TOS PROTOCOL AND PROCEDURE: CORE SAMPLING FOR PLANT BELOWGROUND BIOMASS

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A	03/25/2011	ECO-00148	Initial release
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1 DESCRIPTION

1.1 Purpose

The primary purpose of this document is to provide a change-controlled version of Observatory protocols and procedures. This document provides the content for training and field-based materials for NEON staff and contractors. 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.

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

1.2 Scope

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

- General safety practices
- Site-specific safety practices
- General equipment maintenance

It does identify procedure-specific safety hazards and associated safety requirements such as safe handling of soil coring equipment, or safe use of required chemicals and reagents.

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 information that shall be applied in the current document. Examples are higher level requirements documents, standards, rules and regulations.

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.001155	NEON Training Plan
AD [04]	NEON.DOC.050005	Field Operations Job Instruction Training Plan

2.2 Reference Documents

Reference documents contain information complementing, explaining, detailing, or otherwise supporting the information included in the current document.

RD [01]	NEON.DOC.000008	NEON Acronym List
RD [02]	NEON.DOC.000243	NEON Glossary of Terms
RD [03]	NEON.DOC.000914	NEON Science Design: Plant Biomass and Productivity
RD [04]	NEON.DOC.005003	NEON Scientific Data Products Catalog
RD [05]	NEON.DOC.014051	Field Audit Plan
RD [06]	NEON.DOC.000824	Data and Data Product Quality Assurance and Control Plan
RD [07]	NEON.DOC.005005	Level 0 Data Product Catalog
RD [08]	NEON.DOC.002135	Datasheets for Field and Lab Protocol: Core Sampling for Plant Belowground Biomass
RD [09]	NEON.DOC.001925	NEON Raw Data Ingest Workbook for TOS Belowground Biomass SoilCore
RD [10]	NEON.DOC.014048	TOS Field and Lab Protocol: Soil Physical, Chemical, and Microbial Measurements
RD [11]	NEON.DOC.000987	TOS Protocol and Procedure: Measurement of Vegetation Structure
RD [12]	NEON.DOC.001271	TOS Protocol: Manual Data Transcription
RD [13]	NEON.DOC.001925	NEON Raw Data Ingest Workbook for TOS Belowground Biomass Soil Core
RD [14]	NEON.DOC.001708	TOS Field and Lab Protocol: Soil Pit Sampling for Plant Belowground Biomass

2.3 Acronyms

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

2.4 Definitions

A **protocol** is a formal summary description of a procedure and its related rationale, and includes information on knowledge and resources needed to implement the procedure. A **procedure** is a set of prescribed actions that must take place to achieve a certain result, and can also be called a method. It differs from a science design in that science designs provide a more complete description of the rationale for selecting specific protocols. It differs from a training manual in that training manuals provide materials in support of skills acquisition in the topic areas including information on how to best train staff rather than detailing only the steps of the procedure.

3 BACKGROUND AND OBJECTIVES

3.1 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[14]), 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[10]). 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.

3.2 NEON Science Requirements

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.

3.3 NEON Data Products

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[04]).

4 PROTOCOL

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 SOPs B, C, and 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 D).
- **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 or more 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.

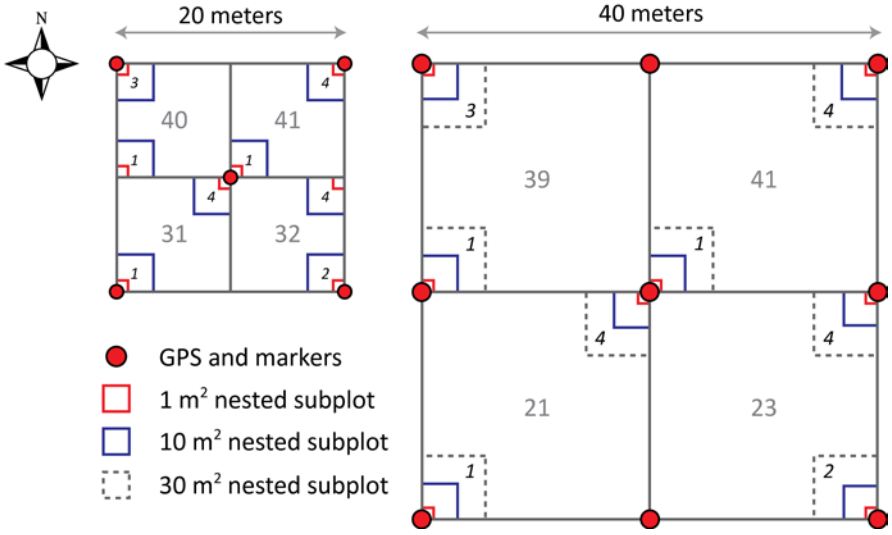


Figure 1. Illustration of two NEON plot sizes used for belowground biomass soil core sampling. Grey numbers indicate subplotIDs, but soil core sampling is only dependent on subplots for 40m x 40m plots. Italic black numbers show the location of nested subplots that are used for % cover and diversity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

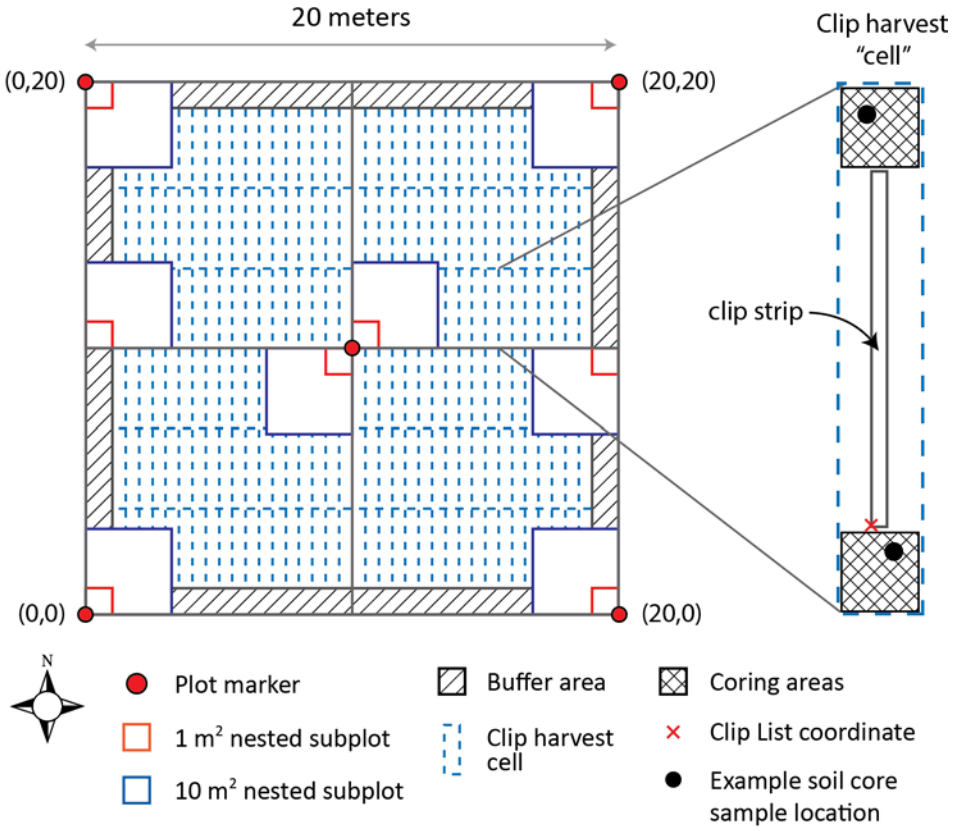


Figure 2. A 20m x 20m Tower plot showing the locations of 0.5m x 3m clip harvest cells used for belowground biomass soil core sampling (*left*). Within a clip harvest cell selected for soil core sampling, one core is collected from each of the areas to the North and South of the clip-strip (*right*). 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…).

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.

5 QUALITY ASSURANCE AND CONTROL

The procedures associated with this protocol will be audited according to the Field Audit Plan (RD[05]). 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 (RD[06]).

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

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

Table 1. Guidance indicating how to respond to unanticipated delays in soil core field sampling, and the consequences of potential delays.

Delay	Action	Adverse Outcome	Outcome for Data Product
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	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.	A finite but indeterminable amount of root decomposition will occur in sampled cores until they are preserved.	Increased uncertainty in belowground biomass estimates.

Delay	Action	Adverse Outcome	Outcome for Data Product
	4. Preserve first core if delay is > 7 days, 5. Resume core sampling in same clip strip ASAP		
	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	If delay prevents completing sampling from all plots or subplots within a 1 month window, belowground biomass may fluctuate substantially.	Potentially substantial increases in uncertainty for belowground biomass estimates.
	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.

6 SAFETY

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 C for best practice procedures to minimize exposure to toxic oils.

7 PERSONNEL REQUIREMENTS

For the field work, a minimum of 2 field technicians are required for harvesting soil cores due to weight of equipment and soil cores.

Required skills:

- 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.

8 TRAINING REQUIREMENTS

All technicians must complete required safety training as defined in the NEON Training Plan (RD[04]). Additionally technicians complete protocol specific training for safety and implementation of protocol as required in Field Operations Job Instruction Training Plan (RD[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!***

9 SAMPLE FREQUENCY AND TIMING

9.1 Criteria for Determining Sampling Dates

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.

9.2 Sampling Frequency

Table 2. 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 immediately following field work.
SOP D	Tower	All	1X per site	NA	"Dilution sampling" occurs only once per site, within the first 5y of Operations.

9.3 Sampling Timing Parameters

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 is still acceptable for terrestrial sampling according to TOS standing water guidelines (RD[XX]), clip location "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 the soil core location is not the same as that of the greatest peak biomass clip.

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.

10 STANDARD OPERATING PROCEDURES

SOP A Preparing for Sampling

SOP 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 3**). A "quick relief" bit is suitable for most soils.

Table 3. 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 materials as specified in **Table 4**.

Table 4. 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 SOP B.

Maximo Number	Item Description	Qty	Conditions Used	Action(s)
Suggested	GPS unit	1	All	• Charge • Load target plot locations
Required	Compass, mirror-sight, adjustable declination	1	All	Check/set correct declination*
MX100322	TruPulse 360R laser rangefinder and clinometer	1	All	• Check battery, charge (if possible) • Clean lenses with lens cloth or lens tissue (if necessary) • Check/set correct declination*. See Appendix B. • Calibrate tilt-sensor (only necessary after severe drop-shock; see Appendix B.
MX103276 MX103279	3" OD (66.5mm ID) soil core tube and bit assembly	1	All	Measure 30 cm from the bottom of the bit, and mark on the tube with electrical tape.
Required	Re-usable cold packs	As needed	All	Place in -20 ˚C freezer
Required	Freezer, -20 ˚C	1-2	When lab processing is delayed after field sampling	Clear sufficient space to freeze core samples after field sampling, and temporarily store until laboratory processing takes place.
Required	Hand clippers	1	All	Clean and sharpen blades (if necessary)
Required	Sand, or other site-specific material	As needed	All	Ensure supply is sufficient for backfilling soil core holes.
Required	Belowground biomass core "Field Coring Datasheet"	As needed	All	Print as needed on waterproof copy paper
Required	Herbaceous biomass Clip Lists	As needed	All	Print as needed on waterproof copy paper
Required	Belowground biomass core "Random Subplot List"	As needed	Tower plots ≥ 1600 m^2	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/

SOP A.2 Preparing for Processing Soil Cores in the Laboratory (SOP C)

1) Prepare equipment and materials as specified in **Table 5**.

Table 5. Actions required to prepare equipment and materials for processing belowground biomass soil core samples in the laboratory (SOP C). Equipment listed here are only those items that require preparation actions before sampling; the full equipment list is provided in SOP C.

Maximo Number	Item Description	Qty	Conditions Used	Action(s)
Required	Root washing station	1	All	Empty and clean sediment traps
MX100230	Drying oven	2	All	• Clear space for drying root samples • Oven 1: Set temperature to 65 ˚C • Oven 2: Set temperature to 105 ˚C
Required	Lab Weighing Datasheet	As needed	All	Print as needed

SOP A.3 Preparing for Dilution Sampling for Fine Root Fragments (SOP D)

1) Prepare equipment and materials as specified in **Table 6**.

Table 6. Actions required to prepare equipment and materials for quantifying fine root fragments with the dilution technique (SOP D). Equipment listed here are only those items that require preparation actions before carrying out the procedure; the full equipment list is provided in SOP D.

Maximo Number	Item Description	Qty	Conditions Used	Action(s)
Required	Root washing station	1	All	Empty and clean sediment traps
Required	French press plunger stick and nut, 23oz press	1	All	Attach French press plunger cross piece to bottom of plunger stick with nut
Required	French press plunger cross, 23oz press	1	All	Attach French press plunger cross piece to bottom of plunger stick with nut
Required	Aluminum weigh tins	200+	All	Label with unique tinID (**Figure 6**)
Required	Lab Dilution Datasheet	As needed	All	Print as needed

SOP B Soil Core Sampling in the Field

Field Equipment and Materials

Table 7. Field equipment and materials required to carry out belowground biomass soil core sampling in the field.

Maximo Number	Item Description	Qty	Conditions Used	Purpose

Maximo Number	Item Description	Qty	Conditions Used	Purpose
MX103276	Soil core sampling tube, 36” length, 3” OD	1	All	Generate soil core sample
MX103277	Soil core drive head assembly	1	All	Works with slide hammer to drive soil core tube into soil
MX103278	Soil core drive head pin, 3” length	1	All	Attach drive head assembly to core tube
MX103279	Soil core quick relief bit, 3” OD*	1	Standard bit for coring most soils	Attach to soil core sampling tube
MX103280	Soil core slide hammer, 16#	1	All	Drive sampling tube into soil
MX103281	Soil core basket retainer, 3” adapter	1	Sandy soils that do not hold together	Attach basket retainer system to sampling tube
MX103282	Soil core basket retainer, 3” basket	2	Sandy soils that do not hold together	Retain sandy soil in sampling tube
MX103283	Soil core basket retainer, 3” bit	1	Sandy soils that do not hold together	Bit that works with basket retainer
Suggested	GPS unit, pre-loaded with plot locations	1	All	Navigate to plots or subplots
MX100322	TruPulse 360R laser rangefinder, current declination entered	1	Slope >10%, brushy	Locate clip strip within a plot or subplot
MX103218	Foliage filter for laser rangefinder	2	Brushy vegetation	Facilitates use of TruPulse in brushy conditions
Required	Reflective surface (bicycle reflector or reflective tape on back of field notebook/clipboard)	1	Used with TruPulse	Accurate location of clip strip with TruPulse in “FLT” mode
Suggested	Extra battery for TruPulse (CR123A type)	2	Used with TruPulse	Battery backup
MX104362	Chaining pins, steel	2	Plot slope <10%; grassland, savannah	Stretching tapes to enable location of target clip strip
Required	Fiberglass meter tape (30m or longer)	1	Plot slope <10%; grassland, savannah	Locate clip strip within plots or subplots
Required	4”x 5” pin flags with PVC stakes	6	All	Accurate location of clip strip; PVC stakes avoid magnetic interference with compass or TruPulse
Required	Hand clippers, fine tip	1	All	Remove aboveground plant parts from soil coring location
MX100721	Soil knife, hori-hori style	1	All	Loosen soil at surface to expose

Maximo Number	Item Description	Qty	Conditions Used	Purpose
				non-root plant parts
Required	Coin envelopes, 3⅜" x 6", Kraft paper (e.g. Uline S-14720)	40+	All	Store and organize non-root below-ground biomass
Required	Plastic freezer bags, 1.5 or 2 gallon	40+	All	Store and organize soil core samples
Required	Large chest-style cooler, with frozen cold packs	2+	All	Keep core samples cool, slow down root decomposition
Required	Sharpies	2	All	Label paper bags
Required	Pencils	2	All	Record sampling metadata
Required	Herbaceous biomass "Clip Lists"	Variable	All	Identify clip strip associated with peak biomass clip harvest
Required	Belowground biomass "Random Subplot Lists"	Variable	Tower plots ≥ 1600 m^2	Identify subplots for soil core sampling
Required	Belowground biomass "Field Coring Datasheets"	Variable	All	Record sampling metadata

* 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.

SOP B.1 Soil Core Sample Collection 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:

- 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.*
- Place a pin flag at the desired relative X-coordinate.
- 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 Appendix B for detailed instructions operating the TruPulse).
- 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 Appendix B for detailed instructions operating the TruPulse).

d) Place a pin flag at the clip-strip (X,Y) location – i.e. the SW corner of the clip-strip.

***BEST PRACTICE TIPS**

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

4) 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**).

- 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.
- Place pin flag "B" 50 cm to the east of pin flag "A"
- Place pin flag "C" 50 cm to the south of pin flag "A"

- Place pin flag “D” 50 cm to the south of pin flag “B”

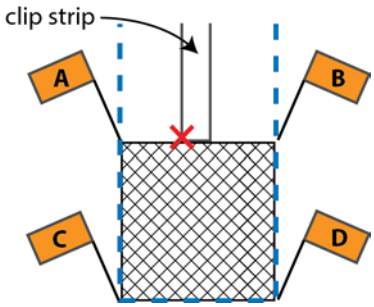


Figure 3. Delineating the southern soil core area (cross hatched) within a clip-harvest “cell” (dashed blue lines) with pin flags. The clip-strip (black lines) lies immediately to the north of the southern soil core area, and the red “x” marks the coordinates provided in the Herbaceous Biomass Clip List.

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**).

- Place pin flag “E” 2 m to the north of pin flag “A”
- Place pin flag “F” 2.5 m to the north of pin flag “A”
- Place pin flag “G” 2 m to the north of pin flag “B”
- Place pin flag “H” 2.5 m to the north of pin flag “B”

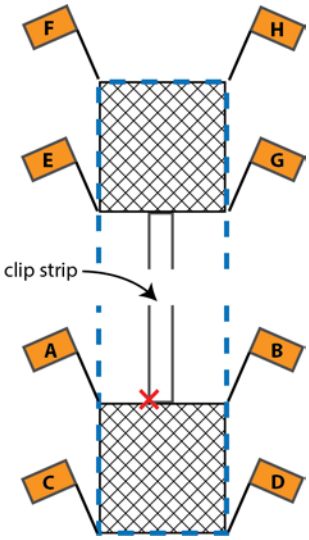


Figure 4. Delineating the northern soil core area (cross hatched) within a clip-harvest “cell” with pin flags (dashed blue lines). The middle of both the cell and the clip-strip have been omitted for clarity.

6) For each of the two soil coreID sampling areas:

- 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*)

- 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[11] for complete definitions of the growth form codes)
        - **sms** = small shrub
        - **sis =** single shrub
        - **sgr =** shrub group
        - **smt =** small tree
        - **sap** = sapling
    - **bareGround** (the percent of the 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 A).

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.

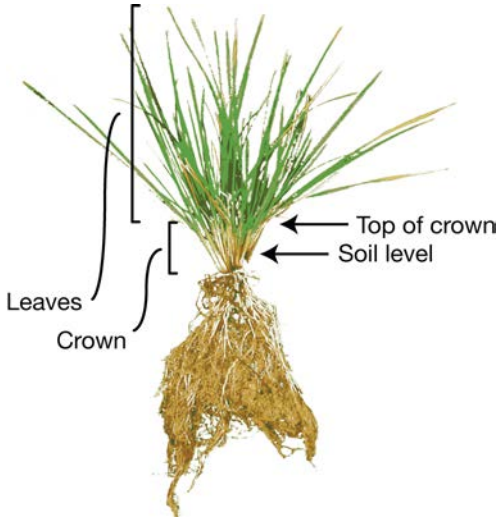


Figure 5. Illustration of a perennial grass, and the location of crown material relative to leaves and the soil surface.

9) For each core, harvest non-root belowground plant parts from the top 3 cm of soil:

- Score the ground with the soil core bit so it is clear exactly where the soil will be cored.
- 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.
- 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.***
- 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:

- 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.
- 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.
- Push the core tube back and forth sharply several times to loosen it within the soil profile.

- Remove the core tube from the ground, and carefully extract the core and lay it on top of the labeled plastic bag.
- 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 D).

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:

a) Wash and sieve soil cores to separate soil from root biomass, and pick apart roots from other soil organic matter
b) Dry belowground biomass parts to constant weight
c) Weigh and record dry weight biomass
d) Grind samples with biomassCode = ROOT for chemical analyses

Laboratory Equipment and Materials

Table 8. Equipment and materials required to sieve belowground biomass soil cores, separate roots from soil organic matter, and dry root samples.

Maximo Number	Item Description	Qty	Conditions Used	Purpose
Required	Root washing station	1	All	Remove mineral soil from organic material
Required	Soil sieve, 2 mm stainless mesh, 8” or 12” diameter	4-5	All	Remove mineral soil from organic material
Required	Soil sieve, 250 µm stainless mesh, 8” or 12” diameter	4-5	All	Remove mineral soil from organic material
Required	Rectangular enamel pan, white (app. 33 cm x 20 cm, or 13”x 9”)	2	All	Facilitates separating roots (which float) from mineral particles
Suggested	5-gallon plastic bucket	2	Cores preserved by drying	Soak core sample prior to sieving to break up cohesive clays and rehydrate roots
Required	Large plastic weigh boats	50+	Large root quantities	Weigh relatively large quantities of dried root samples
Suggested	Small plastic weigh boats	50+	Small root quantities	Weigh relatively small quantities of dried root samples
Required	Forceps, blunt tip, stainless steel	2	All	Separate roots from organic material
Required	* Wire gauge with openings approx. 2mm, 1mm, and 0.5mm	2	All	Sort roots into size classes during sieving and picking
Required	Clasp envelopes, 6”x 9”, Kraft paper	50	Large root quantities	Store and organize sieved roots during and after drying
Required	Coin envelopes, 3⅜”x6”, Kraft paper	50	Small root quantities	Store and organize sieved roots during and after drying
Required	Paper bag, 25# Kraft	20	All	Organize root samples in the drying ovens
Required	Sample microsplitter	1	Large root volumes	Creates identical sub-samples from ground sample
Required	Hi-back pans for sample	2	With micro	2 per splitter; receives split sub-

Maximo Number	Item Description	Qty	Conditions Used	Purpose
	microsplitter		splitter	sample
Required	Scintillation vials with caps, 20 mL volume	Variable	All	Containers for storing ground split sub-samples
Required	Sharpie, extra fine tip	2	All	Labeling envelopes and scint vials
Required	Pencils	2	All	Record dry weight of root samples
Required	Lab Weighing Datasheet	Variable	All	Record dry weight of root samples

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

SOP 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.

SOP 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 PRACTICE TIPS ***

- 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.

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 y 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 strip 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.

Laboratory Equipment and Materials

In addition to equipment listed in **Table 8**, the dilution sampling for fine root fragments procedure requires items listed in **Table 9**.

Table 9. Equipment and materials required to carry out dilution sampling for fine root biomass fragments < 1 cm.

Maximo Number	Item Description	Qty	Conditions Used	Purpose
Required	Soil sieve, 53 µm stainless mesh, 8" or 12" diameter	2	All	Consolidate residual fraction from both cores per clip strip, rinse, and transfer to beaker for dilution
Required	Magnetic mixing plate	1	All	Randomize aqueous suspended residual fraction
Required	Magnetic stir bar, 2" to 3" length	2	All	Randomize aqueous suspended residual fraction
Required	Beaker, 1 L	2	All	Hold smaller aqueous suspended residual fraction volumes
Required	Beaker, 2 L	2	All	Hold larger aqueous suspended residual fraction volumes
Required	Plunger, diameter minimally less than beaker diameter (RD[13])	1	All	Stop mixing vortex, randomize aqueous suspended residual fraction
Required	Syringe, 40 mL, with tip cut off to make a 1 cm diameter aperture	2	All	Aspirate sub-sample from randomized aqueous residual fraction
Required	Plastic laboratory squirt bottle, filled with water	1	All	Rinse syringe following sub-sampling
Required	Aluminum weighing dishes, 65 mL (e.g. Fisher #: 08-732-102)	200	All	Hold and dry root and organic material from sub-samples.
Required	Forceps, fine tip	2	All	Pick small root fragments apart from organic material
Suggested	Sheet tray, baking or equivalent	1	All	Transfer aqueous samples in

Maximo Number	Item Description	Qty	Conditions Used	Purpose
				aluminum dishes to drying ovens

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**).

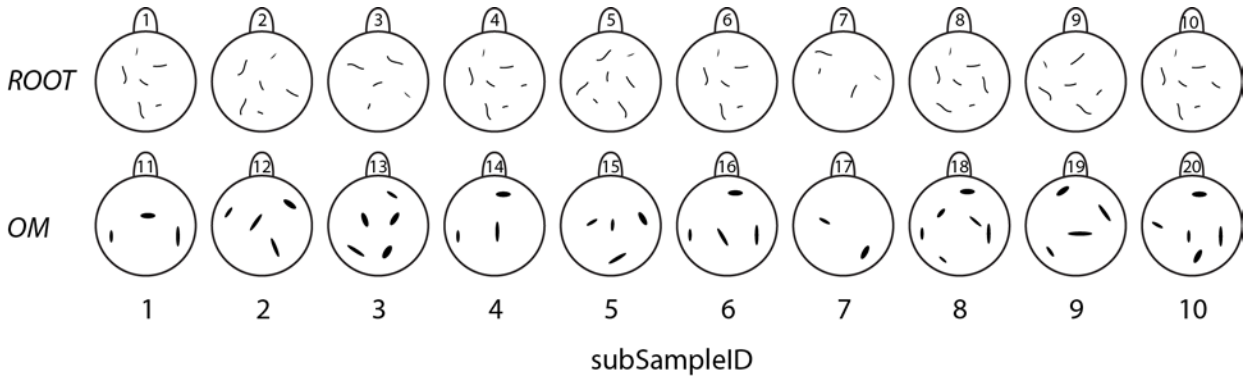


Figure 6. Pairs of labeled aluminum weighing tins for separating roots from OM in residual fraction sub-samples. Mixed sub-samples are initially transferred to the *OM* tins, and roots are then sorted into the *ROOT* tins.

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

Data recorded manually into field and lab datasheets must be transcribed for ingest into NEON's Cyberinfrastructure. This should be no more 14 days after processing samples.

Currently, there is a change-controlled spreadsheet version of the ingest document (RD[13]), 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[12]).

Table 10. 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.

11 REFERENCES

Berhongaray, G., J. S. King, I. A. Janssens, and R. Ceulemans. 2013. An optimized fine root sampling methodology balancing accuracy and time investment. Plant and Soil **366**:351-361.

Burton, A. J., and K. S. Pregitzer. 2008. Measuring forest floor, mineral soil, and root carbon stocks. Pages 129-142 *in* C. M. Hoover, editor. Field measurements for forest carbon monitoring. Springer-Verlag, New York.

Koteen, L., and D. Baldocchi. 2013. A randomization method for efficiently and accurately processing fine roots, and separating them from debris, in the laboratory. Plant and Soil **363**:383-398.

Milchunas, D. G., and W. K. Lauenroth. 2001. Belowground primary production by carbon isotope decay and longterm root biomass dynamics. Ecosystems **4**:139-150.

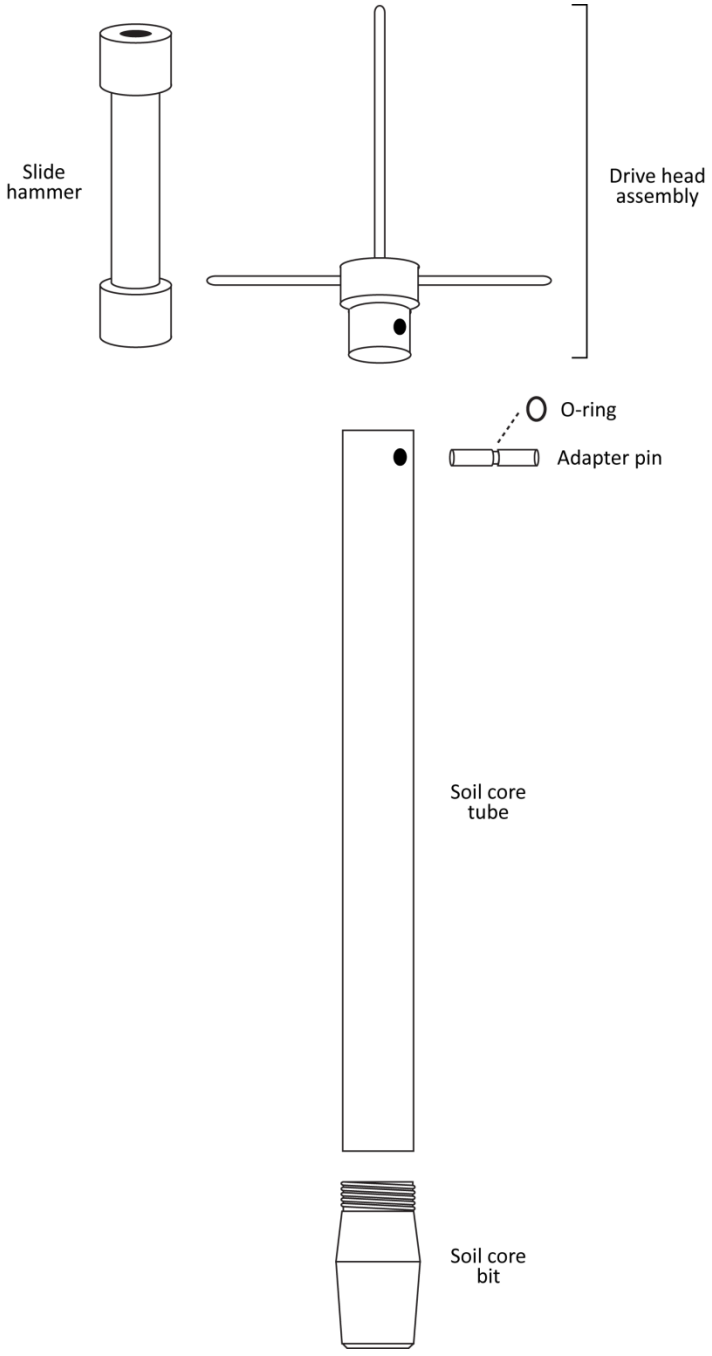
Steinaker, D. F., and S. D. Wilson. 2005. Belowground litter contributions to nitrogen cycling at a northern grassland-forest boundary. Ecology **86**:2825-2833.

Taylor, B. N., K. V. Beidler, E. R. Cooper, A. E. Strand, and S. G. Pritchard. 2013. Sampling volume in root studies: the pitfalls of under-sampling exposed using accumulation curves. Ecology Letters **16**:862-869.

Tierney, G. L., and T. J. Fahey. 2007. Estimating belowground primary productivity. Pages 120-141 *in* T. J. Fahey and A. K. Knapp, editors. Principles and standards for measuring primary production. Oxford University Press, New York.

Appendix A SOIL CORE ASSEMBLY

Component parts of the Giddings soil core assembly:



Appendix B USING AND CALIBRATING THE TRUPULSE 360 LASER RANGEFINDER

Setting the Declination Offset

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

Tilt Sensor Calibration

1) Press the "Power/Fire" button to turn on the unit. The viewfinder will display the main "Measurement Mode" screen.
2) Press and Press and hold ▼ for 4 s to enter "System Setup Mode".
3) Press ▼ until **inC** is displayed in the viewfinder, then press "Power/Fire"..
4) **no** and **CAL** will blink. Press ▼ until **yes** and **CAL** blink, then press "Power/Fire" again.
    a) Calibration can be aborted by pressing "Power/Fire" when **no** and **CAL** are alternately displayed.
5) **C1_Fd** will be displayed in the view finder.
6) Place the TruPulse on a flat, relatively flat surface (within 15deg of level). Follow the sequence outlined in **Figure 7**.
    a) At each step wait approximately 1 second before pressing "Power/fire", then wait another second before moving to the next position. It is important that the unit is held steady when you press "Power/fire".
    b) To abort and return to previous calibration at any point hold ▲ or ▼ for 4 sec.

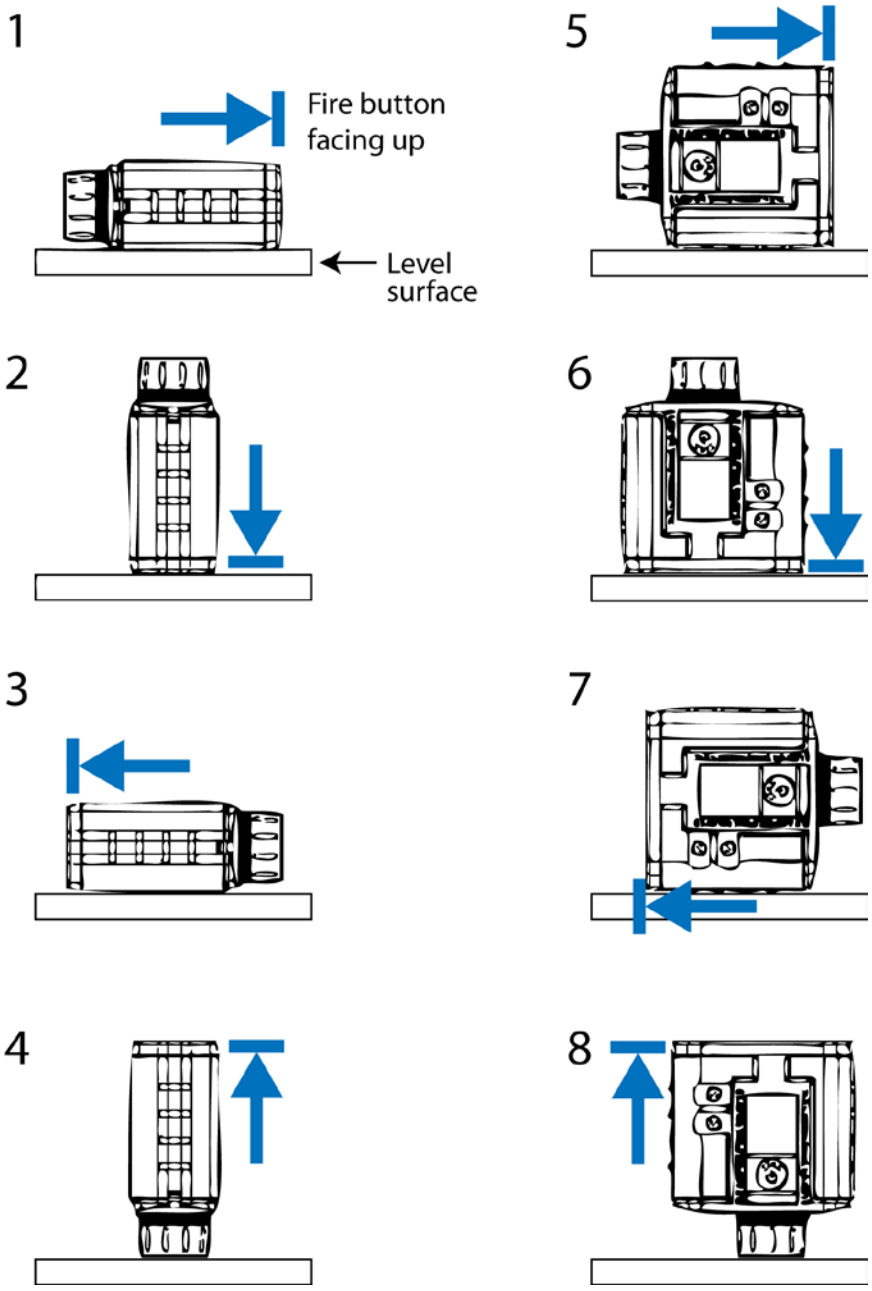


Figure 7. The tilt-sensor calibration routine for the TruPulse 360R laser rangefinder. The blue arrow and line indicate the direction of thelens at each calibration step.

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

Using and Calibrating the TruPulse 360R Compass

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

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

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

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

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

- For increased confidence, repeat the test with a second target at 90˚ to the azimuth of the first target.

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

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

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

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

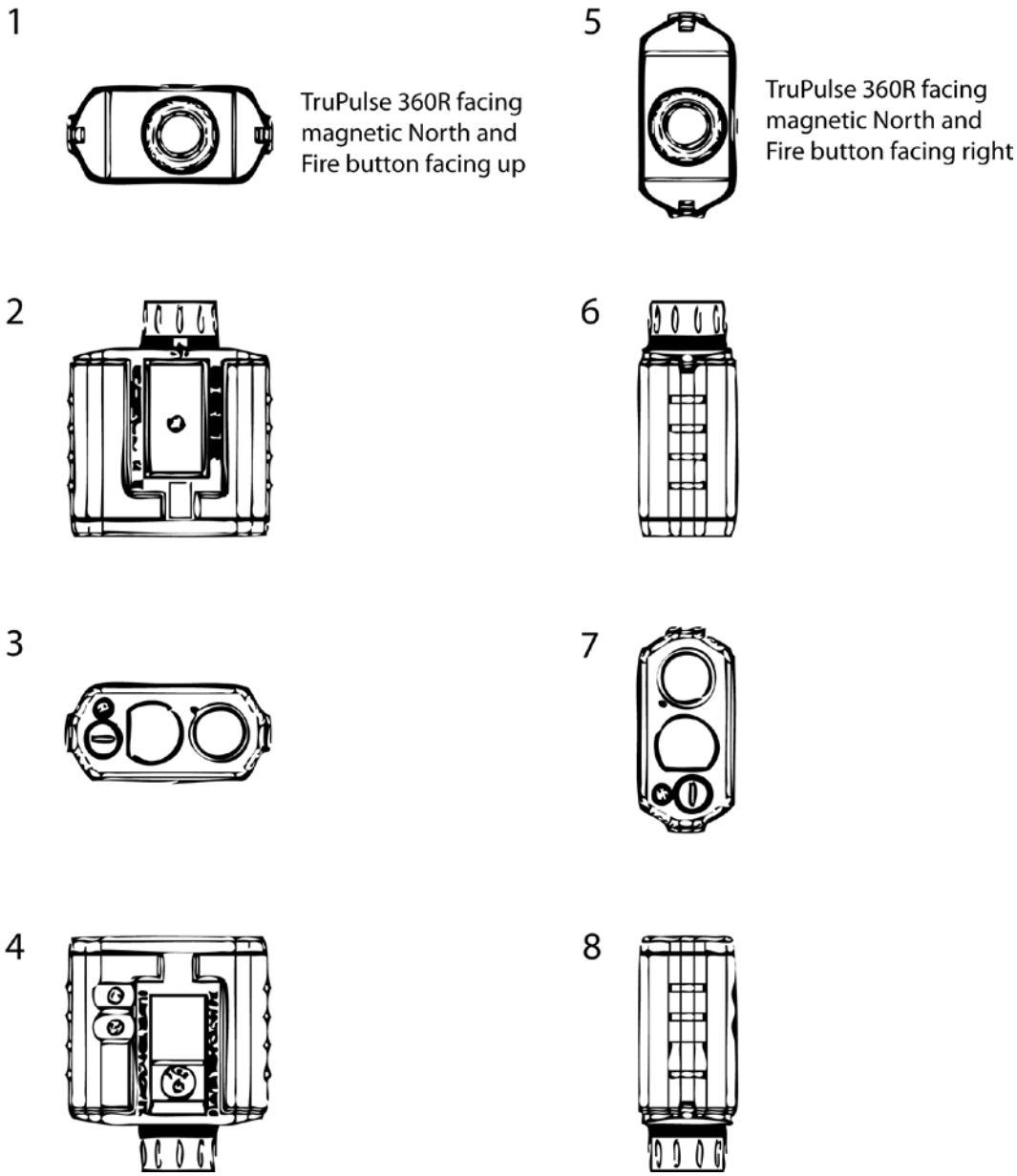


Figure 8. The internal compass calibration routine for the TruPulse 360R laser rangefinder.

Measuring Distance from a Known Point

1) Press "Power/Fire" to turn on the TruPulse.
2) Set the unit to Target Mode = Filter
   a) Press ▲ for 4 seconds. The active Target Mode appears in the viewfinder. Press ▲ or ▼ to cycle through available Target Modes.

      Available Target Modes are:

Table 11. Laser Target Modes available for the TruPulse 360 laser rangefinder/clinometer models.

Target Mode	Definition	When to Use
Std	Standard, single-shot	Clear shot to unobstructed target
Con	Continuous; pressing and holding "Power /Fire" will continuously acquire targets for up to 10 s	Useful for scanning trees in order to find the highest point
CLO	Closest; pressing and holding "Power /Fire" will acquire multiple targets, the viewfinder displays the closest target	Narrow targets in the foreground
FAr	Farthest; identical to CLO, but the view-finder displays the farthest target	• Target partially obscured by brush • Finding highest point of a tree
Flt	Filter; the sensor sensitivity is reduced to only detect laser pulses returned from a reflective surface; ***'F' appears in the viewfinder***	• Measuring targets through thick brush • In very heavy brush, the optional foliage filter can be used in this mode (but is not required)

   b) Choose "Flt" and press "Power/Fire" to make the chosen Target Mode active.
3) Press either the ▲ or ▼ button until **HD** (i.e. Horizontal Distance) appears in the viewfinder.
4) Person 1: Hold the reflective surface at the location for which a distance measurement is required so that it is visible to Person 2.
5) Person 2: Look through the TruPulse viewfinder, aim the crosshairs at the reflective surface held by Person 1, and press and hold "Power/Fire" until the distance is displayed in the viewfinder.

Measuring Azimuth from a Known Point

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

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

Appendix C MANAGING EXPOSURE TO *TOXICODENDRON* SPECIES

Equipment and Materials

Table 12. Equipment and materials required to minimize exposure to toxic oils from roots of *Toxicodendron spp.* that may be encountered.

Item Description	Qty	Example Item	Purpose
Envelopes, pre-weighed, labeled with envelope weight	As needed	Coin or clasp type (see **Table 8**)	*Toxicodendron* roots never handled directly again after they are placed in pre-weighed envelope.
Labeled clippers, dedicated to clipping *Toxicodendron spp.*	1	See **Table 8**	Prevent spread of toxic oils to multiple clippers
Cotton gloves, single use	Box of 12	http://www.globalindustrial.com/p/safety/hands/cotton-canvas-gloves/anchor-4501v-8-oz-cotton-canvas-knit-wrist-1110	Prevent oil contact with hands while collecting cores in the field.
Disposable PPE outer-wear	Case of 24	Coveralls; http://disposable-garments.com/shop/koolguard/koolguard-coveralls/	Prevent oil contact with skin and clothing while collecting cores in the field.
Large, single-use plastic bags	Box	Trash bag or large Ziploc type bag	Transport used gloves and PPE from the field and minimize toxic oil transfer.
Latex gloves, single use	Box		Prevent hand exposure during sieving.
Labeled sieve set(s), dedicated to sieving samples containing *Toxicodendron*	As needed	Sieve set contains: • 2 mm sieve • 250 µm sieve (see **Table 8**)	Prevent spread of toxic oils to multiple sieves.
Labeled forceps, blunt tip, stainless steel; dedicated to *Toxicodendron* samples	As needed	See **Table 8**	Prevent spread of toxic oils to multiple forceps.
Cleanser, urushiol-specific	As needed	Tecnu or equivalent; http://www.teclabsinc.com/products/poison-oak-ivy/tecnu	Clean sieves and forceps after use.

Minimizing Exposure to Toxic Oil in the Field and Lab

The following are best-practice techniques for minimizing exposure to toxic oil during soil core sampling in areas with *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.

Appendix D 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 weighing's 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.