheld compass to check orientation.

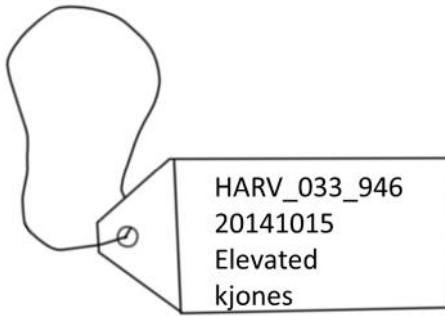
**STEP 4** – Monument clip strip corners with aluminum stakes

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### B.2 Litter trap status codes

Code	Description
OK	Litter collected –Trap in good shape, no issues
TE	Litter not collected – Trap empty
HO	Litter not collected –Holes large enough for leaves to pass through. Holes near the base of the screen (the lowest hanging point) are worse than holes on the side of the screen.
TB	Litter not collected – trap blocked. Large branches or leaves (especially palm fronds) present in the trap which may have prevented trap from collecting litter or diverted falling litter away from the trap
TT	Litter not collected – trap tilted 10° (use clinometer on compass to measure)
RE	Litter not collected – trap broken

### B.3 Example field collection label



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**APPENDIX C    REMINDERS**

N/A

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## APPENDIX D ESTIMATED DATES FOR ONSET AND CESSATION OF SAMPLING

The dates listed here are estimated from satellite imagery (MODIS) averaged Enhanced Vegetation Index (EVI) values from 2001-2013 and are the ‘average Greenness Increase’ date a proxy for the beginning of spring, the time period when sampling for winter litterfall, and the beginning and average end of senescence. The sampling dates in this table are based on MODIS data for an area centered on the NEON flux tower; NLCD vegetation classification listed is based on the dominant vegetation found in the tower airshed.

Sampling schedules may be modified based on local conditions, for example, if the NLCD vegetation class is identified as ‘Mixed Forest’ but plots are almost entirely coniferous trees, sampling may be shifted to ‘Monthly, Year Round’ even though the table specified ‘Spring + Senescence’ or ‘Hybrid’ sampling schedule. Dates are only listed for sites with forests where intensive sampling during fall senescence is anticipated; all other sites will be sampled once a month all year or not at all.

**Table 13.** Estimated sampling dates, in julian days

Domain	Site code	Primary Airshed NLCD	Trap Location Selection	Suggested Sampling Schedule	Average Greenness Increase	Beginning of Senescence	Average End of Senescence
01	BART	Mixed Forest	Random	Hybrid	30-Apr	8-Aug	27-Oct
01	HARV	Mixed Forest	Random	Hybrid	20-Apr	8-Aug	27-Oct
02	BLAN	Deciduous Forest/ Pasture Hay	Random	Spring + Senescence	16-Mar	29-Jul	6-Nov
02	SCBI	Deciduous Forest	Random	Hybrid	26-Mar	30-May	16-Nov
02	SERC	Deciduous Forest	Random	Hybrid	21-Mar	8-Aug	21-Nov
03	DSNY	Grassland Herbaceous		None			
03	JERC	Mixed Forest	Random	Hybrid	1-Mar	9-Jul	17-Sep
03	OSBS	Evergreen Forest	Random	Monthly, Year Round			
04	GUAN	Evergreen Forest	Random	Monthly, Year Round			

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Domain	Site code	Primary Airshed NLCD	Trap Location Selection	Suggested Sampling Schedule	Average Greenness Increase	Beginning of Senescence	Average End of Senescence
04	LAJA	Cultivated Crops		None			
05	STEI	Deciduous Forest	Random	Spring + Senescence	30-Apr	3-Aug	12-Oct
05	TREE	Deciduous Forest	Random	Spring + Senescence	30-Apr	3-Aug	12-Oct
05	UNDE	Deciduous Forest	Random	Spring + Senescence	5-May	3-Aug	12-Oct
06	KONA	Cultivated Crops		None			
06	KONZ	Grassland Herbaceous	Targeted	Spring + Senescence	31-Mar	29-Jul	27-Oct
06	UKFS	Deciduous Forest	Random	Spring + Senescence	16-Mar	29-Jul	26-Nov
07	GRSM	Deciduous Forest	Random	Spring + Senescence	31-Mar	3-Aug	6-Nov
07	MLBS	Deciduous Forest	Random	Spring + Senescence	20-Apr	8-Aug	6-Nov
07	ORNL	Deciduous Forest	Random	Spring + Senescence	31-Mar	29-Jul	11-Nov
08	LENO	Woody Wetlands	Random	Spring + Senescence	11-Mar	19-Jul	1-Dec
08	DELA	Woody Wetlands	Random	Spring + Senescence	1-Mar	24-Jul	26-Nov
08	TALL	Evergreen Forest	Random	Monthly, Year Round	16-Mar	14-Jul	26-Nov
09	DCFS	Grassland Herbaceous		None			
09	NOGP	Grassland Herbaceous		None			
09	WOOD	Grassland Herbaceous		None			
10	CPER	Grassland Herbaceous		None			
10	RMNP	Evergreen Forest	Random	Monthly, Year Round	30-Apr	29-Jul	11-Nov
10	STER	Cultivated Crops		None			

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Domain	Site code	Primary Airshed NLCD	Trap Location Selection	Suggested Sampling Schedule	Average Greenness Increase	Beginning of Senescence	Average End of Senescence
11	CLBJ	Grassland Herbaceous	Random	Spring + Senescence	1-Mar	29-Jun	21-Nov
11	OAES	Grassland Herbaceous		None			
12	YELL	Shrub Scrub	TBD	TBD	30-Apr	9-Jul	7-Oct
13	MOAB	Shrub Scrub	-	None	26-Mar	13-Aug	27-Oct
13	NIWO	Evergreen Forest	Targeted	Monthly, Year Round			
14	JORN	Shrub Scrub	TBD	TBD	21-Mar	2-Sep	16-Nov
14	SRER	Shrub Scrub	Targeted	Monthly, Year Round	30-May	28-Aug	26-Nov
15	ONAQ	Shrub Scrub	-	None	16-Mar	19-Jun	7-Oct
16	ABBY	Evergreen Forest	Random	Monthly, Year Round	20-Apr	24-Jul	27-Oct
16	WREF	Evergreen Forest	Random	Monthly, Year Round			
17	SJER	Evergreen Forest	Targeted	Monthly, Year Round	27-Sep	5-Apr	4-Jun
17	SOAP	Evergreen Forest	Random	Monthly, Year Round	31-Mar	4-Jul	17-Oct
17	TEAK	Evergreen Forest	Random	Monthly, Year Round	30-Apr	24-Jul	27-Oct
18	BARO	Sedge Herbaceous		None			
18	TOOL	Dwarf Scrub		None			
19	BONA	Deciduous Forest	Random	Hybrid	15-May		7-Sep
19	DEJU	Evergreen Forest	Random	Monthly, Year Round			
19	HEAL	Shrub Scrub	Targeted	Monthly, Year Round	15-May	29-Jul	2-Sep
20	PUUM	Evergreen Forest	Random	Monthly, Year Round			



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## APPENDIX E SITE-SPECIFIC INFORMATION

At sites with prescribed burning, collect litter as if conducting a regular sampling bout even if one is not scheduled, prior to scheduled burn. Remove traps then replace as soon as possible following after. Dates of removal and re-setting of litter traps do not need to be recorded as no litter production is expected during this period. Resume prescribed sampling schedule once traps are reset.

Burn sites include, but may not be limited to, the following:

**Table 14.** Burn sites

Domain	Site Code	Site Name
D03	JERC	Jones Ecological Research Center
D03	OSBS	Ordway-Swisher Biological Station
D06	KONZ	Konza Prairie Biological Station (Core)
D08	TALL	Talladega National Forest
D09	WOOD	Woodworth
D11	CLBJ	LBJ National Grassland
D17	SOAP	Soaproot Saddle

Mesh size on elevated trap assembly kits is 1mm. Particles < 1mm diameter may be capable of passing through the standard mesh and may be underestimated in dry mass measurements.

Sites with known coniferous species with needles < 1mm include:

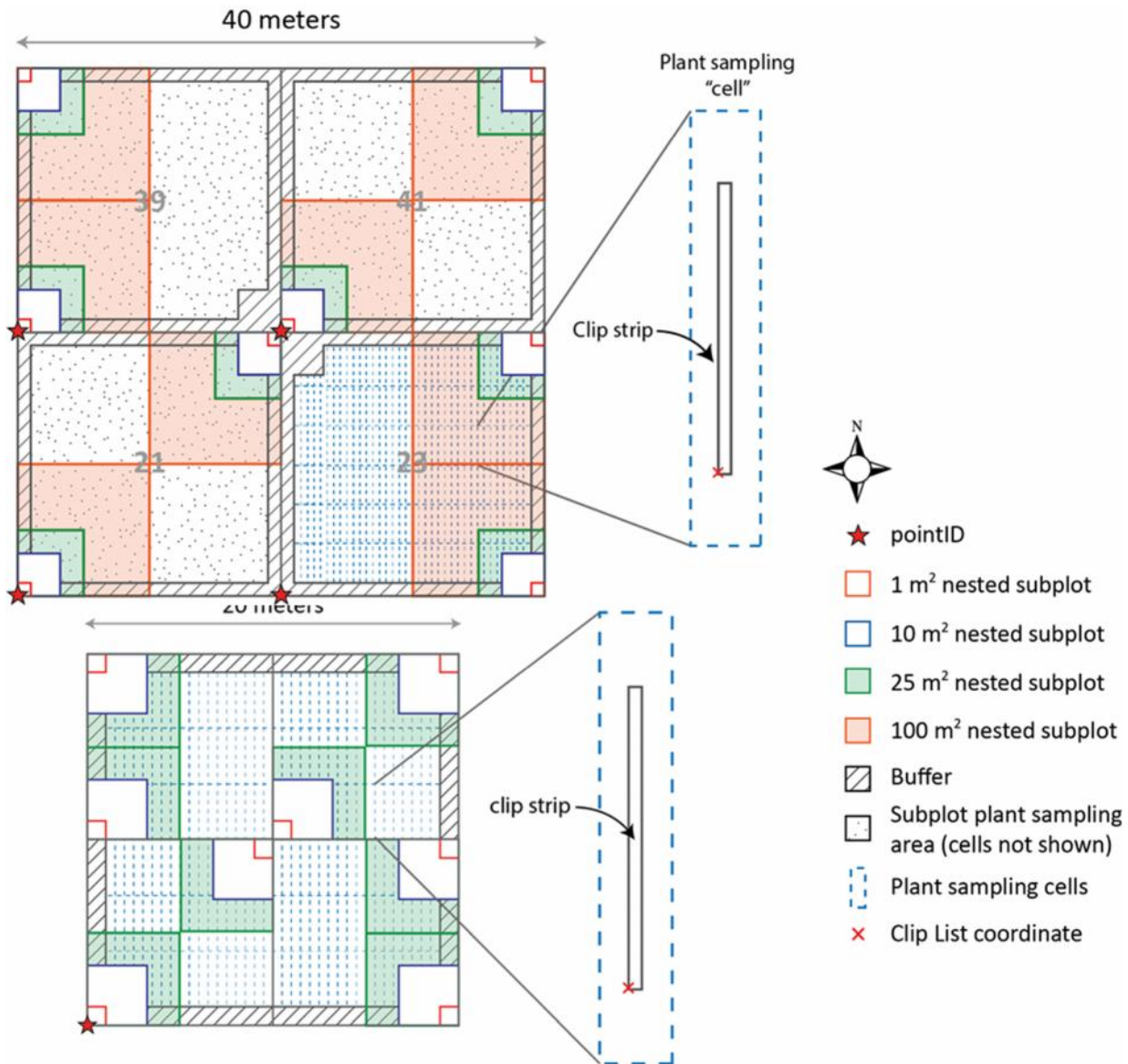
Domain	Site Code	Site Name	Species with needle width < 1mm
D01	HARV	Harvard Forest	<i>Larix decidua</i>
D01	BART	Bartlett Experimental Forest	<i>Picea rubrum, Tsuga canadensis</i>
D05	STEI	Steigerwaldt Land Services	<i>Larix laricina</i>
D05	UNDE	UNDERC	<i>Larix laricina</i>
D05	TREE	Treehaven	<i>Larix laricina</i>
D19	DEJU	Delta Junction	<i>Picea mariana, Larix laricina</i>

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Domain	Site Code	Site Name	Species with needle width < 1mm
D19	HEAL	Healy	<i>Picea mariana, Larix laricina</i>
D19	BONA	Caribou Creek - Poker Flats Watershed	<i>Picea mariana, Larix laricina</i>

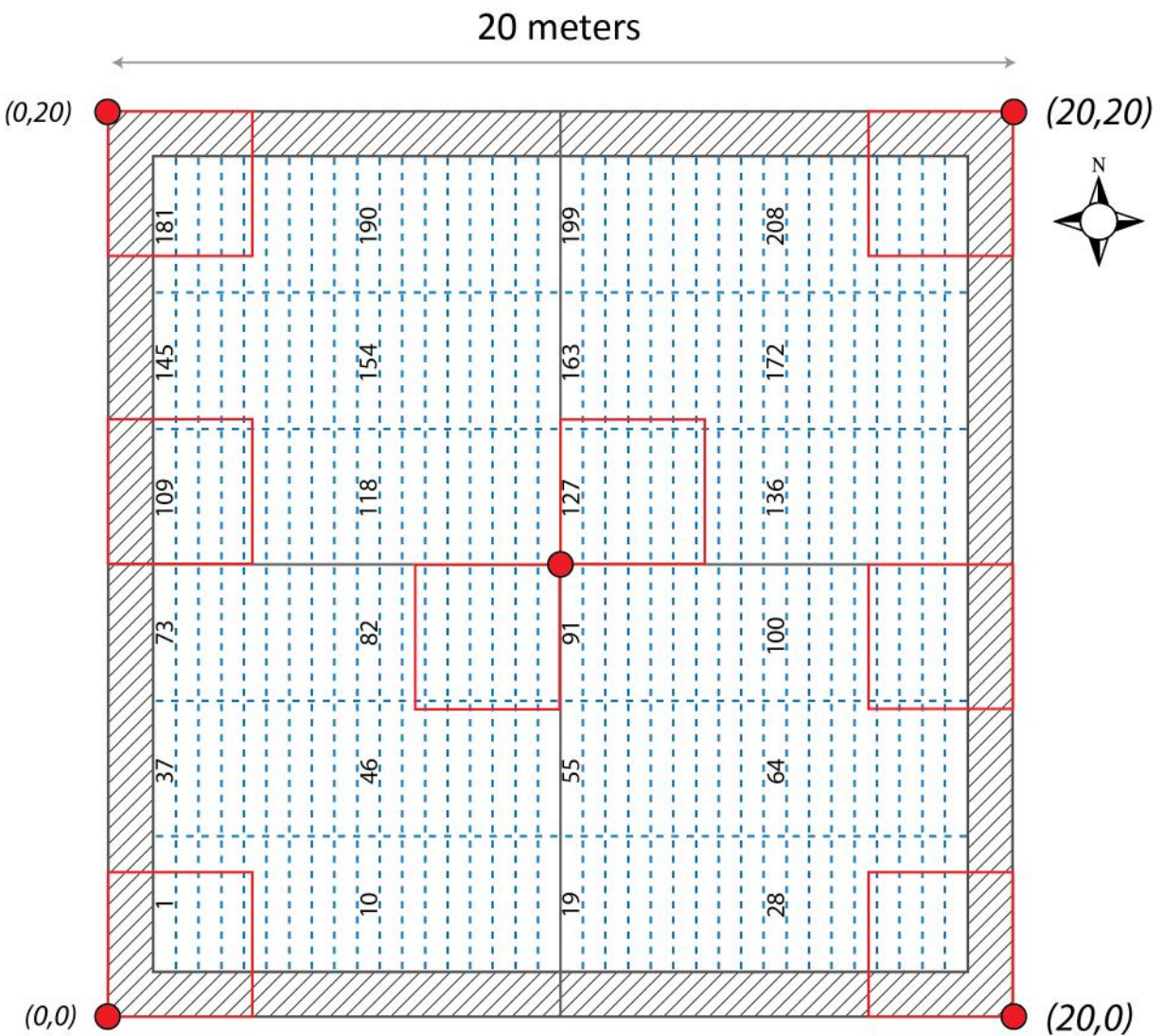
**APPENDIX F PLOT MAPS**

40m x 40m (top image) and 20m x 20m (lower image) Tower Plots showing the location of 0.5m x 3m clip-harvest cells (dashed blue lines). Subplot IDs are listed in gray for the 40m x 40m plot. The clip-strip coordinates provided to technicians are supplied on a per subplot basis (red 'X' in the figures). For plot centroids, navigate 1 m North and 5 cm East from this point. To locate the clip cell / ground trap SW corner, navigate 0.5 m South and 20 cm West from the provided coordinates. Exclusion areas in 40m x 40m Tower Plots selected for Plant Diversity sampling are consistent with a 20m x 20m plot centered on the plot centroid. Clip cells in exclusion areas are not included in the randomized clipLists provided by NEON Science.

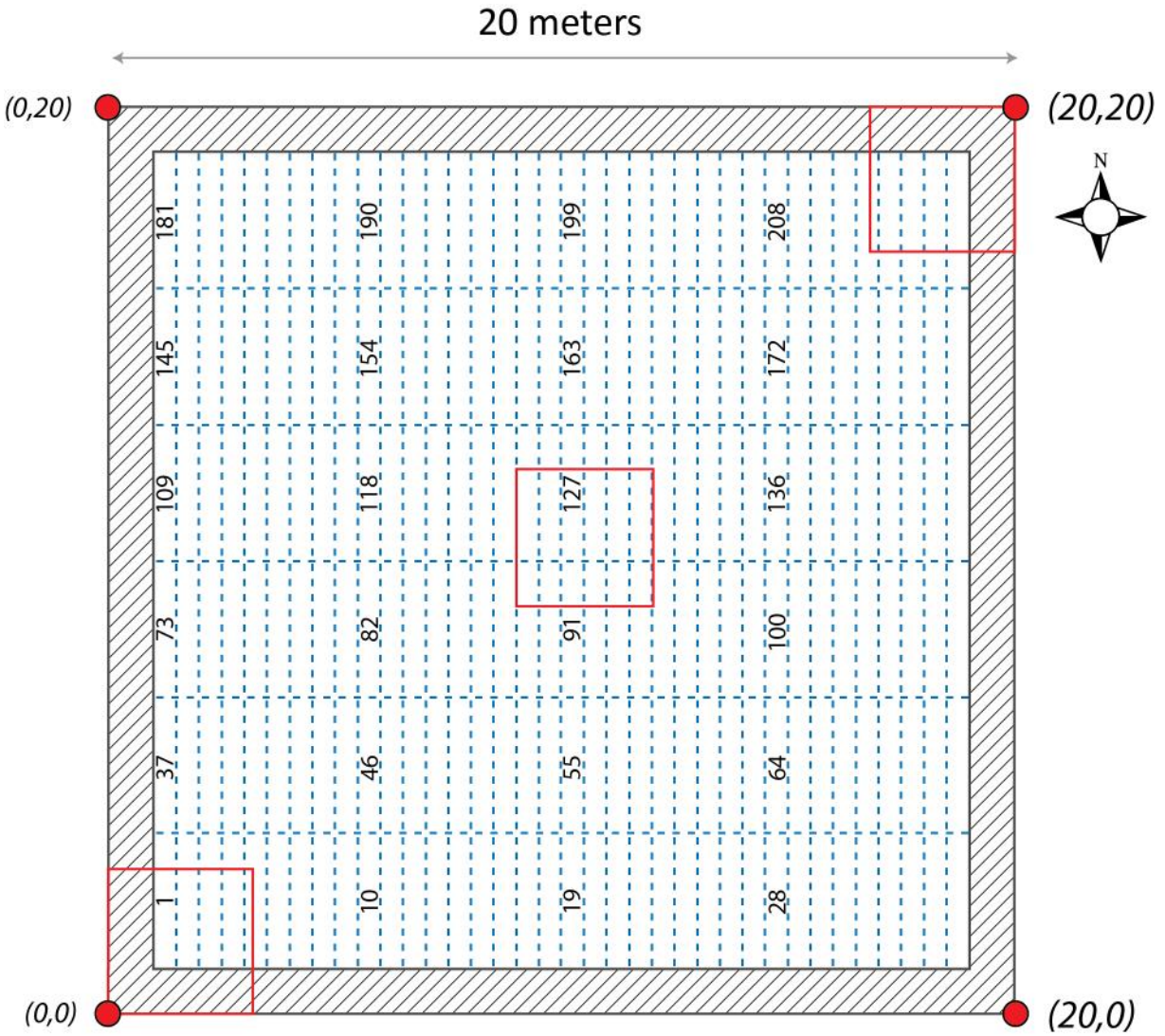


**APPENDIX G CLIPCELLNUMBER COORDINATES AND MAPS**

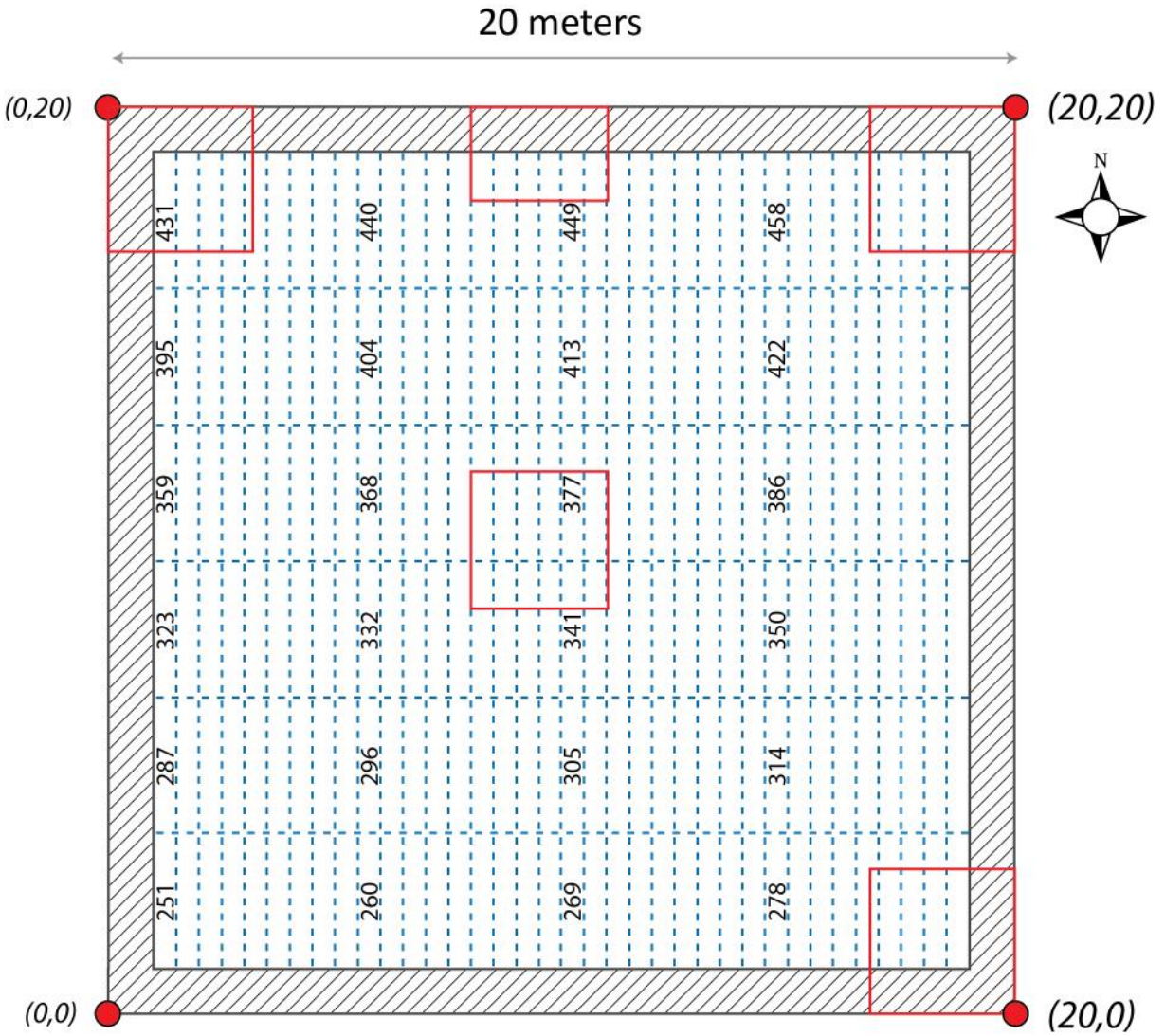
Targeted deployment of ground and elevated litter traps (SOP B) in habitats with non-continuous cover (< 50% of the plot area) of woody vegetation requires locating Clip Strips within “patches” of vegetation with overstory species > 2 m. To identify trap location within woody “patches,” first map out the location of patches within a selected subplot, use a random selection procedure to pick an individual patch then use the appropriate map in this Appendix to determine which clipCellNumber should be sampled. Use Table 15 in to find the easting and northing values associated with that Clip Strip so that it can be delineated at a known location relative to the SW corner of the 20m x 20m plot / subplot.



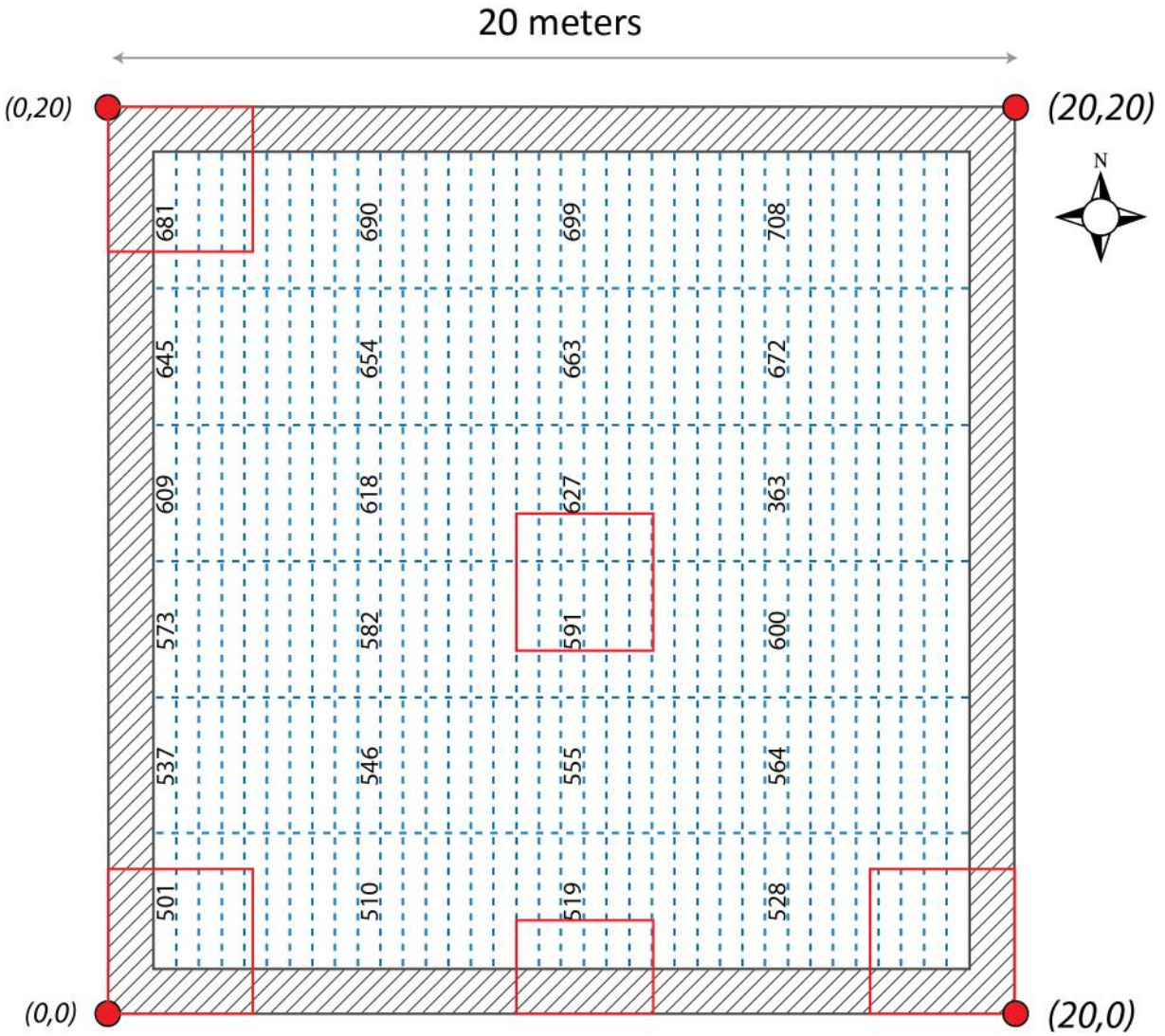
**Figure 7.** Map of clipCellNumbers in a 20m x 20m base plot (subplotID = 31 in provided Clip Lists). Red squares indicate nested subplots used for diversity sampling; clip cells that significantly overlap red squares are not used for litter sampling. However, clip cells with minimal overlap (e.g., 48-54, 68-72, 145-149) are considered for litter sampling.



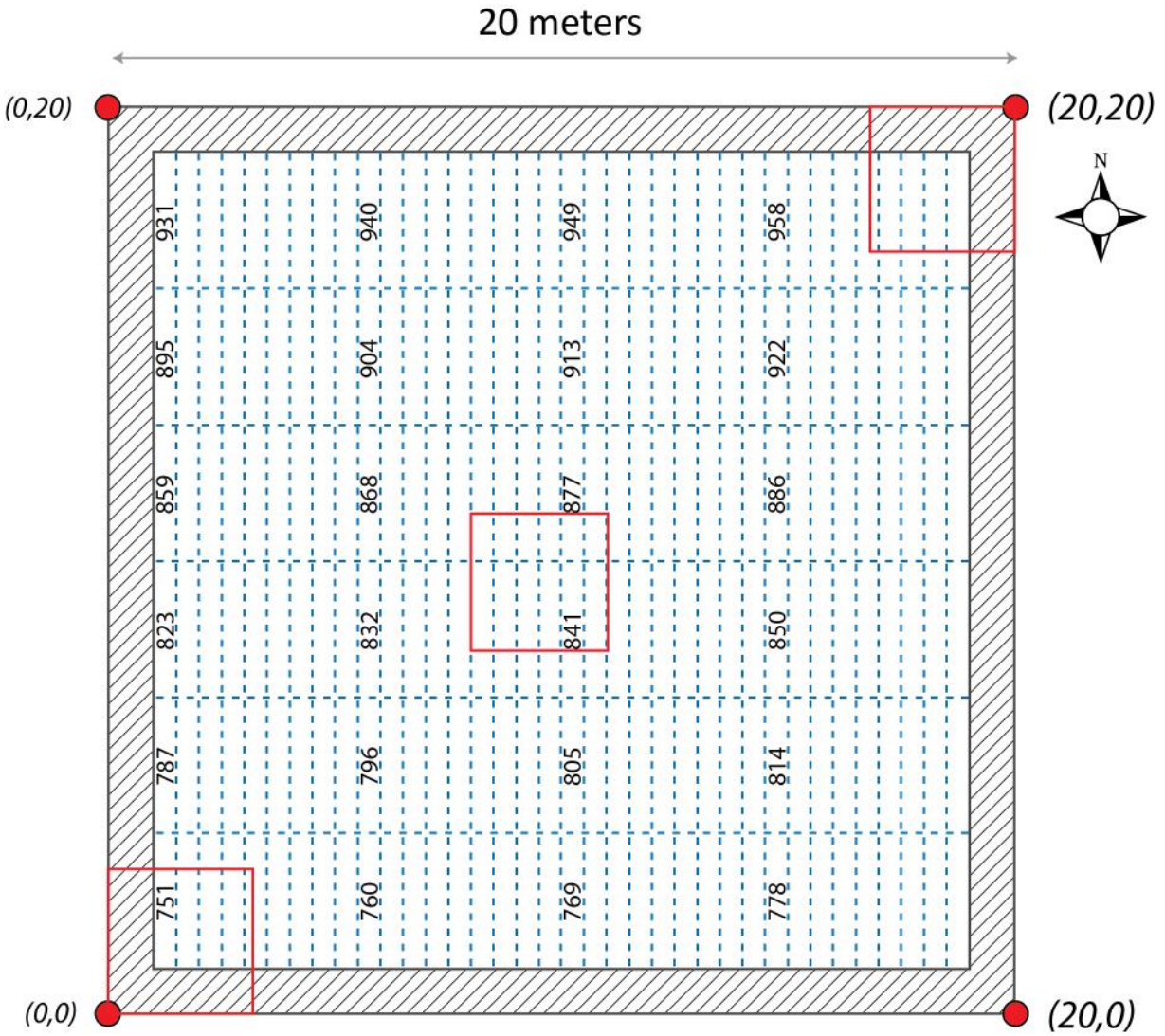
**Figure 8.** Map of clipCellNumbers for subplotID = 21 in a 40m x 40m Tower base plot. Clip cells that overlap nested subplots indicated by red squares are not used for litter sampling.



**Figure 9.** Map of clipCellNumbers for subplotID = 23 in a 40m x 40m Tower base plot. Clip cells that overlap nested subplots indicated by red squares are not used for litter sampling.



**Figure 10.** Map of clipCellNumbers for **subplotID = 39** in a 40m x 40m Tower base plot. Clip cells that overlap nested subplots indicated by red squares are not used for litter sampling.



**Figure 11.** Map of clipCellNumbers for **subplotID = 41** in a 40m x 40m Tower base plot. Clip cells that overlap nested subplots indicated by red squares are not used for litter sampling.



### G.1 Coordinates for litter trap placement by clipCellNumber and subplotID

Table 15. List of clipCellNumbers by subplotID and associated easting and northing coordinates. Coordinates correspond to the either 1) SW corner of the 0.5m x 3m ClipCell for ground trap placement, or 2) the centroid of the clip cell over which an elevated trap would be placed. Offsets indicate the distance in meters relative to the SW corner of the plot (subplotID = 31) or subplot. These are **not** the same coordinates used in the herbaceous clip harvest protocol. Print this Appendix separately for use with this protocol

clipCell Number subplotID = 31	clipCell Number subplotID = 21	clipCell Number subplotID = 23	clipCell Number subplotID = 39	clipCell Number subplotID = 41	Ground Trap easting offset	Ground Trap northing offset	Elevated Trap easting offset	Elevated Trap northing offset
1	1	251	501	751	1	1	1.25	2.5
2	2	252	502	752	1.5	1	1.75	2.5
3	3	253	503	753	2	1	2.25	2.5
4	4	254	504	754	2.5	1	2.75	2.5
5	5	255	505	755	3	1	3.25	2.5
6	6	256	506	756	3.5	1	3.75	2.5
7	7	257	507	757	4	1	4.25	2.5
8	8	258	508	758	4.5	1	4.75	2.5
9	9	259	509	759	5	1	5.25	2.5
10	10	260	510	760	5.5	1	5.75	2.5
11	11	261	511	761	6	1	6.25	2.5
12	12	262	512	762	6.5	1	6.75	2.5
13	13	263	513	763	7	1	7.25	2.5
14	14	264	514	764	7.5	1	7.75	2.5
15	15	265	515	765	8	1	8.25	2.5
16	16	266	516	766	8.5	1	8.75	2.5
17	17	267	517	767	9	1	9.25	2.5
18	18	268	518	768	9.5	1	9.75	2.5
19	19	269	519	769	10	1	10.25	2.5
20	20	270	520	770	10.5	1	10.75	2.5
21	21	271	521	771	11	1	11.25	2.5
22	22	272	522	772	11.5	1	11.75	2.5
23	23	273	523	773	12	1	12.25	2.5
24	24	274	524	774	12.5	1	12.75	2.5
25	25	275	525	775	13	1	13.25	2.5
26	26	276	526	776	13.5	1	13.75	2.5
27	27	277	527	777	14	1	14.25	2.5
28	28	278	528	778	14.5	1	14.75	2.5
29	29	279	529	779	15	1	15.25	2.5
30	30	280	530	780	15.5	1	15.75	2.5
31	31	281	531	781	16	1	16.25	2.5
32	32	282	532	782	16.5	1	16.75	2.5
33	33	283	533	783	17	1	17.25	2.5
34	34	284	534	784	17.5	1	17.75	2.5

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clipCell Number subplotID = 31	clipCell Number subplotID = 21	clipCell Number subplotID = 23	clipCell Number subplotID = 39	clipCell Number subplotID = 41	Ground Trap easting offset	Ground Trap northing offset	Elevated Trap easting offset	Elevated Trap northing offset
35	35	285	535	785	18	1	18.25	2.5
36	36	286	536	786	18.5	1	18.75	2.5
37	37	287	537	787	1	4	1.25	5.5
38	38	288	538	788	1.5	4	1.75	5.5
39	39	289	539	789	2	4	2.25	5.5
40	40	290	540	790	2.5	4	2.75	5.5
41	41	291	541	791	3	4	3.25	5.5
42	42	292	542	792	3.5	4	3.75	5.5
43	43	293	543	793	4	4	4.25	5.5
44	44	294	544	794	4.5	4	4.75	5.5
45	45	295	545	795	5	4	5.25	5.5
46	46	296	546	796	5.5	4	5.75	5.5
47	47	297	547	797	6	4	6.25	5.5
48	48	298	548	798	6.5	4	6.75	5.5
49	49	299	549	799	7	4	7.25	5.5
50	50	300	550	800	7.5	4	7.75	5.5
51	51	301	551	801	8	4	8.25	5.5
52	52	302	552	802	8.5	4	8.75	5.5
53	53	303	553	803	9	4	9.25	5.5
54	54	304	554	804	9.5	4	9.75	5.5
55	55	305	555	805	10	4	10.25	5.5
56	56	306	556	806	10.5	4	10.75	5.5
57	57	307	557	807	11	4	11.25	5.5
58	58	308	558	808	11.5	4	11.75	5.5
59	59	309	559	809	12	4	12.25	5.5
60	60	310	560	810	12.5	4	12.75	5.5
61	61	311	561	811	13	4	13.25	5.5
62	62	312	562	812	13.5	4	13.75	5.5
63	63	313	563	813	14	4	14.25	5.5
64	64	314	564	814	14.5	4	14.75	5.5
65	65	315	565	815	15	4	15.25	5.5
66	66	316	566	816	15.5	4	15.75	5.5
67	67	317	567	817	16	4	16.25	5.5
68	68	318	568	818	16.5	4	16.75	5.5
69	69	319	569	819	17	4	17.25	5.5
70	70	320	570	820	17.5	4	17.75	5.5
71	71	321	571	821	18	4	18.25	5.5
72	72	322	572	822	18.5	4	18.75	5.5
73	73	323	573	823	1	7	1.25	8.5
74	74	324	574	824	1.5	7	1.75	8.5

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75	75	325	575	825	2	7	2.25	8.5
76	76	326	576	826	2.5	7	2.75	8.5
77	77	327	577	827	3	7	3.25	8.5
78	78	328	578	828	3.5	7	3.75	8.5
79	79	329	579	829	4	7	4.25	8.5
80	80	330	580	830	4.5	7	4.75	8.5
81	81	331	581	831	5	7	5.25	8.5
82	82	332	582	832	5.5	7	5.75	8.5
83	83	333	583	833	6	7	6.25	8.5
84	84	334	584	834	6.5	7	6.75	8.5
85	85	335	585	835	7	7	7.25	8.5
86	86	336	586	836	7.5	7	7.75	8.5
87	87	337	587	837	8	7	8.25	8.5
88	88	338	588	838	8.5	7	8.75	8.5
89	89	339	589	839	9	7	9.25	8.5
90	90	340	590	840	9.5	7	9.75	8.5
91	91	341	591	841	10	7	10.25	8.5
92	92	342	592	842	10.5	7	10.75	8.5
93	93	343	593	843	11	7	11.25	8.5
94	94	344	594	844	11.5	7	11.75	8.5
95	95	345	595	845	12	7	12.25	8.5
96	96	346	596	846	12.5	7	12.75	8.5
97	97	347	597	847	13	7	13.25	8.5
98	98	348	598	848	13.5	7	13.75	8.5
99	99	349	599	849	14	7	14.25	8.5
100	100	350	600	850	14.5	7	14.75	8.5
101	101	351	601	851	15	7	15.25	8.5
102	102	352	602	852	15.5	7	15.75	8.5
103	103	353	603	853	16	7	16.25	8.5
104	104	354	604	854	16.5	7	16.75	8.5
105	105	355	605	855	17	7	17.25	8.5
106	106	356	606	856	17.5	7	17.75	8.5
107	107	357	607	857	18	7	18.25	8.5
108	108	358	608	858	18.5	7	18.75	8.5
109	109	359	609	859	1	10	1.25	11.5
110	110	360	610	860	1.5	10	1.75	11.5
111	111	361	611	861	2	10	2.25	11.5
112	112	362	612	862	2.5	10	2.75	11.5
113	113	363	613	863	3	10	3.25	11.5
114	114	364	614	864	3.5	10	3.75	11.5

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115	115	365	615	865	4	10	4.25	11.5
116	116	366	616	866	4.5	10	4.75	11.5
117	117	367	617	867	5	10	5.25	11.5
118	118	368	618	868	5.5	10	5.75	11.5
119	119	369	619	869	6	10	6.25	11.5
120	120	370	620	870	6.5	10	6.75	11.5
121	121	371	621	871	7	10	7.25	11.5
122	122	372	622	872	7.5	10	7.75	11.5
123	123	373	623	873	8	10	8.25	11.5
124	124	374	624	874	8.5	10	8.75	11.5
125	125	375	625	875	9	10	9.25	11.5
126	126	376	626	876	9.5	10	9.75	11.5
127	127	377	627	877	10	10	10.25	11.5
128	128	378	628	878	10.5	10	10.75	11.5
129	129	379	629	879	11	10	11.25	11.5
130	130	380	630	880	11.5	10	11.75	11.5
131	131	381	631	881	12	10	12.25	11.5
132	132	382	632	882	12.5	10	12.75	11.5
133	133	383	633	883	13	10	13.25	11.5
134	134	384	634	884	13.5	10	13.75	11.5
135	135	385	635	885	14	10	14.25	11.5
136	136	386	636	886	14.5	10	14.75	11.5
137	137	387	637	887	15	10	15.25	11.5
138	138	388	638	888	15.5	10	15.75	11.5
139	139	389	639	889	16	10	16.25	11.5
140	140	390	640	890	16.5	10	16.75	11.5
141	141	391	641	891	17	10	17.25	11.5
142	142	392	642	892	17.5	10	17.75	11.5
143	143	393	643	893	18	10	18.25	11.5
144	144	394	644	894	18.5	10	18.75	11.5
145	145	395	645	895	1	13	1.25	14.5
146	146	396	646	896	1.5	13	1.75	14.5
147	147	397	647	897	2	13	2.25	14.5
148	148	398	648	898	2.5	13	2.75	14.5
149	149	399	649	899	3	13	3.25	14.5
150	150	400	650	900	3.5	13	3.75	14.5
151	151	401	651	901	4	13	4.25	14.5
152	152	402	652	902	4.5	13	4.75	14.5
153	153	403	653	903	5	13	5.25	14.5
154	154	404	654	904	5.5	13	5.75	14.5

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155	155	405	655	905	6	13	6.25	14.5
156	156	406	656	906	6.5	13	6.75	14.5
157	157	407	657	907	7	13	7.25	14.5
158	158	408	658	908	7.5	13	7.75	14.5
159	159	409	659	909	8	13	8.25	14.5
160	160	410	660	910	8.5	13	8.75	14.5
161	161	411	661	911	9	13	9.25	14.5
162	162	412	662	912	9.5	13	9.75	14.5
163	163	413	663	913	10	13	10.25	14.5
164	164	414	664	914	10.5	13	10.75	14.5
165	165	415	665	915	11	13	11.25	14.5
166	166	416	666	916	11.5	13	11.75	14.5
167	167	417	667	917	12	13	12.25	14.5
168	168	418	668	918	12.5	13	12.75	14.5
169	169	419	669	919	13	13	13.25	14.5
170	170	420	670	920	13.5	13	13.75	14.5
171	171	421	671	921	14	13	14.25	14.5
172	172	422	672	922	14.5	13	14.75	14.5
173	173	423	673	923	15	13	15.25	14.5
174	174	424	674	924	15.5	13	15.75	14.5
175	175	425	675	925	16	13	16.25	14.5
176	176	426	676	926	16.5	13	16.75	14.5
177	177	427	677	927	17	13	17.25	14.5
178	178	428	678	928	17.5	13	17.75	14.5
179	179	429	679	929	18	13	18.25	14.5
180	180	430	680	930	18.5	13	18.75	14.5
181	181	431	681	931	1	16	1.25	17.5
182	182	432	682	932	1.5	16	1.75	17.5
183	183	433	683	933	2	16	2.25	17.5
184	184	434	684	934	2.5	16	2.75	17.5
185	185	435	685	935	3	16	3.25	17.5
186	186	436	686	936	3.5	16	3.75	17.5
187	187	437	687	937	4	16	4.25	17.5
188	188	438	688	938	4.5	16	4.75	17.5
189	189	439	689	939	5	16	5.25	17.5
190	190	440	690	940	5.5	16	5.75	17.5
191	191	441	691	941	6	16	6.25	17.5
192	192	442	692	942	6.5	16	6.75	17.5
193	193	443	693	943	7	16	7.25	17.5
194	194	444	694	944	7.5	16	7.75	17.5

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195	195	445	695	945	8	16	8.25	17.5
196	196	446	696	946	8.5	16	8.75	17.5
197	197	447	697	947	9	16	9.25	17.5
198	198	448	698	948	9.5	16	9.75	17.5
199	199	449	699	949	10	16	10.25	17.5
200	200	450	700	950	10.5	16	10.75	17.5
201	201	451	701	951	11	16	11.25	17.5
202	202	452	702	952	11.5	16	11.75	17.5
203	203	453	703	953	12	16	12.25	17.5
204	204	454	704	954	12.5	16	12.75	17.5
205	205	455	705	955	13	16	13.25	17.5
206	206	456	706	956	13.5	16	13.75	17.5
207	207	457	707	957	14	16	14.25	17.5
208	208	458	708	958	14.5	16	14.75	17.5
209	209	459	709	959	15	16	15.25	17.5
210	210	460	710	960	15.5	16	15.75	17.5
211	211	461	711	961	16	16	16.25	17.5
212	212	462	712	962	16.5	16	16.75	17.5
213	213	463	713	963	17	16	17.25	17.5
214	214	464	714	964	17.5	16	17.75	17.5
215	215	465	715	965	18	16	18.25	17.5
216	216	466	716	966	18.5	16	18.75	17.5

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## APPENDIX H COLLECTING LITTERFALL FROM *TOXICODENDRON* SPECIES

This appendix deals with protocol-specific mitigation strategies for dealing with *Toxicodendron* in the course of litterfall and fine woody debris sampling. General strategies for preventing *Toxicodendron* exposure are described in detail in the *Toxicodendron* SOP (RD[12]).

### H.1 Equipment and Materials

**Table 16.** Equipment and materials required for a team of two to minimize exposure to toxic oils from *Toxicodendron* spp. during litter collection

Item Description	Qty	Example Item	Purpose
Small paper bags, pre-weighed, labeled with bag weight	Variable	8# or lunch sack type	<i>Toxicodendron</i> biomass never handled directly again after it is placed in pre-weighed bag.
Cotton gloves, single use	Box of 12	<a href="http://www.globalindustrial.com/p/safety/hands/cotton-canvas-gloves/anchor-4501v-8-oz-cotton-canvas-knit-wrist-1110">http://www.globalindustrial.com/p/safety/hands/cotton-canvas-gloves/anchor-4501v-8-oz-cotton-canvas-knit-wrist-1110</a>	Prevent oil contact with skin.
Disposable PPE outer-wear	Case of 24	Coveralls; <a href="http://disposable-garments.com/shop/koolguard/koolguard-coveralls/">http://disposable-garments.com/shop/koolguard/koolguard-coveralls/</a>	Prevent oil contact with skin, normal clothing.
Large, single-use plastic bags	Box	Trash bag or large Ziploc type bag	Transport used gloves and PPE and minimize toxic oil transfer.
Cleanser, urushiol-specific	1	Tecnu or equivalent; <a href="http://www.teclabsinc.com/products/poison-oak-ivy/tecnu">http://www.teclabsinc.com/products/poison-oak-ivy/tecnu</a>	Clean equipment and surfaces after use.

### H.2 Minimizing Exposure to Toxic Oil in the Field and Lab

Plot locations with *Toxicodendron* spp. present will require a modified sampling strategy to collect and weigh litter dry mass. There are two possible approaches to collection, either of which is acceptable from a science perspective.

Option A: sort all litter material in the field.

Field processing litter will require extra time in the field but all functional groups from the trap can then be treated in a similar manner to *Toxicodendron*. That is, weighed and discarded without removing material from bags.

Option B: sort non-*Toxicodendron* material in the lab

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Sort *Toxicodendron* from the trap, bulk the remainder in a cloth collection bag, sort in the lab (optionally on butcher paper) then decontaminate all surfaces with Urushiol-specific cleanser.

The following are best-practice techniques for minimizing exposure to *urushiol* oils during litterfall collection of *Toxicodendron* species.

1. Prior to field work:
  - ) Count out bags for storing and drying *Toxicodendron* biomass **and** other functional groups (include enough for collection of leaves, fruit and stems in separate bags). Don't mix *Toxicodendron* biomass with any other biomass.
  - ) Pre-weigh (to nearest 0.01 g) and label each paper bag that will be used for storing and drying litter material from traps that include *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 *Toxicodendron* biomass in the field:
  - ) Wear cotton gloves and dispose after single use. Toxic oils can pass through nitrile or latex gloves.
  - ) Bring a clean, new plastic bag to the field for storing and transporting contaminated gloves after use.
  - ) Wear a thin outer layer of disposable PPE over clothes and shoes.
3. After field work is complete, wash clothing and collection bags according to these guidelines or similar:
  - ) While handling and loading unwashed clothing exposed to toxic oils, wear gloves or use a clean cloth to prevent direct contact between your skin and the clothing.
  - ) Wash with ordinary laundry detergent at the highest recommended water temperature.
  - ) Do not overload the machine; the clothes must be allowed to agitate freely.
4. To process *Toxicodendron* biomass in the laboratory:
  - ) Wear cotton gloves while handling *Toxicodendron* or any litter material that may have come in contact with *Toxicodendron* litter in traps.
  - ) Disinfect all tools and lab surfaces used in the sorting process with Technu. Discard gloves.
  - ) Minimize potential spread of toxic oil by putting *Toxicodendron* biomass bags into the same drying oven every time.
  - ) When drying is complete, clean drying oven shelves used for drying *Toxicodendron* biomass bags with hot water and Tecnu. Wear appropriate PPE when cleaning.
  - ) Record weight dried biomass, minus weight of the bag, to nearest 0.01 g. Dried *Toxicodendron* biomass should never leave the bag.
  - ) After weighing, dispose of all biomass bags from traps that contained *Toxicodendron*.



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- Tissue from these traps will not be specimen mounted, or processed for Litter bioarchive and leaf chemistry (i.e., archived and sent for external chemical analysis).

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**APPENDIX I TROUBLESHOOTING**

Sampling Challenge	Proposed solution
Plot is seasonally inundated.	<p><i>Deployment</i> – Initial deployment of traps cannot occur while plots are inundated. If a site experiences areas of seasonal flooding, deployment may occur in all dry plots prior to deployment in inundated plots. Trap deployment is not all or nothing though it is preferable, once all traps are deployed, to sample all plots at the same time. Production is reported as per/year mass/area so there is wiggle room on collection dates. Tracking is easier if everything is on the same schedule.</p> <p>Though PVC is not buoyant, a sealed frame of an elevated trap could be lifted and moved by water in the plot. If it appears likely that a plot may occasionally experience periods of inundation act preemptively by weighting the elevated traps (consider bricks or large rocks) or drilling a couple small holes in the top of the frame to allow air to escape, minimizing float potential.</p> <p><i>Collection</i> - Though at the time of deployment of ground traps the clip strip is cleared of all woody material, inundation will move litter laterally across the landscape, it is likely in these plots; total annual production of fine woody debris will be overestimated since material &gt; 1yr will float into the trap area. It is not practical to attempt to distinguish new litter from old, so all qualifying litter present in the trap area should be collected; record trapCondition = PF to indicate that the trap location was previously flooded. This way a user can search records and identify those at which estimates of annual production are affected by flooding.</p>
Atypical structures in litter samples slow down sorting time	At sites with high diversity of species, it may be difficult to identify structures that are only occasionally encountered in litter samples. One solution may be to create a reference collection to make sorting more efficient. Collections may include: pollen cones, seed cones, seeds, or flower parts. Creation of a litter reference collection is at the discretion of domain staff and is not a requirement imposed by Science. For distinguishing structures from flowers vs. fruits, one approach may be to use phenological cues to sort unattached flower/seed structures into the appropriate functional group.
Quarantine in effect at site	Discontinue sampling, document quarantine issues via a problem ticket. Coordinate with Domain Manager, HQ Permitting, and regulatory agency to determine how sampling should proceed.
Elevated traps overtopped by plant growth	Manually remove plants growing up and on elevated litter traps, as well as plants growing beneath and immediately around the trap that are likely to grow up trap legs. Ground tarps may be used to minimize growth of particularly aggressive plants beneath elevated traps. Additionally, adding weights (or a rock) to the screen may help traps remain upright if vegetation does threaten to disturb the trap between maintenance bouts.

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Sampling Challenge	Proposed solution
Unexpected material collected in litter trap	<p>All qualifying plant material present in the elevated traps should be collected. Galls, for example, shouldn't be removed from litter but should be sorted with the functional group from which the source tissue originates; a gall on a twig should be sorted with twigs and branches, a gall on a leaf should be sorted with leaves.</p> <p>Plant material that may not originate from overhanging vegetation but does qualify according to the guidelines provided in this protocol should be collected. For example nest material including grass, twigs, herbaceous plants, and moss, collected and transported by birds or small mammals still represents material produced within a given year, presumably from the plot or nearby areas. Nest material likely contains many different tissue types some of which may not be identifiable, it is therefore acceptable to sort all nest material in the 'other' category.</p> <p>Seeds from fruits consumed elsewhere then deposited by birds represent plant material produced in the current year that would otherwise have landed in the 0.5m<sup>2</sup> patch of ground, these seeds should be collected and sorted in the 'seeds' category.</p> <p>An exception is made for sap. Do not place pieces of sap or any other plant exudate, in the drying ovens, under heat, these materials will be lost to melting or pose safety concerns due to natural flammability. Exudates are not explicitly accounted for in net primary productivity calculations. Small amounts of sap bound in woody seed cones does not generally pose a fire hazard. However, if the volume of exudate is great enough to saturate a paper bag or there is any risk of sap dripping and collecting on heating elements in the oven, exclude the material from the sample and record # of female cones were discarded.</p> <p>Non-plant material, including invertebrates and animal by-products, found in a field trap should be removed when collected and discarded according. Dead vertebrates found in the trap should be collected and processed according to the guidelines in the State Collection Permit.</p>

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**APPENDIX J ALTERNATIVE TRAP MATERIALS**

Based on site conditions, it may be necessary to modify materials used in construction of the elevated litter trap.

Here are suggestions employed by some NEON domains to address specific issues:

- ) **Destruction by bears. Trap frame material.** The design specifies PVC but at some sites, this material may be attractive to bears resulting in widespread damage to traps. Wood traps, constructed at the domain office are an approved alternative (Figure 12 and Figure 13). In addition, use of non-oxidizing metal would also be acceptable, e.g. conduit.



Figure 12. Elevated trap destroyed by bears at SCBI.



Figure 13. Wood elevated trap frame at Konza.

- J **Destruction by rodents.** Application of a non-toxic capsaicin rodent deterrent spray on trap surfaces will render the trap material un-palatable without causing undue harm to surrounding vegetation or wildlife. Spray must be re-applied to maintain efficiency. If zip ties are targeted, mesh may be secured to trap with the aluminum wire used to attach numbered tags to shrubs and saplings.
- J **Removal of material by wind.** Traps may be weighted by placing baseball-size rocks in the elevated trap to prevent wind from disturbing the mesh and forcing collected material out of the trap. Additionally, using a larger piece of mesh than the 4ft x 4ft piece provided in the kits will create more sag, a deeper bowl (i.e., >20 cm specified in SOP A) that may trap material more effectively in windy conditions.

Elements that may **not** be modified:

- J Trap shape, elevated traps must be square
- J Trap size, elevated traps must be 0.5m<sup>2</sup> (70 cm x 70 cm)
- J Use of non-oxidizing materials, if metal is used for any portion of the trap, it must resistant to rust (aluminum, stainless steel...)