TOS PROTOCOL AND PROCEDURE: CORE SAMPLING FOR PLANT BELOWGROUND BIOMASS

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Change Record

REVISION	DATE	ECO #	DESCRIPTION OF CHANGE
A	03/25/2011	ECO-00148	Initial release
B	01/20/2015	ECO-02273	Production release, template change, method improvements
C	02/26/2015	ECO-02702	Migration to new protocol template

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1 OVERVIEW

1.1 Background

Belowground biomass represents a substantial component of the total plant biomass and plant carbon in terrestrial ecosystems, yet belowground biomass stocks and turnover remain very poorly understood both in space and in time. This is in large part due to the inherent difficulties associated with measuring plant parts that are obscured within soil. Developing a better understanding of how much belowground plant biomass there is, as well as how much of that biomass is produced and decomposed within a given year, is therefore crucial to improving our understanding of how terrestrial ecosystems respond to environmental changes. Here, we define fine roots to be roots with diameter ≤ 10 mm (Burton and Pregitzer 2008). In combination with the belowground biomass soil pit sampling conducted during site construction (RD[09]), the soil core sampling described here will enable estimation of the amount of belowground plant biomass ≤ 10 mm diameter associated with the dominant vegetation at a site. In addition, data from the NEON soil array minirhizotrons (MR) will provide an insight into how fast that biomass is produced and decomposed. Taken together, these two NEON datasets will facilitate the calculation of belowground plant productivity on a continental scale.

Fine root frequency, biomass, and turnover rates differ substantially across size classes. In general, larger size classes constitute more of the biomass than smaller size classes, but larger roots also turn over much more slowly and therefore contribute less to annual belowground net primary productivity (BNPP) than fine roots do (Steinaker and Wilson 2005, Tierney and Fahey 2007). The most common and robust method to measure belowground biomass in both forest and grassland ecosystems is via relatively large diameter (5–10 cm) cores (Tierney and Fahey 2007, Burton and Pregitzer 2008). As such, NEON will use the soil coring technique to estimate belowground fine root biomass. Because high-volume coarse roots occur infrequently in the soil, higher volume samples result in more accurate estimates of belowground biomass (Taylor et al. 2013). However, large sample volumes require a significant amount of time to sieve and sort in the laboratory. Given that time is limiting, there is therefore an inherent trade-off between the number and size of samples that must be resolved (Berhongaray et al. 2013). NEON will use a 3-inch outside diameter (66.5mm ID) soil corer for belowground biomass sampling, and samples will be cored to 30 cm depth in order to be consistent with the sampling depth used for soil biogeochemistry and microbe sampling (RD[07]). At each sampling location, two 30 cm cores will be pooled, for a total sample volume of 2722 cm^3 per sampling location. Sample volumes of this size should be sufficient to encounter roots up to 10 mm diameter in the majority of soil samples (Taylor et al. 2013).

To account for differences in BNPP across fine root diameter classes, researchers typically sort roots within core samples into various size classes, and then calculate fine root production separately for each class. Following Burton and Pregitzer (2008), NEON will sort roots within each core into < 0.5 mm, 0.5–1 mm, 1–2 mm, and 2–10 mm categories. Sampled roots > 2 mm diameter will contribute to belowground

biomass estimation, but not estimation of BNPP, because minirhizotron data for roots > 2 mm diameter will likely be insufficient to estimate a turnover coefficient for this size class with reasonable uncertainty.

Soil samples are sieved to remove soil, picked to separate roots from other organic material, and roots are then sorted to diameter size class. Picking and sorting roots is time consuming, and many researchers employ cutoffs to limit the amount of time spent searching for small root fragments – e.g. a common cutoff of 1 cm means that root fragments < 1 cm length are ignored and discarded. However, root fragments < 1 cm length can contribute > 50% of the total root biomass in some samples (Koteen and Baldocchi 2013). To account for the biomass of small root fragments, NEON will perform a one-time assessment of this biomass pool per site during the first 5 y of Operations, and standard root sampling events will adopt a 1 cm length cutoff.

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

The author is grateful for time and detailed advice provided by Daniel Milchunas and Mark Lindquist at the Shortgrass Steppe LTER program. In addition, SOP D "Dilution Sampling for Fine Root Biomass Fragments" is based on the work of Koteen and Baldocchi (2013).

2 RELATED DOCUMENTS AND ACRONYMS

2.1 Applicable Documents

Applicable documents contain higher-level information that is implemented in the current document. Examples include designs, plans, or standards.

AD[01]	NEON.DOC.004300	EHS Safety Policy and Program Manual
AD[02]	NEON.DOC.004316	Operations Field Safety and Security Plan
AD[03]	NEON.DOC.000724	Domain Chemical Hygiene Plan and Biosafety Manual
AD[04]	NEON.DOC.001155	NEON Training Plan
AD[05]	NEON.DOC.050005	Field Operations Job Instruction Training Plan
AD[06]	NEON.DOC.000914	NEON Science Design for Plant Biomass and Productivity
AD[07]	NEON.DOC.014051	Field Audit Plan
AD[08]	NEON.DOC.000824	Data and Data Product Quality Assurance and Control Plan

2.2 Reference Documents

Reference documents contain information that supports or complements the current document. Examples include related protocols, datasheets, or general-information references.

RD[01]	NEON.DOC.000008	NEON Acronym List
RD[02]	NEON.DOC.000243	NEON Glossary of Terms
RD[03]	NEON.DOC.005003	NEON Scientific Data Products Catalog
RD[04]	NEON.DOC.001271	NEON Protocol and Procedure: Manual Data Transcription
RD[05]	NEON.DOC.002135	Datasheets for TOS Protocol and Procedure: Core Sampling for Plant Belowground Biomass
RD[06]	NEON.DOC.001925	NEON Raw Data Ingest Workbook for TOS Belowground Biomass Soil Core
RD[07]	NEON.DOC.014048	TOS Protocol and Procedure: Soil Physical, Chemical, and Microbial Measurements
RD[08]	NEON.DOC.000987	TOS Protocol and Procedure: Measurement of Vegetation Structure
RD[09]	NEON.DOC.001708	TOS Protocol and Procedure: Soil Pit Sampling for Plant Belowground Biomass
RD[10]	NEON.DOC.001717	TOS Standard Operating Procedure: TruPulse Rangefinder Use and Calibration

2.3 Acronyms

Acronym	Definition
BNPP	Belowground net primary productivity
MR	Minirhizotrons
OM	Organic material

3 METHOD

The Standard Operating Procedures (SOPs) presented in this protocol describe tasks that, when taken together, allow estimation of plant belowground root and non-root biomass across four root size classes. These SOPs are:

- **SOP A: Preparing for Sampling.** Instructions to prepare for sampling for SOP B, SOP C, and SOP D.
- **SOP B: Soil Core Sampling in the Field.** Collecting soil core samples from peak herbaceous biomass clip harvest "cells" in the field, and recording required data and metadata. Includes core preservation prior to laboratory processing (preservation methods are described fully in Appendix F).
- **SOP C: Processing Belowground Biomass Samples in the Laboratory.** Steps to wash, sieve, and separate roots from mineral soil and organic matter, and once roots are separated, steps to dry, weigh, grind, and sub-sample roots for chemical analysis.
- **SOP D: Dilution Sampling for Fine Root Fragments.** A one-time per site procedure to quantify the amount of fine root biomass present in small root fragments < 1 cm length. By carrying out this SOP, it is possible to ignore root fragments < 1 cm length in SOP C while still generating accurate fine root biomass estimates, resulting in significant long-term time savings.

Belowground biomass soil core sampling takes place in 400 m^2 sampling units located within Tower plots or subplots (Figure 1). Soil core sampling does not occur in Distributed or Gradient plots. In 20m x 20m Tower plots, there will be one within-plot sampling location per bout. In larger 40m x 40m Tower plots (i.e. four 400 m^2 subplots per plot), two subplots per plot will be randomly chosen for soil core sampling per bout, and there will be one soil core sampling location per subplot. This strategy means that:

- At sites with thirty 20m x 20m Tower plots, there will be n=30 pooled soil core samples.
- At sites with twenty 40m x 40m Tower plots, there will be n=40 pooled soil core samples.

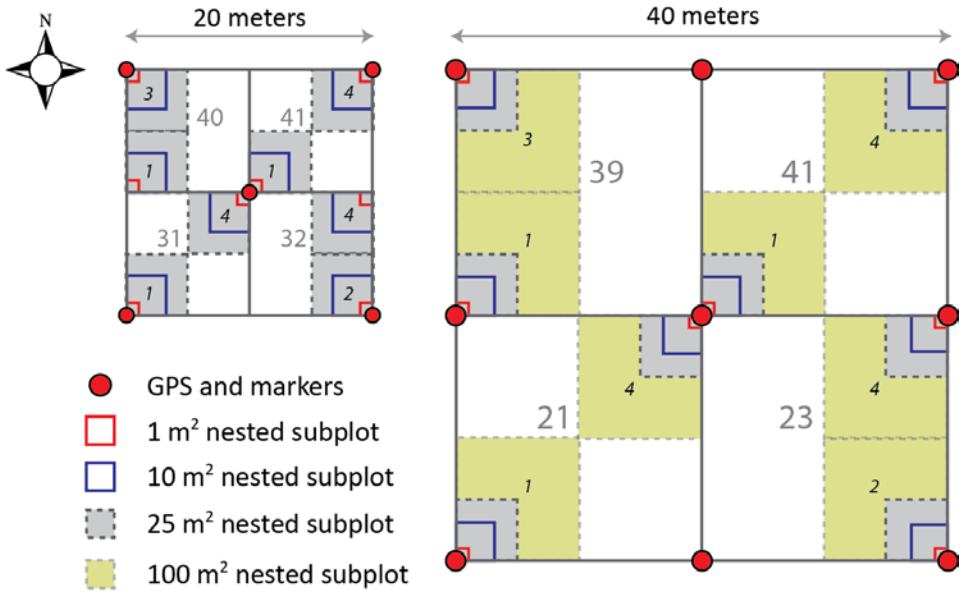

Figure 1. Illustration of two NEON plot sizes used for belowground biomass soil core sampling. Grey numbers indicate subplotIDs, but soil core sampling is only dependent on subplots for 40m x 40m plots. Italic black numbers show the location of nested subplots that are used for % cover and diversity measurements. Soil core sampling is prohibited within 1 m^2 and 10 m^2 nested subplots.

Within each 400 m^2 plot or subplot selected for belowground biomass core sampling, soil sampling locations in a given year are spatially co-located with the clip harvest "cell" used for the peak herbaceous biomass clip-harvest in that year. Clip harvest cells are 0.5m x 3m, are sequentially numbered, and coordinates are assigned to the SW corner of a 0.1m x 2m clip-strip that is centered within each clip harvest cell. These coordinates are relative to the SW corner of the plot or subplot (i.e. the SW corner of the plot or subplot is defined as having coordinates [0,0]) (Figure 2, *left*). To determine soil coring locations, technicians consult a plot-specific "Clip List"to determine which clip harvest cell was (or will be) used for the peak biomass harvest in the current growing season. Within each clip harvest cell, one 66.5mm ID (3" OD) x 30cm length soil core sample should be taken from anywhere within each of the areas to the North AND South of the 0.1m x 2m clip-strip (Figure 2, *right*). These two soil core samples are then pooled into one sample for the plot or subplot. To avoid roots and rocks, technicians may sample from anywhere within the areas shown in Figure 2.

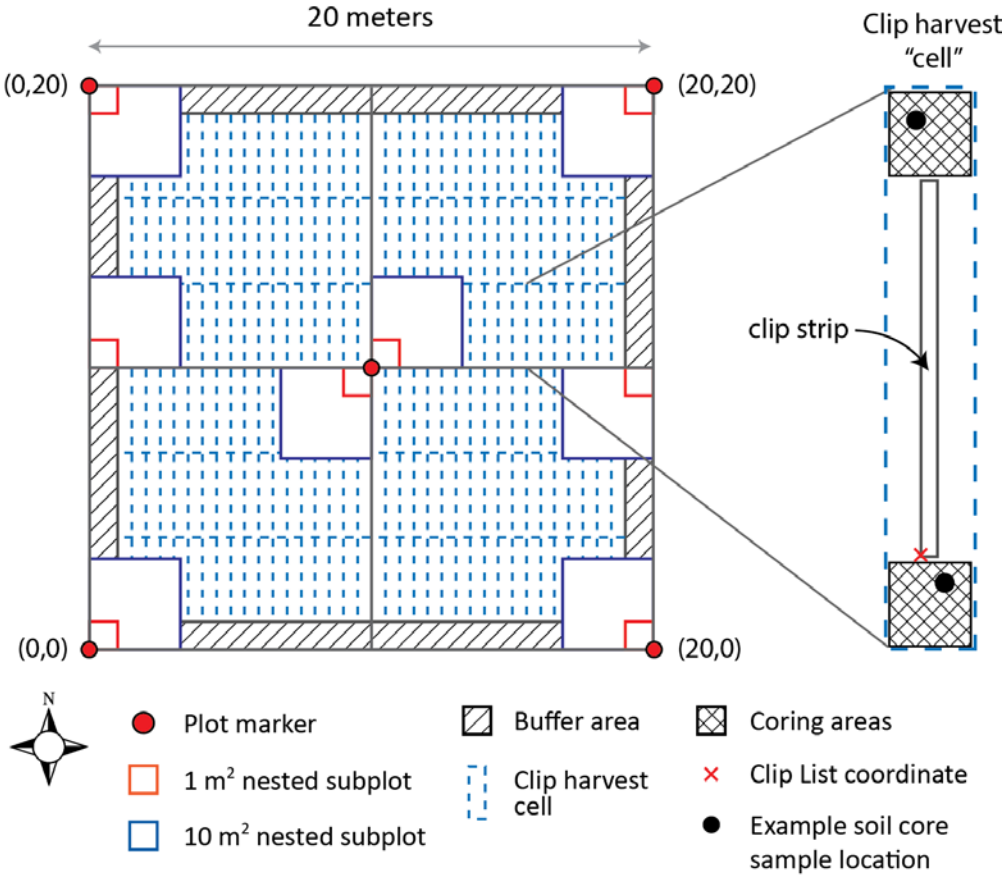

Figure 2. A 20m x 20m Tower plot showing the locations of 0.5m x 3m clip harvest cells used for belowground biomass soil core sampling (*left*); the largest 25 m^2 nested subplot has been omitted for clarity. Within a clip harvest cell selected for soil core sampling, one core is collected from each of the areas to the North and South of the clip-strip (*right*). The red "x" shows the coordinates provided in the Herbaceous Biomass Clip Lists.

Prior to driving the corer into the ground, crowns, corms, rhizomes, and other perennial belowground parts that are not roots are removed from the top 3 cm of soil and placed in a labeled envelope or bag. In some ecosystems, these non-root belowground plant parts may constitute a significant portion of the belowground biomass, but because they typically grow very slowly compared to roots, their biomass is separated from root biomass in order to improve the accuracy of BNPP estimates.

After sampling from a given clip strip is completed, it is necessary to backfill the hole created with a material approved by the site host (e.g. sand, dirt, etc.).

Once soil samples are removed from the ground, they should be stored in a cooler chilled with re-usable cold packs at all times until they are transported back to the laboratory for preserving or processing (sieving, picking, drying, etc.). Coolers may be kept cold with re-usable cold packs, and cold packs should be exchanged for freshly "charged" cold packs every 12 hours. After soil core samples have been transported back to the laboratory, they should be preserved as soon as possible to halt decomposition

of severed roots. Preservation allows cores to be processed at a later date, and is accomplished via either: 1) Drying in a 65 ˚C oven drying oven for 48 h; or 2) freezing indefinitely in a -20 ˚C freezer.

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

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

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

A number of protocol-specific QC checks may be used to ensure that:

- Equipment is used properly in the field
- Plant parts are sorted properly into functional groups in the field
- Soil samples are processed in the lab according to the protocol, and
- Dried root biomass is weighed and ground properly

4 SAMPLING SCHEDULE

4.1 Sampling Frequency and Timing

Table 1. Sampling frequency for belowground biomass soil core procedures on a per SOP basis

SOP	Plot Type	Plot Number	Sampling Events	Yearly Interval	Remarks
SOP B	Tower	All	1X per sampling year	5y	Yearly interval may be more frequent following per site analysis of MR data
	Distributed, Gradient	NA	NA	NA	Distributed and Gradient plots are not cored for belowground biomass.
SOP C	Tower	All	1X per sampling year	Same as SOP B	***Soil core preservation (drying, freezing) should occur as soon as possible following field work.***
SOP D	Tower	All	1X per site	NA	"Dilution sampling" occurs only once per site, within the first 5y of Operations.

A given sampling bout should ideally be concluded within 1 month of initiation so that the belowground standing crop does not change appreciably during the time that all target plots are sampled. This ensures that data collected across all plots within a given sampling bout are as comparable as possible.

At sites where plots may be seasonally submerged (e.g. D03 DSNY), core sampling must be timed to avoid standing water in potential soil core locations. If a plot is partially submerged but still accessible for terrestrial sampling, "cells" that contain standing water must be rejected for soil core sampling, a new clip-location "cell" must be chosen, and it must be noted in the "remarks" field that "soil core not the from peak clip cell."

After soil cores are sampled from a given clip strip, the following points are critical with respect to timing:

- Place soil core samples immediately into a cooler, and keep stored with re-usable cold packs until samples can be preserved by freezing at -20 ˚C or drying.

4.2 Criteria for Determining Onset and Cessation of Sampling

It is theoretically desirable to perform belowground biomass core sampling when the root crop is at peak biomass. However, peak belowground biomass does not necessarily correspond with peak aboveground biomass, and in some ecosystems, does not vary in a predictable manner within a growing season from year to year (Milchunas and Lauenroth 2001). Combined with the fact that belowground biomass timecourse data are unavailable for the majority of NEON sites, the timing of belowground biomass soil core sampling is guided by these two factors, listed in order of importance:

- **Date of peak biomass herbaceous clip harvest**: Perform belowground biomass soil coring either immediately before, during, or immediately after the herbaceous clip harvest associated with the greatest aboveground *peak biomass*.
- **Soil hardness**: At some sites, peak herbaceous biomass occurs during hot, dry parts of the year when soils are extremely hard and veritably impenetrable due to high clay content (e.g. D10 CPER). At sites where these conditions occur, the timing of soil core sampling may be moved to earlier in the growing season when soil moisture is more conducive to core sampling.
    - If soil hardness dictates the timing of core sampling, it is not important exactly when in the growing season sampling occurs, but once an acceptable sampling window is chosen for a given site, all future sampling within that site should occur within ± 2 weeks of that sampling window.

4.3 Timing for Laboratory Processing and Analysis

Because root biomass continues to be biologically active after sampling, and because root structures are delicate and easily decomposed by soil organisms, it is important to preserve soil core samples in the laboratory as soon as possible following collection in the field in order to prevent mass loss. Ideally, soil core samples are preserved within 24 h of collection in the field. In the event that it is not possible to preserve cores within 24 h of collection, it is acceptable to keep soil cores in cold storage for up to a maximum of 72 hours before they are preserved in the laboratory.

Once soil core samples are preserved, there are no scientific limits on the length of time that may pass before samples are put through the laboratory wet-sieving procedure (SOP C).

4.4 Sampling Timing Contingencies

Table 2. Contingent decisions

Delay/Situation	Action	Outcome for Data Products
Hours	If delay prevents sampling both cores from a given clip strip: 1. Bag and label first sampled core, 2. Ensure all small bags of sorted biomass are labeled, 3. Place small bags into a 25# bag, label, and place 25# bag in a cooler. 4. Resume core sampling in same clip strip ASAP	None
	If delay occurs between plots or subplots: Resume core sampling ASAP.	
1-14 days	If delay prevents sampling both cores from a given clip strip: 1. Bag and label first sampled core, 2. Ensure all small bags of sorted biomass are labeled, 3. Place small bags into a 25# bag, label, and place 25# bag in a cooler. 4. Preserve first core if delay is > 7 days, 5. Resume core sampling in same clip strip ASAP	Increased uncertainty in belowground biomass estimates. A finite but indeterminable amount of root decomposition will occur in sampled cores until they are preserved.
	If delay occurs between plots or subplots: 1. Preserve previously sampled cores if delay is > 7 days, 2. Resume core sampling ASAP.	
14+ days	If delay prevents sampling both cores from a given clip strip: 1. Bag and label first sampled core, 2. Ensure all small bags of sorted biomass are labeled, 3. Place small bags into a 25# bag, label, and place 25# bag in a cooler. 4. Preserve first core, 5. Resume core sampling in same clip strip ASAP	Potentially substantial increases in uncertainty for belowground biomass estimates. If delay prevents completing sampling from all plots or subplots within a 1 month window, belowground biomass may fluctuate substantially.
	If delay occurs between plots or subplots: 1. Preserve previously sampled cores 2. Resume core sampling ASAP.	

Once cores are preserved after field sampling, lab processing may occur at any time, and there are no consequences associated with the amount of time elapsed between core preservation and the onset of laboratory work. However, once laboratory processing is initiated on a given sample, processing should be carried all the way through without stopping.

For QA/QC of the weighing and data entry portion of the laboratory work, select 10% of the previously dried, weighed samples for QA/QC per sampling bout. Technicians re-weigh and record the QA mass in the "qaRootMass" field of the "Lab Weighing Datasheet", and then transcribe QA mass data to the "qaRootMass" field in the "bbc_rootmass_in" ingest sheet.

5 SAFETY

This document identifies procedure-specific safety hazards and associated safety requirements. It does not describe general safety practices or site-specific safety practices.

Personnel working at a NEON site must be compliant with safe field work practices as outlined in the Operations Field Safety and Security Plan (AD[02]) and EHS Safety Policy and Program Manual (AD[01]). Additional safety issues associated with this field procedure are outlined below. The Field Operations Manager and the Lead Field Technician have primary authority to stop work activities based on unsafe field conditions; however, all employees have the responsibility and right to stop their work in unsafe conditions.

For the field procedures, safety training is required to properly use the soil corer. Soils may contain fungi that may cause illness. Refer to the Operations Field Safety and Security Plan (AD[02]) for details on locations and appropriate precautions.

For the laboratory procedures, safety training is required to operate drying ovens and the grinding mill.

If soil core samples may contain roots of *Toxicodendron spp.*, consult Appendix G for best practice procedures to minimize exposure to toxic oils.

6 PERSONNEL AND EQUIPMENT

6.1 Equipment

The following equipment is needed to implement the procedures in this document. Equipment lists are organized by task. They do not include standard field and laboratory supplies such as charging stations, first aid kits, drying ovens, ultra-low refrigerators, etc.

Table 3. Equipment list – Soil-core sampling belowground biomass in the field

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
Durable Items						
MX103276	R	Soil core sampling tube, 36” length, 3” OD	Generate soil core sample	All	1	N
MX103277	R	Soil core drive head assembly	Works with slide hammer to drive soil core tube into soil	All	1	N
MX103278	R	Soil core drive head pin, 3” length	Attach drive head assembly to core tube	All	1	N
MX103279	R	Soil core quick relief bit, 3” OD*	Attach to soil core sampling tube	Standard bit for coring most soils	1	N
MX103280	R	Soil core slide hammer, 16#	Drive sampling tube into soil	All	1	N
MX103281	R	Soil core basket retainer, 3” adapter	Attach basket retainer system to sampling tube	Sandy soils that do not hold together	1	N

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
MX103282	R	Soil core basket retainer, 3” basket	Retain sandy soil in sampling tube	Sandy soils that do not hold together	2	N
MX103283	R	Soil core basket retainer, 3” bit	Bit that works with basket retainer	Sandy soils that do not hold together	1	N
	S	GPS unit, pre-loaded with plot locations	Navigate to plots or subplots	All	1	N
MX100322	R	TruPulse 360R laser rangefinder, current declination entered	Locate clip strip within a plot or subplot	Slope >20%, brushy	1	N
MX103218	R	Foliage filter for laser rangefinder	Facilitates use of TruPulse in brushy conditions	Brushy vegetation	2	N
	R	Reflective surface (bicycle reflector or reflective tape on back of field notebook/clipboard)	Accurate location of clip strip with TruPulse in “FLT” mode	Used with TruPulse	1	N
	S	Extra battery for TruPulse (CR123A type)	Battery backup	Used with TruPulse	2	N

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
	R	Fiberglass meter tape (30m or longer)	Locate clip strip within plots or subplots	Plot slope <20%; grassland, savannah	1	N
	R	Hand clippers, fine tip	Remove aboveground plant parts from soil coring location	All	1	N
MX100721	R	Soil knife, hori-hori style	Loosen soil at surface to expose non-root plant parts	All	1	N
	R	Large chest-style cooler, with frozen cold packs	Keep core samples cool, slow down root decomposition	All	2+	N
	R	Sharpies	Label paper bags	All	2	N
Consumable items						
MX104362	R	Chaining pins, steel	Stretching tapes to enable location of target clip strip	Plot slope <20%; grassland, savannah	2	N
	R	4”x 5” pin flags with PVC stakes	Accurate location of clip strip; PVC stakes avoid magnetic interference with compass or TruPulse	All	6	N

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
	R	Coin envelopes, 3⅜" x 6", Kraft paper (e.g. Uline S-14720)	Store and organize non-root below-ground biomass	All	40+	N
	R	Plastic freezer bags, 1.5 or 2 gallon	Store and organize soil core samples	All	40+	N
	R	Pencils	Record sampling metadata	All	2	N
	R	Herbaceous biomass "Clip Lists"	Identify clip strip associated with peak biomass clip harvest	All	Varies	N
	R	Belowground biomass "Random Subplot Lists"	Identify subplots for soil core sampling	Tower plots ≥ 1600 m^2	Varies	N
RD[05]	R	Belowground biomass "Field Coring Datasheets"	Record sampling metadata	All	Varies	N

R/S=Required/Suggested

* Bits with greater relief may be required to prevent soils with high clay content that are prone to expansion from getting stuck in the soil core tube. See http://www.soilsample.com/tooling/soiltubes.htm for available bits and soil core accessories.

Table 4. Equipment list – Sieving belowground biomass cores, separating roots from soil organic matter, and drying root samples

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
Durable Items						
	R	Root washing station	Remove mineral soil from organic material	All	1	N
	R	Soil sieve, 2 mm stainless mesh, 8” or 12” diameter	Remove mineral soil from organic material	All	4-5	N
	R	Soil sieve, 250 µm stainless mesh, 8” or 12” diameter	Remove mineral soil from organic material	All	4-5	N
	R	Rectangular enamel pan, white (app. 33 cm x 20 cm, or 13”x 9”)	Facilitates separating roots (which float) from mineral particles	All	2	N
	R	Forceps, blunt tip, stainless steel	Separate roots from organic material	All	2	N
	R	* Wire gauge with openings approx. 2mm, 1mm, and 0.5mm	Sort roots into size classes during sieving and picking	All	2	N
	R	Grinding mill, Wiley, 20 mesh	Grind fine root samples for chemical analysis	All	1	N
	R	Sample microsplitter	Creates identical sub-samples from ground sample	Large root volumes	1	N
	R	Hi-back pans for sample microsplitter	2 per splitter; receives split sub-sample	With micro splitter	2	N
	R	Sharpie, extra fine tip	Labeling envelopes and scint vials	All	2	N

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
	S	5-gallon plastic bucket	Soak core sample prior to sieving to break up cohesive clays and rehydrate roots	Cores preserved by drying	2	N
Consumable items						
	R	Pencils	Record dry weight of root samples	All	2	N
RD[05]	R	Lab Weighing Datasheet	Record dry weight of root samples	All	Variable	N
	R	Scintillation vials with caps, 20 mL volume	Containers for storing ground split sub-samples	All	Variable	N
	R	Large plastic weigh boats	Weigh relatively large quantities of dried root samples	Large root quantities	50+	N
	R	Clasp envelopes, 6”x 9”, Kraft paper	Store and organize sieved roots during and after drying	Large root quantities	50	N
	R	Coin envelopes, 3⅜”x6”, Kraft paper	Store and organize sieved roots during and after drying	Small root quantities	50	N
	R	Paper bag, 25# Kraft	Organize root samples in the drying ovens	All	20	N
	S	Small plastic weigh boats	Weigh relatively small quantities of dried root samples	Small root quantities	50+	N

R/S=Required/Suggested

* Gauge 12 = 2.05 mm, gauge 18 = 1.02 mm, and gauge 24 = 0.51 mm

Table 5. Equipment list – Dilution sampling for fine root biomass fragments < 1 cm

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable Items					
	R	Soil sieve, 53 µm stainless mesh, 8” or 12” diameter	Consolidate residual fraction from both cores per clip strip, rinse, and transfer to beaker for dilution	2	N
	R	Magnetic mixing plate	Randomize aqueous suspended residual fraction	1	N
	R	Magnetic stir bar, 2” to 3” length	Randomize aqueous suspended residual fraction	2	N
	R	Beaker, 1 L	Hold smaller aqueous suspended residual fraction volumes	2	N
	R	Beaker, 2 L	Hold larger aqueous suspended residual fraction volumes	2	N
	R	Plunger, diameter minimally less than beaker diameter (RD[06])	Stop mixing vortex, randomize aqueous suspended residual fraction	1	N
	R	Syringe, 40 mL, with tip cut off to make a 1 cm diameter aperture	Aspirate sub-sample from randomized aqueous residual fraction	2	N
	R	Plastic laboratory squirt bottle, filled with water	Rinse syringe following sub-sampling	1	N
	R	Aluminum weighing dishes, 65 mL (e.g. Fisher #: 08-732-102)	Hold and dry root and organic material from sub-samples.	200	N

Item No.	R/S	Description	Purpose	Quantity	Special Handling
	R	Forceps, fine tip	Pick small root fragments apart from organic material	2	N
	S	Sheet tray, baking or equivalent	Transfer aqueous samples in aluminum dishes to drying ovens	1	N
Consumable Items					
None					

R/S=Required/Suggested

Table 6. Equipment list – Minimizing exposure to toxic oils from roots of *Toxicodendron spp*. that may be encountered

Item No.	R/S	Description	Purpose	Quantity
Durable Items				
	R	Labeled clippers, dedicated to clipping *Toxicodendron spp.* (see Table 4)	Prevent spread of toxic oils to multiple clippers	1
	R	Labeled sieve set(s), dedicated to sieving samples containing *Toxicodendron.* (Set contains 2mm sieve and 250 µm sieve. See Table 4.)	Prevent spread of toxic oils to multiple sieves.	As needed
	R	Labeled forceps, blunt tip, stainless steel; dedicated to *Toxicodendron* samples	Prevent spread of toxic oils to multiple forceps.	As needed
Consumable Items				
	R	Cleanser, urushiol-specific, Tecnu or equivalent (example)	Clean sieves and forceps after use.	As needed
	R	Cotton gloves, single use (example)	Prevent oil contact with hands while collecting cores in the field.	Box of 12
	R	Disposable PPE outer-wear (example)	Prevent oil contact with skin and clothing while collecting cores in the field.	Case of 24
	R	Large, single-use plastic bags (trash bag or large Ziploc-type bag)	Transport used gloves and PPE from the field and minimize toxic oil transfer.	Box
	R	Latex gloves, single use	Prevent hand exposure during sieving.	Box
	R	Envelopes, pre-weighed, labeled with envelope weight, coin or clasp type (see Table 4)	*Toxicodendron* roots never handled directly again after they are placed in pre-weighed envelope.	As needed

R/S=Required/Suggested

6.2 Training Requirements

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

For the field component of this protocol, technicians must be trained in navigating to points in the field with a GPS and manual methods. Most critically, technicians must be trained to quickly identify commonly encountered types of belowground plant parts at the sites within the region of employment (e.g. crowns, corms, rhizomes, roots, etc.).

Training for both the field and laboratory work must emphasize the importance of consistent, detailed labeling of all samples. ***Improper or inconsistent labeling is the most common and problematic error associated with this work!***

6.3 Specialized Skills

For the field work, a minimum of 2 field technicians are required for harvesting soil cores due to weight of equipment and soil cores. Technicians must possess a demonstrated ability to identify crown material associated with perennial grasses.

For the laboratory work, 1-2 laboratory technicians are required to wash, dry, weigh, grind, and sub-sample belowground biomass samples for shipment to external analytical or archive facilities.

6.4 Estimated Time

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

An experienced two-person team will require approximately 1 hour to locate and delineate the target clip cell from within a given plot or subplot in the field, and extract two soil core samples from the target clip cell. In the laboratory, it requires between 0.5-3 h per soil core to perform wet-sieving and picking of root fragments down to 1 cm length. The exact time depends on soil type and vegetation type, and the average time required is approximately 2 h per soil core.

Time requirements for the laboratory dilution method (SOP D) are based on published values (Koteen and Baldocchi 2013), and range between 1.5-2.5 h per sample.

7 STANDARD OPERATING PROCEDURES

SOP A Preparing for Sampling

A.1 Preparing for soil core sampling in the field (SOP B)

1. Using local knowledge of the soils present at the site, determine the type of soil coring bit that is required for the soil conditions at the site (i.e. the degree of relief needed inside the bit) (Table **7**). A "quick relief" bit is suitable for most soils.

Table 7. Soil core bits and the soil types and conditions in which they should be used.

Bit Type	Intended Soil Conditions or Soil Type
Standard taper	Dry soils
Quick relief	Clay/Loam soils (i.e. "typical soils"); relief inside bit allows for moderate expansion of core inside soil core tube, prevents sample from getting stuck
Heavy duty quick relief	Heavy clay soils; additional relief allows for additional expansion of core inside soil core tube
Extra heavy duty quick relief	Extra heavy expansive clay soils; allows for maximal expansion of core inside soil core tube
Basket retainer bit	Works with basket retainer and basket retainer adapter to retain sandy, non-cohesive soil samples inside the soil core tube

2. Prepare equipment and material according to Table 8 below.

Table 8. Actions required to prepare equipment and materials for belowground biomass soil core sampling in the field (SOP B). Equipment listed here are only those items that require preparation actions before sampling; the full equipment list is provided in Table 3.

Item Description	Action(s)
GPS unit	• Charge • Load target plot locations
Compass, mirror-sight, adjustable declination	Check/set correct declination*
TruPulse 360R laser rangefinder and clinometer	• Check battery, charge (if possible) • Clean lenses with lens cloth or lens tissue (if necessary) • Check/set correct declination*. See RD[10]. • Calibrate tilt-sensor (only necessary after severe drop-shock; see RD[10]).
3" OD (66.5mm ID) soil core tube and bit assembly	Measure 30 cm from the bottom of the bit, and mark on the tube with electrical tape.
Re-usable cold packs	Place in -20 ˚C freezer
Freezer, -20 ˚C	Clear sufficient space to freeze core samples after field sampling, and temporarily store until laboratory processing takes place.
Hand clippers	Clean and sharpen blades (if necessary)
Sand, or other site-specific material	Ensure supply is sufficient for backfilling soil core holes.
Belowground biomass core "Field Coring Datasheet"	Print as needed on waterproof copy paper
Herbaceous biomass Clip Lists	Print as needed on waterproof copy paper
Belowground biomass core "Random Subplot List"	Print as needed on waterproof copy paper

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

A.2 Preparing for processing soil cores in the laboratory (SOP C)

1. Empty and clean root washing station sediment traps.
2. Clear space in drying oven for drying root samples.
    a. Set oven 1 temperature to 65°C.
    b. Set oven 2 temperature to 105°C.
3. Print lab weighing datasheets as necessary.

A.3 Preparing for dilution sampling for fine root fragments (SOP D)

1. Empty and clean root washing station sediment traps.
2. Attach French press plunger cross piece to bottom of plunger stick with nut.
3. Label aluminum weigh tins with unique tinID (Figure 6).
4. Print lab dilution datasheets as necessary.

SOP B Soil Core Sampling in the Field

1. Navigate to the plot or subplot to be sampled.
2. Use the plot or subplot-specific Herbaceous Clip List to identify the clip strip that was (or will be) used for the peak herbaceous biomass clip harvest.
   - The Clip List provides the randomized list of potential clip-strip locations per plot or subplot.
   - Coordinates provided for each clip strip correspond to the SW corner of the clip-strip – i.e. the area from which herbaceous biomass is harvested (Figure 2).
   - The Clip List also indicates which clip strips have already been harvested or rejected.
   - If the desired clip-strip is submerged by standing water, reject and work down the Clip List to choose an acceptable clip-strip, and record "peak biomass clip-strip submerged" in the "remarks" field of the "Field Coring Datasheet."
3. Locate the relative X,Y-coordinates of the SW corner of the clip-strip within the target clip strip "cell". The procedure used to locate the X-coordinate depends on the value of the relative Y-coordinate:

If the Y-coordinate is < 10:

a. Run a tape East/West along the south edge of the plot or subplot between the (0,0) → (20,0) plot markers (Figure 2), 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, use the TruPulse in **HD** mode with a reflective surface to locate the Y-coordinate.
   - Make sure the azimuth is 0˚ (True North) when shooting the TruPulse to find the Y-coordinate (see RD[10] for detailed instructions for operating the TruPulse).
d. Place a pin flag at the clip-strip (X,Y) location – i.e. the SW corner of the clip-strip.

If the Y-coordinate is > 10:

a. Run a tape* East/West from the plot or subplot centroid (10,10) to either the (0,10) position or the (20,10) position (Figure 2).

X-coordinate	Tape Layout[1]
1 < X < 10	From (10,10) → (0,10)
10 < X < 20	From (10,10) → (20,10)

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

b. Place a pin flag at the desired relative X-coordinate.
c. Standing directly over the pin flag that was just placed, use the TruPulse in **HD** mode with a reflective surface to locate the Y-coordinate.
   - Make sure the azimuth is 0˚ (True North) when shooting the TruPulse to find the Y-coordinate (see RD[10] for detailed instructions for operating the TruPulse).
d. Place a pin flag at the clip-strip (X,Y) location – i.e. the SW corner of the clip-strip.

TIPS

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

4. Mark the four corners of the southern soil core sampling area within the clip strip "cell" with pin flags to delineate where the first of the two soil cores should be harvested (Figure 3).
   a. Place pin flag "A" 20 cm to the west of the coordinates provided in the Clip List (i.e. the red "x" in Figure 3) – use a meter tape to be accurate.
   b. Place pin flag "B" 50 cm to the east of pin flag "A"
   c. Place pin flag "C" 50 cm to the south of pin flag "A"
   d. Place pin flag "D" 50 cm to the south of pin flag "B"

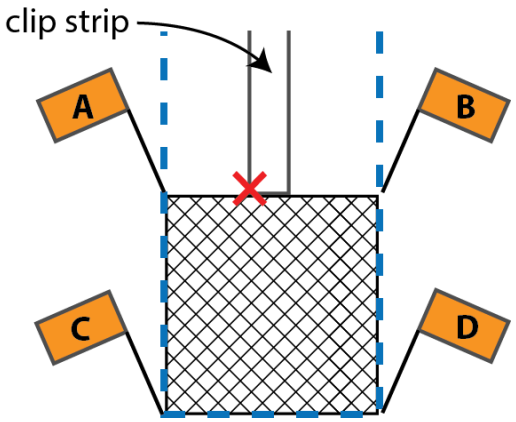

Figure 3. Delineating the southern soil core area (cross hatched) within a clip-harvest "cell" (dashed blue lines) with pin flags. The clip-strip (black lines) lies immediately to the north of the southern soil core area, and the red "x" marks the coordinates provided in the Herbaceous Biomass Clip List.

5. Mark the four corners of the northern soil core sampling area within the clip strip "cell" with pin flags to delineate where the second of the two soil cores should be harvested (Figure 4).
   a. Place pin flag "E" 2 m to the north of pin flag "A"
   b. Place pin flag "F" 2.5 m to the north of pin flag "A"
   c. Place pin flag "G" 2 m to the north of pin flag "B"
   d. Place pin flag "H" 2.5 m to the north of pin flag "B"

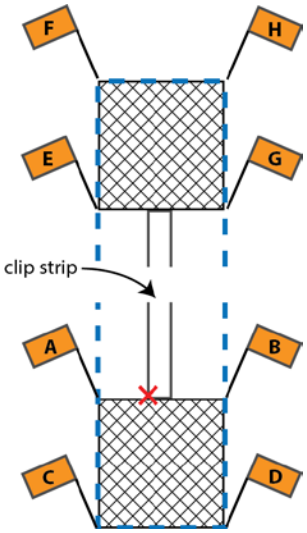

Figure 4. Delineating the northern soil core area (cross hatched) within a clip-harvest "cell" with pin flags (dashed blue lines). The middle of both the cell and the clip-strip have been omitted for clarity.

6. For each of the two soil coreID sampling areas:
   a. Label one large plastic freezer bag to hold the core, and a coin envelope to hold any non-root belowground plant parts. Label bags and envelopes with:
      - **date** (*YYYYMMDD* format)
      - **clipID** (*plotID_NNN* format; e.g. ONAQ_042_047)
      - **coreID** (*N* or *S*)
      - **biomassCode** (*ROOT* or *OTHR*)
   b. Record on the "Field Coring Datasheet":
      - **clipID** (*plotID_NNN* format)
      - **coreID** (*N* or *S*)
      - **ltrDepth** (average litter depth for coreID sampling area; measured to nearest 1 cm)
      - **wstDist10cm** (distance to closest woody stem with DBH ≥ 10 cm; measured to nearest 0.1 m with TruPulse)
      - **wstDist1cm** (distance to closest woody stem with DBH < 10 cm and ddh ≥ 1 cm; measured to nearest 0.1 m with TruPulse)
      - **wstGForm1cm** (the growth form code of 'wstDist1cm' individual; see RD[08] for complete definitions of the growth form codes)
        - **sms** = small shrub
        - **sis =** single shrub
        - **sgr =** shrub group
        - **smt =** small tree
        - **sap** = sapling
      - **bareGround** (% of coreID sampling area that is bare ground; estimated to nearest 10%)

7. Assemble the soil core tube, bit, retainer basket (if necessary), and drive head (see Appendix E).
8. Use hand clippers to remove aboveground plant leaves and stems from the exact 3-inch diameter area to be cored, and remove litter down to the soil surface.
    - Clip forbs and sub-shrubs at the soil surface.
    - Clip perennial graminoids just above the crown (Figure 5); DO NOT clip crown biomass at this point.

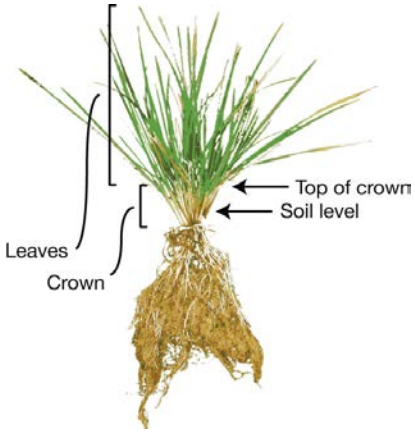

Figure 5. A perennial grass, and the location of crown material relative to leaves and the soil surface

9. For each core, harvest non-root belowground plant parts from the top 3 cm of soil:
    a. Score the ground with the soil core bit so it is clear exactly where the soil will be cored.
    b. Loosen the soil with a soil knife, and remove the soil from around any perennial non-root plant parts growing within the scored area (e.g. corms, rhizomes, crowns, etc.).
        - If perennial graminoid crowns are present, remove soil until the transition from crown to root is visible.
    c. Clip non-root material from within the bit-scored area, and place into the appropriate labeled coin envelope. ***Remove as much soil as possible from plant parts before placing in the coin envelope.***
    d. Place envelopes containing non-root biomass into a cooler with cold packs.
10. For each coreID sampling area, harvest a 66.5 mm ID (3-inch OD) soil core sample to 30 cm maximum depth:
    a. Position the soil core bit back over the scored area, and make sure the soil core assembly is vertical. If the plot is sloped, the soil core assembly should still be vertical.
    b. Use the slide hammer to pound the soil core tube to 30 cm maximum depth (*which should be marked on the soil core tube with electrical tape or similar*).

Once the soil corer is in the ground, do not turn the unit counter-clockwise, as this will unscrew the bit from the core tube underground, resulting in loss of the bit.

c. Push the core tube back and forth sharply several times to loosen it within the soil profile.
d. Remove the core tube from the ground, and carefully extract the core and lay it on top of the labeled plastic bag.
e. Backfill the hole with site-host approved material.

If obstacles are encountered that prevent coring to 30 cm depth, a minimum core depth of 20 cm is acceptable. In the event that a minimum 20 cm depth core cannot be obtained, select another location from within the target coreID sampling area but do not attempt more than 5 alternate locations. If it is impossible to obtain a miminum 20 cm depth core from anywhere within the target coreID, record "20 cm coreDepth not achieved" in the "remarks" field of the "Field Coring Datasheet".

11. Record on the "Field Coring Datasheet":
    - **coreDepth** (the total length of the core; measured to the nearest 1 cm)
    - **time** (the time the core will be placed into the cooler in the field; *HHmm* 24-h format)
    - **remarks** (e.g. "20 cm coreDepth due to root")
12. Place the core in the pre-labeled plastic bag, and put into the cooler immediately for cold storage until cores can be preserved in the laboratory (Appendix F).
13. Record on the "Field Coring Datasheet":
    - **storageHours** (amount of time a given sample was stored in a refrigerated cooler or at 4 ˚C before preservation; nearest 1 h)
14. Dry non-root biomass samples for 48 h in a 65 ˚C drying oven.
    - Once dried, these samples may be stored temporarily at room temperature in a sealed plastic bag until they are weighed (SOP C).

SOP C Processing Belowground Biomass Samples in the Laboratory

Goals for processing belowground biomass samples in the laboratory include:

1. Wash and sieve soil cores to separate soil from root biomass, and pick apart roots from other soil organic matter
2. Dry belowground biomass parts to constant weight
3. Weigh and record dry weight biomass
4. Grind samples with biomassCode = ROOT for chemical analyses

C.1 Wet-sieving soil cores for fine root biomass

1. Prepare preserved cores for wet sieving:
   - For cores preserved by drying, soak in water for 30 minutes in a 5 gallon plastic bucket to break up clays and rehydrate roots. Water depth should be sufficient to cover the cores.
   - If processing soil cores preserved by freezing at -20 ˚C, remove from the freezer and allow to thaw.
2. Group together the two soil core samples that originated from the same clip strip in the field. Roots from these two samples will be pooled following sieving.
3. For each group of two soil core samples, label 5 coin envelopes with the information below. If there is a large amount of root biomass within a given size class, use a clasp envelope instead.
   - **dateSampled** (date roots were sampled in the field; *YYYYMMDD* format)
   - **clipID** (unique ID of the clip grid "cell" from which roots were sampled; *plotID_NNN* format)
   - **status** ("live" or "dead"; if only part of a root is alive, categorize the entire root as "live")
   - **sizeClass** (<0.5, 0.5-1, 1-2, 2-10; can be "NULL" if status="dead")
4. For the first core from a given clip strip, use the root washing station, a 2 mm sieve, and a 250 µm sieve to separate mineral soil from roots and other organic material.
   a. Stack the 2 mm sieve on top of the 250 µm sieve, and place the sieve stack over an available aperture on the root washing station.
   b. Wash the mineral soil through the sieve stack using the hose. Mineral soil particles > 250 µm diameter, roots, and organic matter should be retained in both sieves.
      - Break up aggregates and organic matter pieces using gentle manual pressure.
      - For soils with high clay content – i.e. the mineral soil does not readily wash through the sieve stack – soak the sample in a 5 gallon plastic bucket and gently massage the soil to break it up in the water. The resulting suspension can then be poured carefully through the sieve stack.
   c. Manually remove larger rocks from the top of the 2 mm sieve – but don't spend more than several minutes.

5. Separate roots from particulate organic matter for each sieve, one at a time.
   a. Turn the 2 mm sieve upside down over one of the enamel pans, and use a root washer hose to transfer material from the 2 mm sieve to the pan. Roots often float, and mineral particles sink.
   b. Place the 2 mm sieve back on top of the 250 µm sieve, and decant the sample from the enamel pan back through the sieve stack, retaining mineral particles in the enamel pan.
   c. Discard mineral particles retained in the enamel pan, and rinse the pan.
   d. Repeat (a) to (c) until only roots and organic material remain in the enamel pan.
   e. Use forceps to pick all roots > 1 cm length from the enamel pan, sorting to size class with the wire gauge, and sorting "live" from "dead" roots as you go. The largest diameter of a root fragment should be used to classify the size; it is not necessary to sort "dead" roots to size class. ***Discard root fragments < 1 cm length*** – these will be quantified one time per site according to SOP D.
   f. Place sorted roots into the pre-labeled envelopes created in step (3).
   g. Rinse out the enamel pan, and repeat (a) to (e) for the 250 µm sieve.

Live roots are most readily distinguished from dead roots on the basis of color and friability. Dead roots are often dark brown or black, and are brittle; live roots are often lighter in color and flexible – i.e. they can typically be bent into a "U" shape without breaking.

6. Thoroughly clean the sieves and enamel pan with water between core samples.
7. Check sediment traps in the root washing station; if traps are full, dispose of sediment in an approved receptacle.
8. Repeat steps 4-7 for the second core from the current clip strip.
   - Place sorted roots from both core samples originating from the same clip "cell" into the same set of pre-labeled envelopes created in step (3). That is, pool the roots from both cores from the same clip "cell".
9. Place envelopes containing root samples into a large 25# paper bag to keep samples organized.
10. Dry root samples ≤ 2 mm diameter in a 65 ˚C drying oven for a minimum of 48 h.
    - Dry roots > 2 mm diameter in a 105 ˚C drying oven for 24 h.
11. Remove envelopes containing dried root biomass from the drying oven and place into a large plastic bag, seal the bag, and let dried biomass come to room temperature.
    - Sealing samples in a bag helps reduce water absorption from the air as they cool, particularly in humid environments.
12. Dried root samples may be placed in temporary storage at room temperature prior to weighing.

C.2 Weighing and processing dried belowground biomass samples

Both root biomass, as well as non-root biomass dried in SOP B, are weighed and processed here.

1. Weigh sorted, dried belowground biomass from each clip strip to the nearest 0.01 g using a mass balance and a large weigh boat.
    - For large quantities of biomass that do not readily fit into a large weigh boat, use the following strategies:
        - Substitute a paper bag or a cardboard box lid (or equivalent) for the weigh boat.
        - Crush or chop the biomass to reduce volume so it will fit into a weigh boat.
        - *Avoid splitting the biomass into sub-groups for weighing, as uncertainty is additive for each subgroup created.*

    - For very small quantities of biomass, use a microbalance and a small weigh boat.
2. Record in the "Lab Weighing Datasheet":
    - **dateSampled** (date belowground coring occurred in the field; *YYYYMMDD* format)
    - **clipID** (unique ID of the clip grid "cell" from which roots were sampled; *plotID_NNN* format)
    - **biomassCode** (*ROOT* or *OTHR*)
    - **status** (*LIVE* or *DEAD*)
    - **sizeClass** (<0.5, 0.5-1, 1-2, 2-10; can be NULL if **status** = *DEAD*)
    - **rootMass** (oven-dry mass; nearest 0.01 g)
    - **remarks** (free-form input < 250 characters)
3. Perform QA on the weighing of dried biomass samples. To quantify uncertainty associated with weighing, a portion of dried samples are re-weighed by a different technician than the person who originally weighed the samples.
    a. Per sampling bout, randomly select 10% of dried, previously weighed samples for re-weighing.
    b. Record the QA mass data in the **qaRootMass** field of the "Lab Weighing Datasheet."
4. Once QA masses have been recorded, grind dried samples for chemical analysis with biomassCode = *ROOT* and status = *LIVE* (0.85 mm mesh, mesh size = 20).
    - Samples with biomassCode = *OTHR* may be discarded after weighing.
    - Samples with biomassCode = *ROOT* and status = *DEAD* may be discarded after weighing.
5. Place the ground sample into a 20 mL scint vial for shipment to an external analytical facility for chemical analysis.

BEST PRACTICES

- If the ground sample is > 20 mL volume, use the sample microsplitter to generate a sample volume that will fit into the scint vial.
- Continue splitting as needed until a sub-sample of the desired volume is generated.
- Always transfer the entirety of a split sub-sample to the scint vial, and **DO NOT** transfer portions of a split sub-sample with a scoopula or spatula. These tools should only be used to transfer an ENTIRE sub-sample into a vial.

6. Label scint vials with:
   - **dateSampled** (date sampling occurred in the field; *YYYYMMDD* format)
   - **clipID** (unique ID of the clip grid "cell" from which roots were sampled; *plotID_NNN* format)
   - **sizeClass** (<0.5, 0.5-1, 1-2, 2-10 mm)
7. Discard any excess ground biomass.
8. Clean grinding mill and microsplitter thoroughly with compressed air (20-30 psi) between samples.

C.3 Equipment maintenance

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

SOP D Dilution Sampling for Fine Root Fragments

The dilution sampling for fine root fragments procedure begins following step (5) of SOP C.1, and can be considered an add-on to SOP C. Instead of ignoring and discarding organic material and root fragments < 1 cm length – hereafter referred to as the "residual fraction," the steps below describe how to separate roots from the residual fraction, and quantify them with a time-efficient technique.

SOP D is carried out only one time per site, within the first 5 years of a given site becoming operational. Soil core samples from a random subset of 20 clip strips should be chosen for dilution sampling; Science Operations cannot provide these clip strips ahead of time, as it is not known which clip strip will be associated with peak herbaceous biomass at a site in a given year.

1. Wet sieve two cores per clip strip, removing and processing roots > 1 cm length as per SOP C. In step (5) of SOP C.1, consolidate the "residual fraction" from the 2 mm and 250 µm sieves from both cores per clip strip into one 53 µm sieve.
2. Carefully wash the residual fraction with the root washer hose: the residual fraction sample should be free from mineral soil particles at this point.
3. Transfer the consolidated residual fraction – i.e. all roots < 1 cm length from a given clip strip – to a beaker so the sample may be randomly dispersed in water:
   a. Based on the size of the residual fraction, choose either a 1 or 2 L beaker.

The goal is to sufficiently dilute the residual fraction so that not too many roots need to be picked and sorted, but not dilute so much that there are too few roots to weigh accurately once they are dry. If in doubt, use the 1 L beaker.

   b. With the hose set to a low flow rate, use ≤ 500 mL of water to transfer the residual fraction from the 53 µm sieve to the beaker.
   c. Carefully fill the beaker to ¾ full (750 mL or 1.5 L). Be as accurate as possible, as this volume will be used to estimate the total mass of root fragments < 1 cm length.
   d. Record in the "Lab Dilution Datasheet":
      - **dateSampled** (date roots were sampled in the field; *YYYYMMDD* format)
      - **clipID** (*plotID_NNN* format)
      - **residVolume** (volume of water used to suspend residual fraction in beaker; nearest 10 mL)

   The values recorded above will apply to all of the 10 pairs of aluminum weighing tins in the next step.

4. Label 10 pairs (n=20 total) of aluminum weighing tins to hold 10 sub-samples of the aqueous residual fraction suspension.
   - For each pair of tins, one is for root fragments, and the other is for organic material (OM).
   - Tins should be pre-numbered with a unique tinID (e.g. 1, 2, 3,…, 20, etc.). The tinID is tracked on the datasheet, rather than labeling each tin with the clip strip.
   - Pre-weigh each tin with a microbalance, and record in the "Lab Dilution Datasheet":
     - **subSampleID** (technician assigned number from 1-10)
     - **tinID** (the unique number assigned to the tin)
     - **emptyTinMass** (the mass of the clean, dry, empty tin; record to nearest 0.1 mg)
5. Work in pairs to generate 10 sub-samples from the aqueous suspended residual fraction in the beaker:
   a. Turn the plate mixer on high, and vortex the aqueous suspended residual fraction thoroughly (approx. 10 s). [Person 1]
   b. Turn off the mixer, and quickly plunge the suspension to stop the vortex and randomize the sample in the water. [Person 1]
   c. Take a 20 mL sub-sample from the middle of the water volume in the beaker using the customized 40 mL syringe, and transfer to an aluminum weighing tin. [Person 2]
   d. Back off the plunger in the syringe to the 5 mL mark. Rinse the interior of the syringe with the squirt bottle, and transfer the rinse to the same aluminum weighing tin. [Person 2]
   e. For each tinID, record in the "Lab Dilution Datasheet":
      - **ssVolume** (the volume of the sub-sample taken from the beaker; nearest 1 mL; this will be the same number for both tinIDs in a pair)
      - **ssType** (the type of material the tinID will hold after picking and sorting is complete; the tin initially receiving the mixed sub-sample should be ssType = *OM*, and the tin into which roots are sorted should be ssType = *ROOT*)
   f. Repeat until 10 sub-samples have been transferred to 10 tins (Figure 6).

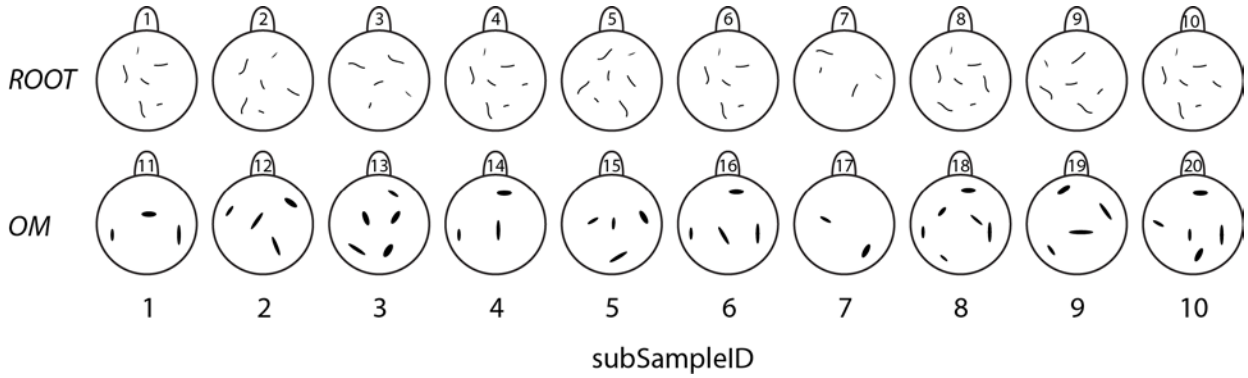

Figure 6. Pairs of labeled aluminum weighing tins for separating roots from OM in residual fraction sub-samples. Mixed sub-samples are initially transferred to the *OM* tins, and roots are then sorted into the *ROOT* tins.

6. For each of the 10 sub-samples, carefully pick and sort root fragments from organic material, and transfer the roots to the empty tin in the pair (Figure 6).
7. Using a sheet tray, carefully transfer tins to a 65 ˚C drying oven for 48 h. Do not leave tins on the sheet tray while drying, as sheet trays occasionally twist when heated, which could spill samples.
8. Repeat for additional pairs of cores from other sampled clip strip.
9. Once tins are dry, weigh each tin+root or tin+OM with a microbalance. Record in the "Lab Dilution Datasheet":
    - **sampleTinMass** (the mass of the tin + dried root or OM material; record to nearest 0.1 mg)
    - **remarks** (free-form field for technician comments, e.g. "spilled small volume"; 250 character limit)

SOP E Data Entry and Verification

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

Currently, there is a change-controlled spreadsheet version of the ingest document (RD[06]), as well as a working database version of the document. The data entry tables are the same in both the spreadsheet and database versions, but the change-controlled spreadsheet version contains summary information about each table, as well as summary information about each field.

Follow QA/QC procedures for ensuring accurate transcription of data (RD[04]).

Table 9. Data ingest tables and their corresponding field and/or lab datasheets; the "DB" column indicates whether a given table is in the working database version of the ingest document.

Ingest Table Name	Purpose	DB
bbcTableSummary_in	Table describing each ingest table, and the corresponding measurement resolution of ingested data	No
bbcFieldSummary_in	Table summarizing each data ingest table, and defining table field names and ingest rules	No
bbc_percore_in	Contains field-collected data and metadata from the "Field Coring Datasheet"	Yes
bbc_mass_in	Contains lab-collected oven-dry root mass data from the "Lab Weighing Datasheet"	Yes
bbc_dilutionmass_in	Contains lab-collected data for < 1 cm root fragments collected with the dilution technique in the "Lab Dilution Datasheet"	Yes

Consult the "bbcFieldSummary_in" table to determine the appropriate data type and format for each field.

SOP F Sample Shipment

F.1 Procedure

1. Take scintillation vial box containing processed samples out of the cabinet for shipment.
2. Wrap the box in bubble wrap and tape securely, then place in a FedEx box for shipment.
3. Include a copy of the USDA letter pertaining to shipment of dried plant sample material in the box and affix any labels required by the permit, if necessary.
4. Include cover letter explaining shipment, and spreadsheet detailing sample inventory (these forms supplied for each specific contract by NEON Headquarters staff).
5. Address shipping label appropriately and ship ground.

F.2 Timelines

Ship samples as soon as possible following processing steps (i.e., within 24 h). Samples that have been air-dried or oven-dried prior to shipment do not "expire", but to decrease build-up of samples in the domain facility, it is better to ship quickly so that samples are not lost or damaged. However, if there is an issue with receiving contracted laboratory being able to accept samples (e.g., contract not established, problem with soil permit), the shipment may have to be held back. In this case, please submit a Jira ticket.

F.3 Laboratory Contact Information and Shipping/Receipt Days

See the CLA shipping document on CLA's NEON intranet site.

8 REFERENCES

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APPENDIX A DATASHEETS

The following datasheets are associated with this protocol:

Table 10. Datasheets associated with this protocol

NEON Doc. #	Title
NEON.DOC.002135	Datasheets for TOS Protocol and Procedure: Core Sampling for Plant Belowground Biomass
NEON.DOC.001925	NEON Raw Data Ingest Workbook for TOS Belowground Biomass Soil Core

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

APPENDIX B QUICK REFERENCES

N/A

APPENDIX C REMINDERS

N/A

APPENDIX D ESTIMATED DATES FOR ONSET AND CESSATION OF SAMPLING

Belowground biomass soil core sampling is ideally timed to roughly coincide with the peak aboveground biomass clip harvest, due to the scientific utility of relatively coincident estimates of both aboveground and belowground biomass. As such, dates listed in Table 11 below are the estimated dates after which greenness begins to decrease at each site, and in theory, after which the majority of above and belowground biomass has been produced. Dates are averages of 2001-2009 MODIS-EVI satellite phenology data. However, soil moisture also influences the timing of sampling, and as such, dates below may need to be adjusted at a given site based on soil moisture conditions within a given year. Soil core sampling should be concluded within 1 month of the actual start date.

Dates are provided in day-of-year (DOY) format. Conversions to MM-DD are provided in Table 12.

Table 11. Estimated average peak greenness dates of for each NEON site based on MODIS-EVI phenology data. Ideally, soil core sampling and aboveground biomass clip harvests should place on or near these dates.

Domain	Site	Approx. Start Date (Day-Of-Year)
01	BART	220
	BURL	220
	HARV	220
02	BLAN	210
	SCBI	220
	SERC	220
03	DSNY	190
	JERC	220
	OSBS	190
04	GUAN	
	LAJA	
	MAME	
05	STEI	215
	TREE	215
	UNDE	215
06	KONA	
	KONZ	90
	KUFS	
07	GRSM	215
	MLBS	220
	ORNL	210
08	CHOC	200
	DELA	205
	TALL	195
09	DCFS	120
	NOGP	115
	WOOD	210

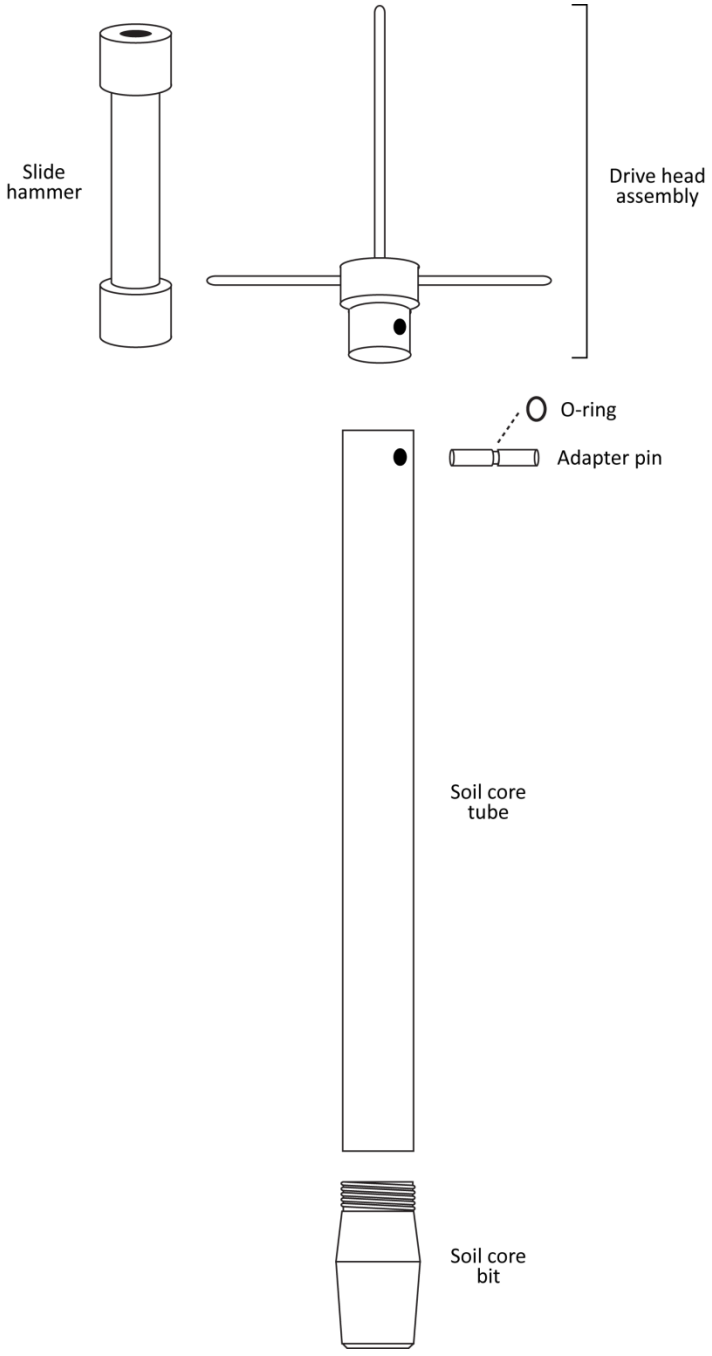
Domain	Site	Approx. Start Date (Day-Of-Year)
10	CPER	90
	RMNP	180
	STER	2-4 wks before crop harvest
11	CLBJ	
	KLEM	75
	TBD	
12	BOZE	
	PARA	
	YELL	190
13	MOAB	85
	NIWO	220
	TBD	
14	JORN	245
	SRER	240
	TBD	
15	ONAQ	75
	RBUT	
	TBD	
16	ABBY	
	THAY	
	WREF	210
17	SJER	270
	SOAP	185
	TEAK	205
18	BARO	210
	TOOL	205
	TBD	
19	DEJU	210
	HEAL	210
	POKE	205
20	OLAA	
	PUFO	
	PUGR	

Table 12. Day-of-year calendar for non-leap years.

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

APPENDIX E SOIL CORE ASSEMBLY

Component parts of the Giddings soil core assembly:



APPENDIX F SOIL CORE PRESERVATION FOLLOWING FIELD SAMPLING

Preserving soil cores as soon as possible after field sampling halts decomposition and loss of biomass from root samples, and allows the field crew to focus on harvesting all cores from the field in as short an amount of time as possible.

1. Following field collection, samples must be kept cool until they are preserved in the laboratory. To ensure samples stay cool, exchange cold packs in coolers for freshly frozen cold packs every 12 h until cores and non-root biomass can be processed in the laboratory. Samples may also be placed into a 4 ˚C refrigerator, if available.
2. Following sampling in the field, preserve soil cores ASAP until further laboratory processing takes place (Table 13).
    - Cores may be preserved either via drying, or via freezing.
    - In general, it is preferable to preserve cores via drying rather than freezing, due to the fact that freezing roots makes identifying dead roots very difficult, but both methods are acceptable.

Table 13. Description of soil core preservation methods, conditions in which each method is preferred, and advantages and disadvantages associated with each method.

Method	Description	Conditions Used	Advantages/Disadvantages
Drying	Oven dry at 65 ˚C to constant weight (i.e. the difference between consecutive weighings is zero ± 5%)	Relatively dry soils that are not muddy to the touch.	• Pro: Cores may be stored at room temperature following drying. • Pro: Live/dead classification easier than when soil is frozen. • Con: Raising temperature increases decomposition until moisture is low, particularly problematic for soils with high field moisture. • Con: Dried cores must be soaked prior to sieving to break up hard aggregates and to rehydrate roots for accurate diameter classification.
Freezing	Freeze at -20 ˚C	Relatively wet soils that are muddy to the touch	• Pro: Faster than drying when soils are wet. • Pro: For wet soils, avoids increased decomp during long drying times. • Con: Requires significant freezer space until soils can be sieved. • Con: Live/dead classification can be difficult or impossible when roots are frozen.

APPENDIX G MANAGING EXPOSURE TO *TOXICODENDRON* SPECIES

1. **Prior to field work:**
   a. Count out coin envelopes or clasp envelopes for storing and drying root samples that will likely contain *Toxicodendron* biomass. Don't mix samples containing *Toxicodendron* biomass with any other samples.
   b. Pre-weigh (to nearest 0.01 g) and label each envelope that will be used for storing and drying cores containing *Toxicodendron* biomass. Once the weight of each empty envelope is written on the envelope, the biomass inside the bag will never have to be touched after it is initially placed in the bag.

2. **To collect soil cores containing *Toxicodendron* biomass in the field:**
   - Wear cotton gloves and dispose after single use.
   - Before collecting the core sample, use a pair of clippers dedicated solely to clipping *Toxicodendron spp* to clip and remove any aboveground *Toxicodendron* biomass that would be contacted while coring.
   - Bring a clean, new plastic bag to the field for storing and transporting contaminated gloves, soil coring equipment, and clippers after use.
   - Wear a thin outer layer of disposable PPE over clothes and shoes.
   - Upon returning to the laboratory, wear fresh latex gloves and clean clippers and soil coring equipment with Tecnu (or equivalent) after each use. Store exposed equipment separate from other laboratory equipment to prevent accidental contact.
   - After field work is complete, wash clothing according to these guidelines or similar:
   - http://laundry.about.com/od/removeoutdoorstains/a/poisonivylaundry.htm

3. **To process *Toxicodendron* biomass in the laboratory:**
   a. Use sieves and forceps dedicated to processing root samples containing *Toxicodendron* biomass. Wash sieves and forceps with Tecnu (or equivalent) following each use.
   b. Minimize potential spread of toxic oil by putting envelopes containing *Toxicodendron* roots into the same drying oven every time.
   c. When drying is complete, clean drying oven shelves used for drying *Toxicodendron* biomass with hot water and Tecnu. Wear appropriate PPE when cleaning.
   d. Record weight of bag + dried biomass to nearest 0.01 g, and also record weight of individual empty bag (to 0.01 g) on data sheets. Dried *Toxicodendron* biomass should never leave the bag.

4. After weighing, dispose of root samples containing *Toxicodendron* biomass. At this point in time, *Toxicodendron* tissue will not be ground for chemical analysis or archived.