

<i>Title:</i> TOS Protocol and Procedure: Core Sampling for Plant Belowground Biomass		<i>Date:</i> 02/17/2017
<i>NEON Doc. #:</i> NEON.DOC.014038	<i>Author:</i> C. Meier	<i>Revision:</i> E

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
D	1/28/2016	ECO-03547	<p>Major changes to protocol include:</p> <ul style="list-style-type: none">) All SOPs now implemented together every time protocol is executed, previously SOP D implemented 1X per site) Timing information updated, and preservation of cores prior to core processing eliminated.) Equipment list updates for lab work) SOP C.1 sieving methods updated based on megapit sampling experience) Roots from 2 cores within a clipCell are now pooled <i>after</i> weighing takes place and prior to grinding for chemical analysis / archive.) "other" non-root biomass no longer quantified) Method for calculating core `storageHours` now consistent with Herbaceous Biomass protocol.) Updated Sample Shipment procedure (SOP F) to be consistent with Herbaceous Biomass protocol.) To aid co-locating herbaceous clip and fine root coring, added maps of clip cells within plots to appendix G.) References to mini-rhizotrons removed after descope.
E	02/17/2017	ECO-0443	<ul style="list-style-type: none">) Added table of common terms and definitions to Section 2.4) Toxicodendron material condensed and removed when possible, now reference RD[12]) Added 'Estimated Time' required for protocol sub-tasks to Section 6.4 based on Field Ops experience.) Updated field and lab equipment list based on feedback from Field Ops prototype.) SOP B: Added 'Linked Protocol' call-out box to highlight connection with Herbaceous Biomass.) SOP B: Added `coringPossible` to better document sample collection effort, and added `coreDiameter` to

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			<p>allow future changes in equipment.</p> <p>) SOP C: Cores may be soaked overnight prior to wet-seiving.</p> <p>) SOP C: Added instructions for using the wire gauge properly to sort roots by diameter.</p> <p>) SOP C: Simplified pooling instructions, and changed minimum mass of pooled sample from 0.250 g to 0.02 g; removed grinding of samples < 1 g (change from 0.75 g).</p> <p>) SOP C/D: Changed all mass measurement requirements to grams, rather than mix of grams and milligrams.</p> <p>) SOP C/D: Changed timing to allow for overnight pause between SOP C and SOP D.</p> <p>) SOP D: Clarified that `sampleVolume` and `subsampleVolume` can be adjusted on a per core basis to optimize root material mass for sorting.</p> <p>) SOP D: Clarified anticipated effort for sorting root/OM aliquots.</p> <p>) Appendix D: Changed dates from DOY to MM/DD format, and updated Ops-IPT approved missing dates.</p>

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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 within the same landsurface area from which NEON Tower eddy covariance data are derived; at many sites this will also be the dominant vegetation type(s).

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). NEON will employ the most common and robust method to measure belowground biomass in both forest and grassland ecosystems: relatively large diameter (5–10 cm) cores (Tierney and Fahey 2007, Burton and Pregitzer 2008). Because large 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 (66.5 mm inside diameter) 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]). Within each clip “cell” selected for belowground biomass sampling, two 30 cm cores will be generated, for a total sample volume of 2722 cm³ per clip cell. If roots up to 10 mm diameter exist at the site, sample volumes of this size should be sufficient to encounter them in the majority of soil samples (Taylor et al. 2013).

To account for differences in BNPP across the spectrum of fine root diameters, researchers typically sort roots within core samples into various size categories, and then calculate fine root production separately for each size category. Following Burton and Pregitzer (2008), NEON will sort roots within each core to the following **sizeCategory** bins: < 0.5 mm, 0.5–1 mm, 1–2 mm, and 2–10 mm.

Soil samples are sieved to remove soil, picked to separate roots from other organic material, and roots are then sorted to diameter size category. Picking and sorting roots is time consuming, and similar to other researchers, NEON will use a 1 cm length cutoff to limit the time spent searching for small root

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fragments – i.e., 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 root fragments < 1 cm length, NEON will employ a dilution technique on a subsample of cores every time fine root sampling occurs.

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	EHSS Policy, Program and Management Plan
AD[02]	NEON.DOC.004316	Operations Field Safety and Security Manual
AD[03]	NEON.DOC.000724	Domain Chemical Hygiene Plan and Biosafety Manual
AD[04]	NEON.DOC.050005	Field Operations Job Instruction Training Plan
AD[05]	NEON.DOC.000914	NEON Science Design for Plant Biomass and Productivity
AD[06]	NEON.DOC.004104	NEON Science Performance QA/QC Plan

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2.2 Reference Documents

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

RD[01]	NEON.DOC.000008	NEON Acronym List
RD[02]	NEON.DOC.000243	NEON Glossary of Terms
RD[03]	NEON.DOC.002652	NEON Level 1, Level 2 and Level 3 Data Products Catalog
RD[04]	NEON.DOC.001271	NEON Protocol and Procedure: Manual Data Transcription
RD[05]	NEON.DOC.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 Biogeochemical 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
RD[11]	NEON.DOC.014037	TOS Protocol and Procedure: Measurement of Herbaceous Biomass
RD[12]	NEON.DOC.001716	TOS Standard Operating Procedure: Toxicodendron Biomass and Handling
RD[13]	NEON.DOC.001710	TOS Protocol and Procedure: Litterfall and Fine Woody Debris
RD[14]	NEON.DOC.001024	TOS Protocol and Procedure: Canopy Foliage Chemistry and Leaf Mass per Area Measurements

2.3 Acronyms

Acronym	Definition
BNPP	Belowground net primary productivity
OM	Organic material

2.4 Definitions

Common terms used throughout this document are defined here, in alphabetical order.

Table 1. Definitions for common terms used throughout the Core Sampling for Plant Belowground Biomass protocol.

Term	Definition
clip cell	A 0.5m x 3m rectangular area within a plot that supports plant below-ground biomass core sampling; the long-edge of the cell is always oriented north/south.
clip list	A randomized list of clip cells for each 20m x 20m plot or subplot, provided by NEON Science. Working down the list through time ensures that selected coring locations will generate an unbiased estimate of plant belowground biomass for every bout.

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Term	Definition
clip strip	A 0.1m x 2m rectangular area, typically centered within each clip cell, that is avoided during plant belowground biomass coring. Coordinates provided in clip lists correspond to the SW corners of clip strips.
coring area	Two 0.5m x 0.5m areas that exist to the north and the south of the clip strip within a given clip cell.
organic material	For the purposes of this protocol, particulate soil organic matter made up of decayed plant parts of unrecognizable origin – i.e., it is not possible to discern leaf, twig, needle, root origin, etc.
residual fraction	The mixture of organic material and root fragments < 1 cm length that is left in the bottom of the sieve after root fragments ≥ 1 cm length have been picked out of the sample. For a subset of cores, root fragments in the residual fraction are quantified via the dilution technique.

3 METHOD

The Standard Operating Procedures (SOPs) presented in this protocol describe tasks that, when taken together, allow estimation of plant belowground fine root biomass across four diameter 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.
-) **SOP C: Processing Belowground Biomass Samples in the Laboratory.** Steps to wash, sieve, and separate roots ≥ 1 cm length 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 sub-sampling 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 time savings.

Belowground biomass soil core sampling takes place in 400 m² 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, two soil cores are sampled from one clip “cell” per bout. In larger 40m x 40m Tower plots (i.e. four 400 m² subplots per plot), soil core sampling occurs in each of the two subplots randomly assigned by Science Operations for sampling, and two soil cores are sampled from one clip cell per subplot per bout. This strategy means that:

-) At sites with thirty 20m x 20m Tower plots, there will be n=60 soil core samples.
-) At sites with twenty 40m x 40m Tower plots, there will be n=80 soil core samples.

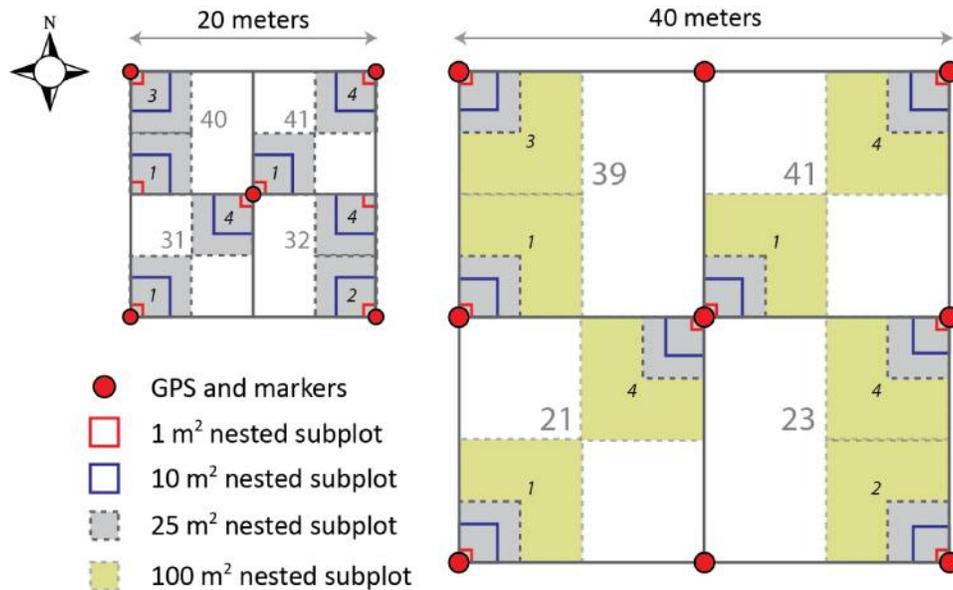


Figure 1. Illustration of two NEON plot sizes used for belowground biomass soil core sampling. Grey numbers indicate subplotIDs, and soil core sampling is only dependent on subplots for 40m x 40m plots. Italic black numbers show the location of nested subplots that are used for % cover and diversity measurements. Soil core sampling is prohibited within 1 m² and 10 m² nested subplots.

SPATIALLY LINKED PROTOCOLS: PLANT BELOWGROUND BIOMASS AND HERBACEOUS BIOMASS

-) In Tower Plots, the Plant Belowground Biomass Core protocol and the Herbaceous Biomass protocol (RD[11]) are spatially linked, and should occur in the same cell in a given sampling year (**Figure 2, right**).
-) In an 'on' year for Belowground Biomass Core sampling, the Clip List should indicate whether the Herbaceous Biomass protocol was performed prior to core sampling; always attempt to acquire core samples from the same cell used for clip harvesting.
-) When accepting/rejecting cells for potential sampling, be sure to consider suitability and representativeness with respect to **both** protocols.



Within each 400 m² plot or subplot, clip cells are 3m x 0.5m, and are sequentially numbered. Coordinates are assigned to the SW corner of a 2m x 0.1m clip strip that is centered within each clip 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 cell was (or will be) used for the peak biomass harvest in the current growing season. Within each clip cell two 66.5 mm diameter (3" OD) x 30 cm length soil core samples will be harvested: one from each of the areas to the North AND South of the 2m x 0.1m clip strip (**Figure 2, right**). To avoid roots and rocks, technicians may sample from anywhere within the North and South sampling areas shown in **Figure 2**.

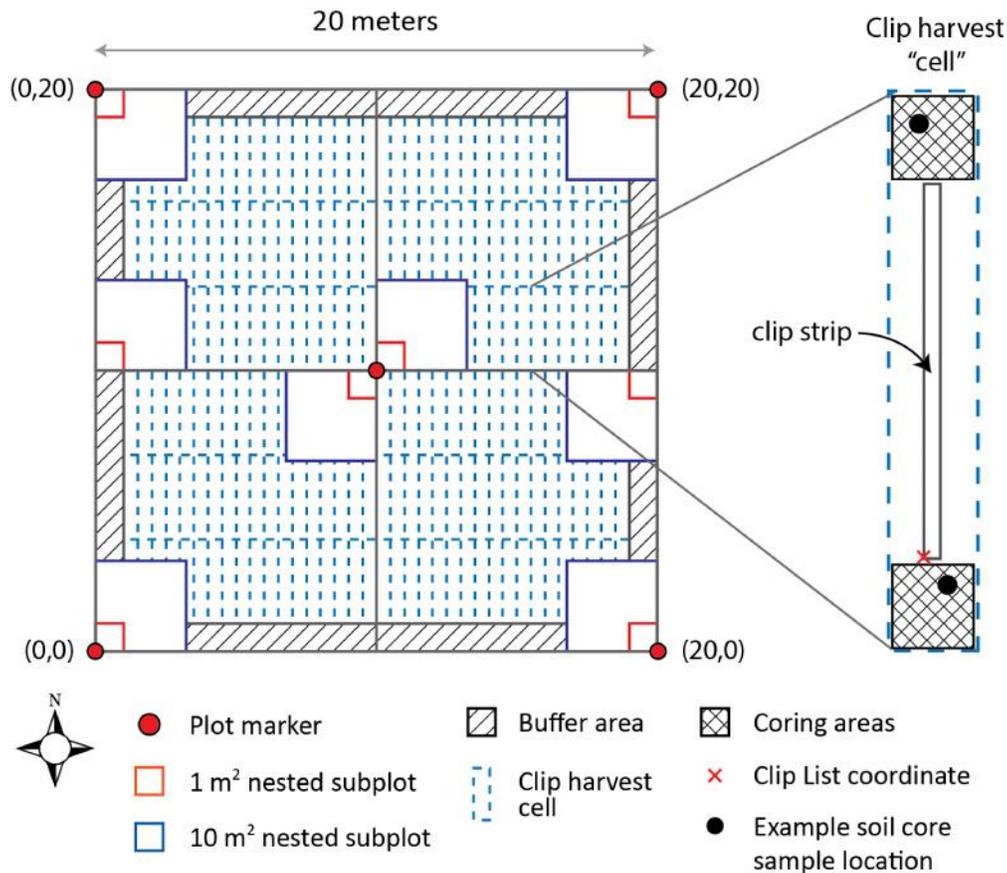


Figure 2. A 20m x 20m Tower Plot showing the locations of 3m x 0.5m clip cells used for belowground biomass soil core sampling (*left*); the largest 25 m² nested subplot has been omitted for clarity. Within a clip cell selected for soil core sampling, one core is collected from each of the areas to the 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 discarded. In some ecosystems, these non-root belowground plant parts may constitute a significant portion of the belowground biomass; however, the NEON protocol is solely focused on measuring fine root biomass.

After sampling from a given clip strip is completed, it is necessary to backfill the holes with a material approved by the site host (e.g. purchased sand, soil from another site-host approved location, etc.).

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.

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

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

4 SAMPLING SCHEDULE

4.1 Sampling Frequency and Timing

Table 2. Sampling frequency for belowground biomass soil core procedures on a per SOP per plot type basis.

SOP	Plot Type	Plot Number	Sampling Events	Yearly Interval	Remarks
SOP B	Tower	All	1X per sampling year	5y	
	Distributed, Gradient	NA	NA	NA	Distributed and Gradient plots are not cored for plant belowground biomass.
SOP C	Tower	All	1X per sampling year	Same as SOP B	SOP quantifies roots ≥ 1 cm length
SOP D	Tower	All	1X per sampling year	Same as SOP B	Dilution sampling quantifies mass of root fragments < 1 cm length.

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, and a new clip-location “cell” must be chosen.

COORDINATING WITH REQUIRED TOS PROTOCOLS

Synchronized Biogeochemistry Protocols

The plant Belowground Biomass Core protocol should be completed as part of a scheduled suite of synchronized TOS measurements aimed at characterizing plant and soil biogeochemical dynamics.

Synchronized protocols and SOPs include:

-) TOS Protocol and Procedure: Soil Biogeochemical and Microbial Sampling, including the N-transformations SOP (RD[07])
-) TOS Protocol and Procedure: Litterfall and Fine Woody Debris, litter chemistry component (RD[13])

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-) TOS Protocol and Procedure: Canopy Foliage Chemistry and Leaf Mass per Area Measurements (RD[14])

Plant Diversity

Three Tower Plots are randomly selected for annual Plant Diversity measurements. If plant diversity sampling is scheduled to occur prior to plant Belowground Biomass Coring in a given sampling year, it may be helpful to identify and demarcate a suitable clip cell prior to performing Plant Diversity sampling. This will ensure that the clip cell is not trampled during diversity sampling. Should plant Belowground Biomass Coring occur before Plant Diversity sampling, take care to avoid trampling 1 m² nested subplots used for Plant Diversity % cover measurements.

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 processed in the laboratory.

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.
 - o 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 be initiated within ± 2 weeks of that sampling window.

4.3 Timing for Scheduling Field Work and Laboratory Processing

Because root biomass continues to be biologically active after sampling, and because root structures are delicate and decompose easily, once soil samples are removed from the ground they must be kept cold at all times until they are processed in the laboratory according to SOP C. Acceptable methods include storing samples in:

-) Coolers kept cold with re-usable cold packs. Cold packs should be exchanged for fresh cold packs every 12 hours.
-) Refrigerator, 4–8 °C

Ideally, soil cores are processed in the laboratory within 24 h of collection in the field. However, it is acceptable to keep soil cores in cold storage for up to a maximum of 72 hours. Once laboratory processing is initiated on a given sample, processing should be carried all the way through without stopping.

Scheduling sieving (SOP C) and Dilution Sampling (SOP D):

-) It is acceptable to pause overnight between execution of SOP C and SOP D. Store labeled residual fractions overnight at 4 °C in a sealed container (e.g., labeled 50 mL tube).

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4.4 Sampling Timing Contingencies

Table 3. Contingency decisions for belowground biomass fine root core sampling.

Delay/ Situation	Action	Outcome for Data Products
Hours	If delay prevents sampling the second core from a given clip cell: 1. Bag and label first sampled core, 2. Place labeled bags into a cooler. 3. Resume core sampling in same clip cell ASAP	None
	If delay occurs between plots or subplots: Resume core sampling ASAP.	
1-14 days	If delay prevents sampling second core from a given clip cell: 1. Bag and label first sampled core, 2. Place labeled bags into a cooler. 3. Process first core within 72 hours of sampling. 4. Resume sampling for second core in same clip cell ASAP	Increased uncertainty in belowground biomass estimates.
	If delay occurs between plots or subplots: 1. Process sampled cores within 72 hours. 2. Resume core sampling at additional required plots ASAP.	
14+ days	If delay prevents sampling second core from a given clip cell: 1. Bag and label first sampled core, 2. Place labeled bags into a cooler. 3. Process first core within 72 hours of sampling, 4. Resume core sampling in same clip cell 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. Process sampled cores within 72 hours. 2. Resume core sampling ASAP.	

4.5 Criteria for Reallocation of Sampling Within a Site

Plant Belowground Biomass Core sampling will occur on the schedule described above at up to 30 Tower Plots per site. Ideally, sampling will occur at these sampling locations for the lifetime of the Observatory (core sites) or the duration of the site’s affiliation with the NEON project (relocatable sites). However, circumstances may arise that require that sampling within a site be shifted from one particular location to another. In general, sampling is considered to be compromised when sampling at a location becomes

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so limited that data quality is significantly reduced. If sampling at a given plot becomes compromised, a problem ticket should be submitted by Field Operations to Science.

There are two main pathways by which sampling can be compromised. Sampling locations can become inappropriately suited to answer meaningful biological questions (e.g., a terrestrial sampling plot becomes permanently flooded). Alternatively, sampling locations may be located in areas that are logistically impossible to sample on a schedule that that is biologically meaningful.

For plant Belowground Biomass Core sampling, criteria for considering a plot compromised include:

-) If sampling cannot be completed in a plot or 2 consecutive bouts.

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 EHSS Policy, Program and Management Plan (AD[01]), and the Operations Field Safety and Security Manual (AD[02]). 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 (e.g., use of gloves and ear plugs). 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. In addition, a laser rangefinder/hypsometer/compass instrument may be used to navigate to cells within plots. Safety considerations for this instrument include:

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

For the laboratory procedures, safety training is required before operating the grinding mill.

Additional safety issues associated with this field procedure include potential exposure to oils from roots of *Toxicodendron spp.* (discussed in Appendix F, AD[02] and RD[12]).

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6 PERSONNEL AND EQUIPMENT

6.1 Equipment

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

Table 4. SOP B equipment list – Soil-core sampling plant 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	2	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

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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
	R	Fiberglass meter tape (30m or longer)	Locate clip strip within plots or subplots	Plot slope <20%; grassland, savannah	1	N

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Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
	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; one cooler per 8 cores sampled.	All	2+	N
	R	Sharpies	Label paper bags	All	2	N
MX104362	R	Chaining pins, steel	Stretching tapes to enable location of target clip strip	Plot slope <20%; grassland, savannah	2	N
	R	Measuring device, with 1 cm demarcations (e.g., tape, ruler, collapsible measuring stick, etc.)	Measure depth of the litter layer and depth of soil core bore hole	All	1	N
	S	Length of dowel, 1" PVC or equivalent (36" total length)	Push soil core sample out of soil core sampling tube	Soil core sticks to tube	1	N
	S	Heavy duty work gloves	Protect hands during soil core sampling	All	1 pair/person	N
Consumable items						

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Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
	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
	R	Heavy duty freezer bags, 1.5 or 2 gallon	Store and organize soil core samples	All	40+	N
	S	Ear plugs	Prevent hearing damage from use of slide hammer.	All	As needed	N
	R	Pencils	Record sampling metadata	All	2	N
	R	Waterproof paper, Rite-in-the-Rain or equivalent	Material for making labels to record soil core metadata in the field	All	10+ sheets	N
	R	Clip Lists	Identify clip cell associated with peak biomass clip harvest	All	Varies	N
	R	Random Tower 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
	S	Horticultural grade sand	Backfill core holes at sites where specified by site host	As specified	4-5 lbs per core	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.

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Table 5. SOP C equipment list – Sieving belowground biomass cores, separating roots from soil organic matter, and drying root samples. Equipment listed is for 3 people working independently at a root washing station.

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
	S	Plastic bucket, bin, or equivalent (5 gallon, 20 L, etc.)	Soak core sample prior to sieving to break up cohesive clays and rehydrate roots	All	6	N
	R	Soil sieve, 2 mm stainless mesh, 8" or 12" diameter	Remove mineral soil from organic material	All	6	N
	S	Soil sieve, 1 mm stainless mesh, 8" or 12" diameter	Remove mineral soil from organic material	Sandy soil sieving	6	N
	R	Soil sieve, 250 µm stainless mesh, 8" or 12" diameter	Remove mineral soil from organic material	All	6	N
	S	Rubber or silicone spatula	Transfer soil and roots from bucket to sieve(s).	All	3	N
	R	Rectangular enamel pan or equivalent, white (app. 30 cm x 20 cm, or 13"x 9")†	Facilitates separating roots (which float) from mineral particles	All	6	N
	R	Forceps, blunt tip, stainless steel	Separate roots from organic material	All	3	N
	R	* Wire gauge with openings approx. 2mm, 1mm, and 0.5mm	Sort roots into size classes during sieving and picking	All	3	N

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Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
	S	Small wire clippers	Clip and separate smaller diameter roots that emerge or fork from bigger roots	Multiple sizeCategories exit	2	N
	R	Grinding mill, Wiley, 20 mesh	Grind larger fine root sample volumes	Sample masses > 750 mg	1	N
	S	Porcelain mortar, 65 mL capacity, with pestle,	Grind smaller fine root sample volumes, avoid loss of small samples in mill	Sample masses < 750 mg	1 set	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
	S	Toothbrush	Clean soil corer threads in field, if changing bit is required.	Field	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 ground split sub-samples	All	Variable	N

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Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
	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	480-640	N
	R	Coin envelopes, 3 $\frac{3}{8}$ "x6", Kraft paper	Store and organize sieved roots during and after drying	Small root quantities	50	N
	R	Paper bag, 8# 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; while not *exactly* the diameters desired, the gauges listed here are acceptable for this protocol.

† Note: the exact dimensions of the pan/tub are not critical, it serves as an aid for more easily spotting roots suspended in water. The only requirement is that it can safely contain liquid; a white material also makes identifying roots easier.

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Table 6. SOP D 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 μ m 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, stir range 60 to 1200 rpm, 4 x 4 inch stirring surface	Randomize aqueous suspended residual fraction	1	N
	R	Magnetic stir bar, 2" to 3" length	Randomize aqueous suspended residual fraction	2	N
	S	Beaker, 1 L	Hold smaller volumes of aqueous suspended residual fraction	2	N
	S	Beaker, 2 L	Hold large volumes of aqueous suspended residual fraction	2	N
	S	Beaker, 4 L	Hold very large volumes of aqueous suspended residual fraction; e.g., for soils with thick O horizon	2	N
	R	Plunger, diameter approx. 1 cm less than beaker diameter	Stop mixing vortex, randomize aqueous suspended residual fraction	1 per per beaker size	N
	R	Syringe, 40 – 60 mL, with tip cut off to make a 1 cm diameter aperture	Aspirate sub-sample from randomized aqueous residual fraction	2	N

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Item No.	R/S	Description	Purpose	Quantity	Special Handling
	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
	R	Forceps, fine tip	Pick small root fragments apart from organic material	2	N
	S	Heavy duty sheet tray, baking or equivalent	Transfer aqueous samples in aluminum dishes to drying ovens; hold and protect samples throughout oven drying procedure.	1	N
	R	Threaded Rod, 12" x ¼", zinc	Plunger device for dilution sampling, rod	1	N
	R	Semi-rigid or rigid waterproof material (e.g., vinyl laminate wall base moulding)	Plunger device for dilution sampling, plunger base	1	N
	R	Wood Dowel, 12" by ¾" diameter	Plunger device for dilution sampling, plunger handle	1	N
	R	Hex Nuts, ¼"	Plunger device for dilution sampling, fastening	4	N
Consumable Items					
None					

R/S=Required/Suggested

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6.2 Training Requirements

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

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. When perennial grasses are present, technicians must possess a demonstrated ability to identify crown material associated with these plants.

For the laboratory work, 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. Please note that if sampling at particular locations requires significantly more time than expected, NEON Science may propose to move these sampling locations.

The optimal strategy for allocating labor to field sampling and laboratory processing tasks, and completing the fine root biomass coring effort within 1 month, depends on the number of people available to complete the work:

Option 1: One crew (minimum of 2 people) first performs the field sampling, then returns to the laboratory to process the cores just sampled in the field. Bear in mind that the number of consecutive field days is limited by the requirement that core samples do not remain in cold storage longer than 72 hours. Once cores have been processed in the laboratory and roots are in the drying oven, the crew returns to the field to sample more cores and repeat the cycle. Several sampling strategies have been successfully employed by NEON Field Operations:

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-) 20m x 20m plots: Sample 3-4 plots in the morning (6-8 cores), then afternoon and entire next day spent processing cores in the laboratory. Dilution SOP performed on 2 cores at a time, to accommodate bench and oven space limitations.
-) 40m x 40m plots: Sample 3-4 plots at a time (12-16 cores), then return to the laboratory for processing all cores.
-) 40m x 40m plots at 'away' site with lodging: Sample 6 plots (24 cores), then return to the laboratory for processing all cores. Work over the weekend to meet requirement that all cores are processed within 72 h of sampling in the field.

Option 2: One crew performs field sampling and initial processing, and another person performs additional laboratory processing. For example:

-) 20m x 20m plots: Sample 2 plots in the morning (4 cores), perform sieving in the afternoon; dilution sampling performed next day by a different person.

Time estimates for various components of the field and laboratory work are below. If you find that time required to complete a given task differs significantly from these estimates, submit a problem ticket to NEON Science:

-) **Field sampling:** 0.5 – 1 h 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.
-) **Sieving:** Typically requires no more than 60 minutes per core.
-) **Sorting:** Typically requires between 1-3+ hours per core, depending on the number of very small roots. Most time is spent sorting ≤ 0.5 mm from '0.5 to ≤ 1 mm' size categories.
-) **Dilution:** Between 1.5-2.5 h per sample (Koteen and Baldocchi 2013).

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7 STANDARD OPERATING PROCEDURES

SOP A Preparing for Sampling

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

1. Make waterproof labels for tracking soil core sampling metadata in the field.
 -) Cut waterproof paper (Rite-in-the-Rain or equivalent) into approx. 3"x5" rectangles.
 -) Write metadata on the labels with Sharpie in the field, and place the labels inside the plastic bags with the cores.
 -) The outside of plastic bags may also be labeled with Sharpie for easy visibility, but do **NOT** rely only on labeling the outside of bags; Sharpie can smear and become unreadable.

2. Use local knowledge of the soils present at the site, and 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**).

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

3. 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 4**.

Item Description	Action(s)
GPS unit	<ul style="list-style-type: none">) Charge) Load target plot locations

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Item Description	Action(s)
Compass, mirror-sight, adjustable declination	Check/set correct declination*
TruPulse 360R laser rangefinder and clinometer	<ul style="list-style-type: none">) 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
Hand clippers	Clean and sharpen blades (if necessary)
Sand, or other site-specific material	Check with the site host to determine the desired back-fill material. Ensure supply is sufficient for backfilling soil core holes.
Belowground biomass core "Field Coring Datasheet"	Print as needed on waterproof copy paper
Clip Lists	Print as needed on waterproof copy paper
Tower Plot "Random Subplot List"	Print as needed on waterproof copy paper; only needed for 40m x 40m Tower Plots.

* 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)

Item Description	Action(s)
Dilution Sampling Plunger	<ul style="list-style-type: none">) Assemble plunger from items listed in Table 6. SOP D equipment list – Dilution sampling for fine root biomass fragments < 1 cm

1. Assemble a plunger (**Figure 3**), with diameter suitable for the size of beaker selected from **Table 6**; plunger pieces can be assembled from locally available hardware store parts.

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- a. Use scissors, a utility knife, or other appropriate tool to cut a circular section out of a piece of acrylic, polycarbonate, or vinyl. The diameter of the circle should be approx. 1 cm less than the diameter of the beaker.
- b. Create a small hole in the center of the circle just large enough to fit the threaded rod zinc rod through (hole is approx. ¼”).
- c. Tighten on one nut <1” from the bottom. Then slide the cut disk on, and fasten with another nut.
- d. Drill a ¼” hole completely through the wooden dowel and cut length to a preferred size.
- e. Repeat step 3 to attach the dowel using two nuts.

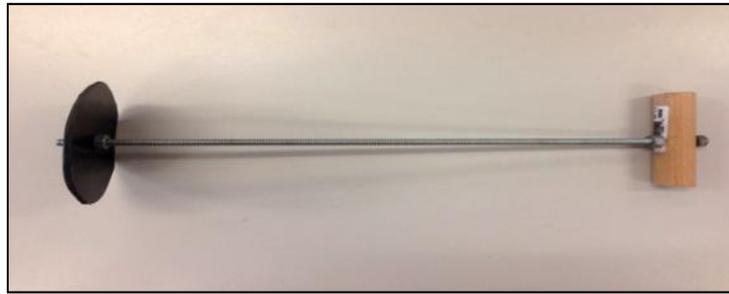


Figure 3. Assembled plunger used to randomize root fragment samples < 1 cm length as part of dilution sampling (SOP D).

2. Label aluminum weigh tins with unique tinLabels.
3. Print lab dilution datasheets as necessary.

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SOP B Soil Core Sampling in the Field

SPATIALLY LINKED PROTOCOL: HERBACEOUS BIOMASS CLIP HARVEST

-) If plant Belowground Biomass Core sampling is scheduled prior to Herbaceous Biomass clip harvest sampling, accepting/rejecting clip strips must be done with both protocols in mind.
-) It is highly desirable for accepted clip strips to support both protocols.
-) If Herbaceous Biomass sampling is scheduled before plant Belowground Biomass Core sampling, be sure to consult each per plot Clip List to enable cross-protocol co-location of sampling.
-) If Herbaceous Biomass sampling is scheduled immediately before Belowground Biomass Core sampling, be sure to stagger the sampling activities to ensure sufficient oven space for all samples.

In addition:

-) Plant Diversity sampling occurs in 3 randomly selected Tower Plots each year. In these plots, identify and demarcate a suitable clip strip prior to performing Plant Diversity sampling.
-) This will ensure that the clip strip is not trampled during Plant Diversity sampling.

B.1 Sample Collection in the Field

1. Navigate to the plot or subplot to be sampled.
2. Use the plot or subplot-specific Clip List to identify the clip cell that was (or will be) used for the peak herbaceous biomass clip harvest in the current year. If the site host allows, a pin flag may be left behind at the SW corner of the clip strip to aid co-location across protocols.
 -) The Clip List provides the randomized list of potential clip cells per plot or subplot.
 -) Coordinates provided for each clip cell correspond to the SW corner of the clip-strip – i.e. the area from which herbaceous biomass is harvested (**Figure 2**).
 -) The Clip List indicates which clip cells have already been harvested or rejected; on the Clip List, mark cells selected for Belowground Biomass Core sampling with **status** = 5.
 -) If the desired peak biomass clip cell is submerged by standing water, reject and work down the Clip List to choose an acceptable clip cell, and record “peak biomass cell submerged” in the “remarks” field.
3. Locate the relative offsetEasting and offsetNorthing coordinates of the SW corner of the clip-strip within the target clip “cell”. The procedure used to locate the offsetEasting coordinate depends on the value of the relative offsetNorthing coordinate:

If the ‘offsetNorthing’ 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.*

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- 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 'offsetNorthing' 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**).

offsetEasting 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 offsetEasting 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 SW corner of the Clip Strip.

TIPS



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

- 4. Assess whether the clip cell is representative of the plot, and accept or reject the location.

Remember that you must consider both this protocol, and the Herbaceous Biomass protocol.

 -) Obstacles, disturbances, and/or irregularities may lead to a cell being unrepresentative, and these may include trees, large rocks, ant nests, downed logs, etc.
 -) If > 3 consecutive potential cells are rejected as 'unrepresentative,' it is necessary to recalibrate the working definition of 'representative.'

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5. Mark the four corners of the South 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 4**).
 - 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 4**) – use a meter tape or ruler 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”
 - e. Domain staff may also pursue building a 50 cm by 50 cm square frame from small diameter PVC piping to reduce pin flag setup time.

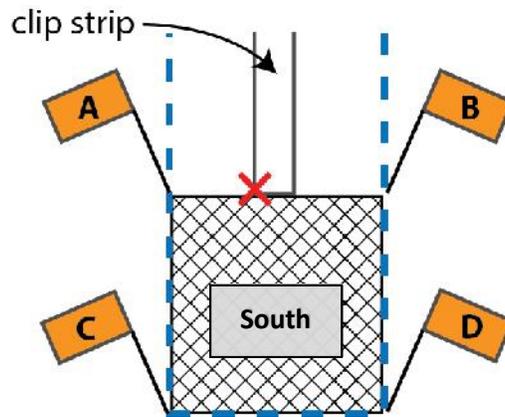


Figure 4. Delineating the South soil core sampling area (cross hatched) within a clip “cell” (dashed blue lines) with pin flags. The clip-strip (black lines) lies immediately to the north of the South soil core sampling area, and the red “x” marks the coordinates provided in the Herbaceous Biomass Clip List.

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6. Mark the four corners of the North 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 5**).
 - 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”

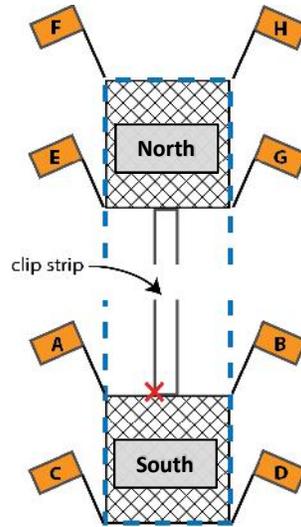


Figure 5. Delineating the North soil core sampling area with reference to the previously delineated South soil core sampling area (cross hatched) within a clip “cell” using pin flags (dashed blue lines indicate the clip cell boundary). The middle of both the cell and the clip-strip have been omitted for clarity.

7. For each of the ‘N’ and ‘S’ soil core sampling areas:
 - a. Create a label on waterproof paper with the information below. The label and the core will then be placed in a large plastic freezer bag.
 -) **coreDate**, YYYYMMDD format
 -) **plotID** and **subplotID**,
for 20m x 20m plots, subplotID = 31
for 40m x 40m plots, subplotID = 21, 23, 39, or 41
 -) **clipCellNumber**, the 3 digits to the right of the last “_” in the clipID on the Clip List
 -) **coreID**, N or S
 - b. Record on the “Field Coring Datasheet”:
 -) **plotID**
 -) **subplotID**
 -) **clipCellNumber**
 -) **coreDiameter**; This is typically 6.65 cm, but may vary if equipment changes through time.
 -) **coreID**; N or S

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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 into a labeled plastic bag.

TROUBLESHOOTING



-) If obstacles are encountered that prevent coring to 30 cm depth, a minimum core depth of 20 cm is acceptable.
 -) If a minimum 20 cm depth core cannot be obtained, select another location from within the target 'N' or 'S' sampling area, but do not attempt more than 3 alternate locations.
 -) If a minimum 20 cm depth core cannot be sampled within the target 'N' or 'S' sampling area, record "20 cm coreDepth not achieved" in the **remarks** field of the 'Field Coring' datasheet, and collect a core with the greatest depth possible.
 -) If no core can be obtained from a representative cell, record **`coringPossible = N'**, and move on to the next plotID or subplotID on the list.
-

- 12. Place the core into the cooler immediately for cold storage until cores can be processed in the laboratory.
 -) Remember to refresh cold packs every 12 h or transfer cores to a refrigerator in the lab.
- 13. Measure the average depth of the bore hole; to do this, push past any loose soil that fell back into the hole, and measure once at what appears to be the correct representative depth. Record in the "Field Coring" ingest:
 -) **coreLength**, the total length of the core; nearest 1 cm
 -) **time**, the time the core is placed into the cooler in the field; *HHmm*, 24-h format
 -) **remarks**, e.g. "20 cm coreLength due to root"
- 14. Backfill the bore hole with site-host approved material.

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B.2 Sample Preservation

-) Keep samples in a cooler with cold packs to minimize cellular activity, reduce decomposition, and preserve sample mass.
-) Change cold packs for fresh ones every 12 h or transfer to a 4 °C refrigerator prior to laboratory processing.

IMPORTANT: Record the **coreDate** and **time** in the “Field” ingest AND **ovenStartDate** and time in the “Root Mass” ingest table so that the number of hours the samples were stored cold can be automatically calculated.

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SOP C Processing Belowground Biomass Samples in the Laboratory

Overview

Fine root samples generated from this procedure are analyzed for isotopes (^{13}C and ^{15}N); as such, disposable latex or nitrile gloves are required during sieving, sorting, and grinding tasks to prevent contamination of the sample with your hands.

Soil cores must be sieved in the laboratory within 72 h of sampling in the field. Use time estimates for lab processing steps provided in Section 4 to plan field work so that a backlog of cores does not develop, and the 72 h requirement can be met. Time sensitive processing steps include:

1. Wash and sieve soil cores to separate mineral soil from root biomass and organic matter, and separate roots ≥ 1 cm length from the residual fraction.
2. Set aside the residual fraction from a random subset of 20 cores for processing with SOP D.
 -) See SOP D, step (1) for guidance on randomly selecting cores for dilution sampling.
 -) It is acceptable to pause overnight between execution of SOP C and SOP D. Store labeled residual fractions overnight at 4 °C in a sealed container (e.g., labeled 50 mL tube).
3. Dry fine root biomass ≥ 1 cm length to constant weight.

Once roots are dry, time is no longer of the essence, and the following may be completed as time allows:

4. Weigh and record dry weight biomass.
5. Grind fine root samples for chemical analyses.

C.1 Sieving soil cores for fine root biomass

Prepare a soil core for sieving:

1. If the soil cores have a large amount of root mass, finely textured soils, or the soil is difficult to break apart by hand without fragmenting roots, wet-sieving may be the most efficient procedure for separating roots from soil; follow instructions in the wet sieving section below.
2. If the soil cores have little root mass, are coarsely textured, or the roots are very brittle then dry-sieving soils may be the most efficient procedure; follow instructions in the dry sieving section below.

The goal for the sieving procedure is to isolate fine roots and sort to **sizeCategory**. Non-root material encountered during sieving is discarded (rhizomes, corms, bulbs, perennial graminoid crowns, etc.).

C.1.1 Wet Sieving Soils

1. For wet sieving:
 -) Soak the core in water for a minimum of 1 hour in a 5 gallon plastic bucket to facilitate breaking up clays. Water depth should be sufficient to cover the core.

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-) Soil cores may also be soaked overnight in the bucket to facilitate workflow scheduling.
- 2. For each soil core sample, label up to 8 coin envelopes with the information below. You will not need all 8 envelopes if all **rootStatus** x **sizeCategory** combinations are not present in the core sample. If there is a large amount of root biomass within a given size class, use a clasp envelope instead.
 -) **coreDate**, date roots were sampled in the field; *YYYYMMDD* format
 -) **plotID** and **subplotID**,
 - for 20m x 20m plots, subplotID = 31;
 - for 40m x 40m plots, subplotID = 21, 23, 39, or 41
 -) **clipCellNumber**, the 3 digits to the right of the last “_” in the clipID on the Clip List
 -) **coreID**, either ‘N’ or ‘S’
 -) **rootStatus**, ‘Live’ or ‘Dead’
 -) **sizeCategory** (<0.5, 0.5-1, 1-2, 2-10)
- 3. Use a 250 µm sieve, a white enamel pan, and the root washing station to begin separating roots and organic material from mineral soil particles in the bucket.
 - a. Massage the sample in the bucket with gentle manual pressure to break up large aggregates and organic matter pieces.
 - b. Thoroughly mix the slurry in the bucket by hand to separate small roots from mineral soil particles. At this point, roots and small pieces of organic material (OM) should be floating on the surface.
 - c. Remove and sort floating roots from the surface of the slurry.
 - i. Skim the surface of the slurry with the 250 µm sieve, then rinse the sieve contents with the root washer.
 - ii. Transfer the sieve contents to the enamel pan by inverting the sieve over the pan and rinsing with the root washer nozzle. Be careful not to overfill / overflow the pan!
 - iii. In the pan, pick and separate root fragments from organic material, sorting to **rootStatus** and **sizeCategory** combinations as you go.
- 4. Separate fine roots remaining in the bucket from mineral soil and organic matter. Use the root washing station, a 2 mm sieve, a 250 µm sieve, and a white plastic or enamel tray.
 - a. Pass no more than 10%-20% of the slurry through the top of the sieve stack.
 -) You must avoid overloading / overflowing the 250 µm sieve.
 - b. Wash fine mineral soil particles through the sieve stack using the root washer nozzle; 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.
 -) BE CAREFUL NOT TO OVERFLOW THE 250 µm SIEVE!

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- c. Manually remove larger rocks from the top of the 2 mm sieve – but don't spend more than several minutes.
 - d. Turn the 2 mm sieve upside down over one of the enamel pans, and use the root washer nozzle to transfer material from the 2 mm sieve to the pan. Roots often float, and mineral particles sink.
 - e. Place the 2 mm sieve back on top of the 250 μ m sieve, and decant the sample from the enamel pan back through the sieve stack, retaining mineral particles in the enamel pan.
 - f. Discard mineral particles retained in the enamel pan, and rinse the pan.
 - g. Repeat (d)-(f) until enough mineral particles have been removed from the sample that it is possible to begin picking ≥ 1 cm root fragments from remaining organic matter.
 - h. Use forceps to pick all roots ≥ 1 cm length from the enamel pan, sorting to **sizeCategory** and **rootStatus** as you go; alternatively, you may sort to **sizeCategory** and **rootStatus** after all roots > 1 cm in length have been picked.
 -) Use a wire gauge to determine the **sizeCategory**; the largest diameter of a root fragment should be used to classify the size.
 -) **VERY IMPORTANT:** To determine root diameter, you must pass the root through the gap *in the side* of the wire gauge; DO NOT insert the root through the larger hole.
 -) The wire gauge may be mounted on the side of the sieve using one of the larger gaps, enabling quick access for size classification.
 -) If only part of a root is alive, categorize the entire root as "live." Dead roots are most often dark brown or black and brittle, while live roots are often lighter in color and flexible – i.e., they can typically be bent into a "U" shape without breaking.
 - i. Place sorted roots into the pre-labeled envelopes created in step (2).
 - j. Rinse out the enamel pan, and repeat steps (d) to (i) for the 250 μ m sieve.
 - k. **If the sample has been randomly selected for dilution sampling**, set aside the residual fraction (i.e., root fragments < 1 cm mixed with organic material) for processing via SOP D.
 -) See SOP D, step (1) for guidance on randomly selecting cores for dilution sampling.
 - l. Clean the 250 μ m sieve, mix the remaining slurry in the bucket by hand, and repeat all of step (4) until the entire sample has been processed through the sieve stack.
5. Thoroughly clean the sieves and enamel pan with water between core samples.
 6. Check sediment traps in the root washing station; if traps are full, dispose of sediment in an approved receptacle.
 7. Gather samples from the same core together to keep them organized. For example:
 -) Place envelopes containing root samples into a paper bag to keep samples organized (lunch sack size works well); OR



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) If there are very few roots, coin envelopes may be paper clipped together.

C.1.2 Dry Sieving Soils

1. Process soils using the different sized sieves as you would with the wet-sieving procedure, but do not apply water.
2. For each soil core sample, label up to 8 coin envelopes with the information below. You will not need all 8 envelopes if all **rootStatus** x **sizeCategory** combinations are not present in the core sample. If there is a large amount of root biomass within a given size class, use a clasp envelope instead.
 -) **coreDate**, date roots were sampled in the field; YYYYMMDD format
 -) **plotID** and **subplotID**,
 - o for 20m x 20m plots, subplotID = 31;
 - o for 40m x 40m plots, subplotID = 21, 23, 39, or 41
 -) **clipCellNumber**, the 3 digits to the right of the last “_” in the clipID on the Clip List
 -) **coreID**, either ‘N’ or ‘S’
 -) **rootStatus**, ‘Live’ or ‘Dead’
 -) **sizeCategory** (<0.5, 0.5-1, 1-2, 2-10)
3. Use a 2 mm sieve, a 250 µm sieve, and a pan bottom. Pass the sample through the sieve stack to separate roots from mineral soil and soil organic matter.
 - a. The 2 mm sieve is useful for catching and removing large rocks from the sample, as well as larger roots
 - b. The 250 µm sieve is useful for capturing any roots that have passed through the 2 mm sieve. Roots ≥ 1 cm in length are not likely to pass through this finer mesh.
 - c. The white pan can be used to more easily differentiate small roots in the 250 µm soil fraction.
4. From each sieve and the enamel pan, separate fine roots ≥ 1 cm in length from mineral soil and organic matter.
 - a. It is often helpful to pass no more than 10 – 20% of the sample through the sieve stack, as it makes it easier to spot roots.
 - b. Break up aggregates and organic matter pieces using gentle manual pressure.
 - c. Manually remove larger rocks from the top of the 2 mm sieve – but don’t spend more than several minutes.
5. Use forceps to pick all roots ≥ 1 cm length from the enamel pan, sorting to **sizeCategory** and **rootStatus** as you go; alternatively, you may sort to **sizeCategory** and **rootStatus** after all roots ≥ 1 cm in length have been picked.
 - a. Use a wire gauge to determine the **sizeCategory**; the largest diameter of a root fragment should be used to classify the size.



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- b. **VERY IMPORTANT:** To determine root diameter, you must pass the root through the gap *in the side* of the wire gauge; DO NOT insert the root through the larger hole.
 - c. The wire gauge may be mounted on the side of the sieve using one of the larger gaps, enabling quick access for size classification.
 - d. If only part of a root is alive, categorize the entire root as “live.” 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 brittle, while live roots are often lighter in color and flexible – i.e., they can typically be bent into a “U” shape without breaking.
 - e. Place sorted roots into the pre-labeled envelopes created in step (2).
 - f. **If the sample has been randomly selected for dilution sampling**, set aside the residual fraction (i.e., root fragments < 1 cm mixed with organic material) for processing via SOP D.
 - g. Clean the 250 µm sieve and repeat all of step (4) until the entire sample has been processed through the sieve stack.
6. Once all roots >1 cm in length have been picked and sorted, wash sediment from roots by using a clean 250 um sieve. Sediment clinging to roots can significantly inflate weighed root biomass; thus the importance of gently washing dry roots once they are sieved.
 - a. Place a sorted group of roots into the 250 um sieve and gently run water over the roots.
 - b. Use forceps to transfer the roots to a labeled coin envelope.
 - c. Repeat the above steps (a-b) for the remaining root samples.
 7. Thoroughly clean the sieves and enamel pan with water between core samples.
 8. Gather samples from the same core together to keep them organized. For example:
 -) Place envelopes containing root samples into a paper bag to keep samples organized (lunch sack size works well); OR
 -) If there are very few roots, coin envelopes may be paper clipped together.

C.2 Drying, weighing, and processing belowground biomass samples

Washed roots should be placed in the drying oven as soon as possible following sieving.

1. Label each bag containing washed root samples with the date and time it is placed in the drying oven.
 -) These data are the **ovenStartDate** and time required during data entry.
 -) **Critical step:** Labeling bags allows assessment of how long different batches of bags have been in the oven, especially when roots sampled on different days occupy the same oven.
2. Place labeled bags into a drying oven for a minimum of 48 h (longer is okay, but not required).
 -) Dry all root diameters at 65 °C.

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3. Remove bags of dried biomass from the drying oven, and label with **ovenEndDate** / Time.
 -) Dried plant material should be weighed immediately after removing from the drying oven, as it will absorb moisture from the air if left in ambient room conditions (particularly in humid environments).
 - o If using this method, it is helpful to remove bags from the oven and weigh one at a time.
 -) Dried samples may also be stored for up to 30 days in ambient room conditions prior to weighing. Samples treated in this manner must be returned to the drying oven for 24 h prior to weighing, and must be weighed as above after removal from the oven.
4. Organize all samples from the same **plotID**, and weigh each fine root sample using a mass balance and a weigh boat. Weighing samples from the same plotID in sequence for this step will greatly facilitate assigning pooling for chemical analysis and potential archiving in SOP 0.
 -) Record **dryMass** on the 'Lab Weighing' datasheet; nearest 0.0001 g, plant material ONLY (without the bag).
 -) For large quantities of biomass that do not readily fit into a large weigh boat, use the following strategies:
 - o Crush or chop the biomass to reduce volume so it will fit into a weigh boat.
 - o *Avoid splitting the biomass into subgroups for weighing, as uncertainty values must be added each time a subgroup is created.*
5. Record required metadata for the sample in the 'Lab Weighing' datasheet.
 -) **coreDate**, date fine roots were sampled in the field
 -) **plotID**, unique ID of the sampled plot
 -) **subplotID**, unique ID of the sampled subplot
 -) **clipCellNumber**, the last three digits of the sampled clipID
 -) **coreID**, either 'N' or 'S'
 -) **ovenStartDate/ Time**, date and time sample was placed in drying oven; 24 h format
 -) **ovenEndDate/ Time**, date and time sample was removed from drying oven; 24 h format
 -) **rootStatus**, 'live' or 'dead'
 -) **sizeCategory**, diameter category of the sorted sample; <0.5, 0.5-1, 1-2, or 2-10 mm
6. Once all masses have been recorded for a given sampling bout, QA will be performed on a subset of samples (SOP 0), or return dried fine roots to temporary storage at ambient conditions. Samples in temporary storage can then be weighed for QA as time permits.

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C.3 Data Quality Assurance

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

1. For each sampling event at a given site, select 10% of dried, previously weighed samples for re-weighing.
 -) If QA weighing does not occur within several hours of the initial weighing, return the selected samples to the drying oven for 24 h prior to QA weighing. In humid environments, samples will pick up moisture from the atmosphere.
2. Record QA weight data to the nearest 0.0001 g in the **qaDryMass** field of the 'Lab Weighing' datasheet.

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C.4 Grinding Fine Root Biomass for Archive and Chemical Analysis

Overview

Once QA masses have been recorded, samples with **rootStatus** = 'live' must be processed for chemical analysis, and possibly archive. Samples with **rootStatus** = 'dead' may be discarded once data have been successfully entered to the NEON database and have been checked for data entry errors.

To create a composite root sample for grinding, live roots within the same **sizeCategory** are pooled across the two 'N' and 'S' cores that originate from the same **clipCellNumber**. This means a maximum of 4 pooled root samples are ground per unique **clipCellNumber** (one sample for each **sizeCategory**).

The samples that are pooled and shipped for chemical analysis and archive must have **sampleID** information linked back to root mass data and per core field data in order to be meaningful. To do this, you will use the 'Chemistry Pooling' ingest table, and indicate which subsamples from the 'Root Mass' table were combined to create the pooled sample. The data ingest application will then automatically create the required chemistry analysis ID, needed for labeling the shipped chemistry analysis sample, and archive ID, if there is sufficient sample available.

Procedural steps:

1. Based on the total mass of each **pooled** sample, decide whether enough sample is available for chemical analysis only, or whether sufficient mass is available for both chemical analysis and archive (**Table 9**).

Table 9. Splitting and processing guidelines for fine root samples, based on pooled sample mass.

dryMass	Samples to create		Processing guidelines
	C:N sample	Archive sample	
<0.02 g	-	-	Do not process sample for C:N analysis or archive.
0.02 – 1 g	X		Do not grind; place entire pooled sample in scint vial. Use gloved hand to crush if necessary.
> 1 g	X	X	Grind sample; use splitter to allocate a minimum of 0.2 g for C:N analysis, and remainder to archive sample.

2. Enter the mass data from the completed 'Lab Weighing' datasheet into the 'Root Mass' data ingest table.
 -) Review the entered data for errors.
3. Enter subsampleID and pooling data into the 'Chemistry Pooling' ingest table.
 -) If the total pooled sample mass is < 0.02 g:

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- In the 'Chemistry Pooling' ingest, select the two parent subsamples that did not generate enough mass for analysis, and select "No pooled sample generated."
 -) If the total pooled sample mass is ≥ 0.02 g but < 1 g:
 - In the 'Chemistry Pooling' ingest, select the two parent subsamples, and select "Chemistry analysis only."
 -) If the total pooled sample mass is ≥ 1 g:
 - In the 'Chemistry Pooling' ingest, select the two parent subsamples, and select "Chemistry analysis and archive."
4. Use the **cnSampleID** and **bgcArchiveID** information automatically generated by the 'Chemistry Pooling' data ingest application to create vial labels and an inventory sheet for the samples that will be shipped.
 -) Pre-label the required number of 20 mL plastic scint vials with the **cnSampleIDs** or **bgcArchiveIDs**.
 -) Use a printed label or laboratory tape wrapped around the entire vial so that it overlaps itself. **Do not write directly on the vial, as it will rub off.**
 5. Grind oven-dried pooled samples with total pooled mass ≥ 1 g.
 -) Use the Wiley Mill with the 20-mesh attachment (0.84 mm mesh).
 -) If total sample mass is < 1 g: Ship the roots as is, and do NOT grind the sample. The analytical facility will grind the sample upon receipt.
 6. When the pooled sample mass is ≥ 1 g, split the sample to generate a subsample for C:N analysis, and another for archive.
 -) Mix the ground sample thoroughly with a spatula, and use an appropriately sized splitter or microsplitter to generate two representative subsamples.
 -) Transfer each subsample to the appropriate pre-labeled 20 mL scint vials created above.
 -) If there is > 1 g of sample, use a splitter and allocate a minimum of 0.2 g for C:N analysis, and send the remainder to archive.

BEST PRACTICE TIPS

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

7. Clean grinding tools thoroughly between samples.
 -) For a grinding mill, clean with compressed air.
 -) Clean mortar and pestle with a kimwipe and ethanol.

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8. Once pooled samples have been shipped, discard excess ground biomass from each sample.

C.5 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.

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SOP D Dilution Sampling for Fine Root Fragments

The dilution sampling for fine root fragments procedure begins part-way through SOP C.1, after all roots ≥ 1 cm length have been picked from the sample. SOP D can be considered an add-on to SOP C that is performed on 20 randomly selected cores each time the fine root biomass protocol is implemented. 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 relatively time-efficient technique.

1. Randomly select 20 cores for processing according to this SOP.
 -) First randomly select 20 clipIDs for dilution sampling, then randomly select either the ‘N’ or ‘S’ coreID from the selected clipIDs.
 -) It is not possible to provide this list ahead of time because coring success in a given clip cell is not guaranteed.
 -) Excel may be used to generate random lists; if you are not familiar with how to generate a random list, consult an experienced lead technician, or your Domain Manager.
2. Take the residual fraction still in the 250 μ m sieve from SOP C, and carefully wash with the root washer nozzle. The residual fraction 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 core – to a beaker, and suspend the sample in water:
 - a. Based on the size of the residual fraction, choose either a 1 L, 2 L, or 4 L beaker. Note that the size of the beaker can be varied from core to core, depending on the size of the residual fraction.



TIP: 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, and dilute further if necessary.

- b. With the root washer nozzle *on a low flow rate*, use ≤ 500 mL of water to transfer the residual fraction from the 250 μ m sieve to the beaker.
 - i. Note: using high pressure water may further disintegrate root fragments
- c. Carefully fill the beaker to approximately $\frac{3}{4}$ full (e.g., 750 mL, 1.5 L, or 3 L). It is helpful to fill up to one of the pre-marked graduations on the beaker, as an accurate volume at this step will be used to estimate the total mass of root fragments < 1 cm length.
- d. Record required metadata in the ‘Lab Dilution’ datasheet. Values will apply to all of the 10 pairs of aluminum weighing tins in the next step (20 tins total).
 -) **coreDate**, date fine roots were sampled in the field, YYYYMMDD format
 -) **processedDate**, date dilution sampling is carried out, YYYYMMDD format
 -) **plotID**, unique ID of the sampled plot

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-) **subplotID**, unique ID of the sampled subplot
 -) **clipCellNum**, the last three digits of the sampled clipID
 -) **coreID**, either 'N' or 'S'
 -) **sampleVolume**, volume of water used to suspend residual fraction in beaker; nearest 10 mL
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.
 -) Tins should be pre-numbered with a unique **tinLabel** (e.g. 1, 2, 3,..., 20, etc.). The **tinLabel** is tracked on the datasheet, rather than labeling each tin with the clipCellNumber.
 -) Pre-weigh each tin with a microbalance, and record in the 'Lab Dilution' datasheet:
 - o **subSampleNumber**, technician assigned number from 1 to 10
 - o **tinLabel**, the unique number assigned to the tin
 - o **tinEmptyMass**, the mass of the clean, dry, empty tin; nearest 0.0001 g
5. Work in pairs to generate 10 sub-samples from the aqueous suspended residual fraction in the beaker. Consult the training video for a visual demonstration of the following steps:
- a. [Person1] Turn the plate mixer on high, and vortex the aqueous suspended residual fraction thoroughly (approx. 10 s from the start of vortexing).
 - b. [Person1] Turn off the mixer, and quickly plunge the suspension to stop the vortex and randomize the sample in the water.
 - c. [Person2] Take a 20 mL sub-sample from the middle of the water volume in the beaker using the customized syringe, and transfer to one of the 'OM' tins.
- NOTE:** In addition to adjusting the **sampleVolume** in step (3) above, the **subsampleVolume** can also be adjusted from 20 mL to optimize the amount of material needed for sorting and weighing. For example, subsample 10 mL if the suspension is particularly dense.
- d. [Person2] 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 tin.
6. Record in the 'Lab Dilution' Datasheet:
-) **subsampleVolume**, the volume of the sub-sample taken from the beaker; nearest 1 mL (this volume will be the same number for both tinIDs in a pair)
 - o The volume of water from the squirt bottle should not be added to this number.
 -) **subsampleType** (data sheet only field), the type of material the tin will hold after picking and sorting is complete; the tin initially receiving the mixed sub-sample should be

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subsampleType = 'OM', and the tin into which roots are sorted should be **subsampleType** = 'ROOT.'

- The **subsampleType** field does not exist in the 'Dilution' ingest table; enter tin mass and tin+mass data from each subsampleType into either **dryMass** fields (for *ROOT* mass) or **omDryMass** fields (for *OM* mass).
7. Repeat steps (5) and (6) until 10 sub-samples have been transferred to 10 '*OM*' tins (**Figure 6**).
 8. For each of the 10 sub-samples, carefully pick and sort root fragments from organic material, and transfer the roots to the '*ROOT*' tin of the pair (**Figure 6**).
 -) A small amount of water in the '*ROOT*' tin aids in transferring root material.
 -) **Aim for approximately 10-15 min sorting time per tin pair**; adjust the **sampleVolume** in the beaker and the **subsampleVolume** in the syringe as necessary.

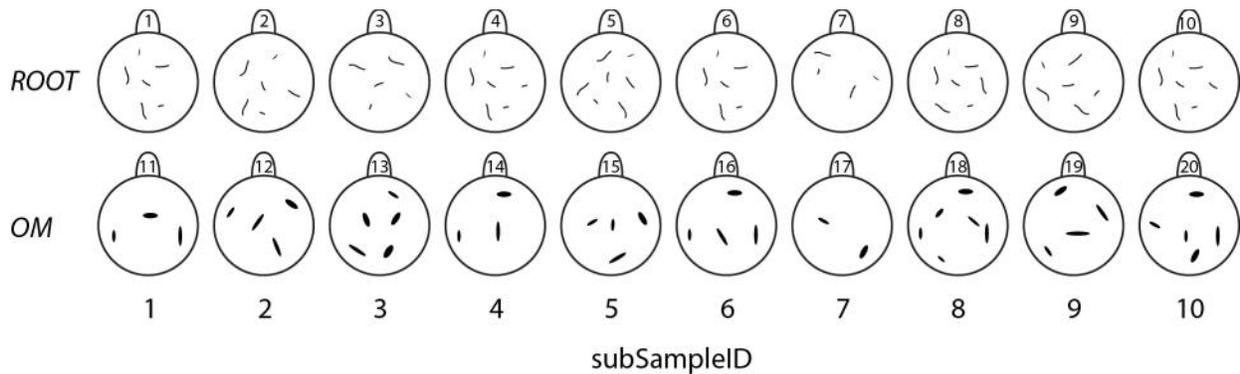


Figure 6. Pairs of labeled aluminum weighing tins for separating roots from OM in residual fraction sub-samples. Mixed sub-samples are initially transferred to the *OM* tins, and roots are then sorted into the *ROOT* tins.

9. Carefully transfer tins to a 65 °C drying oven for 48 h. Record:
 -) **ovenStartDate/Time**, the date and time the samples were placed in the drying oven.

Tips:

 -) Use a tray to move batches of tins in the laboratory.
 -) Heavy duty metal trays may be placed directly in the drying oven with all of the samples.
 -) Do not leave samples on light-weight trays in the drying oven. Light-weight metal trays occasionally twist when heated, which will cause samples to spill.
10. Repeat steps (2) – (9) for additional cores.
11. Once tins are dry, weigh the total mass of each 'tin+*ROOT*' or 'tin+*OM*' with a microbalance. Record in the 'Lab Dilution' datasheet:
 -) **ovenEndDate/Time**, the date and time samples were removed from the drying oven.
 -) **tinSampleMass**, the mass of the dry 'tin+*ROOT*' or 'tin+*OM*' material; nearest 0.0001 g

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SOP E Data Entry and Verification

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

However, given the potential for mobile devices to fail under field conditions, it is imperative that paper datasheets are always available to record data. Paper datasheets should be carried along with the mobile devices to sampling locations at all times. As a best practice, field data collected on paper datasheets should be digitally transcribed within 7 days of collection or the end of a sampling bout (where applicable). However, given logistical constraints, the maximum timeline for entering data is within 14 days of collection or the end of a sampling bout (where applicable). See RD[04] for complete instructions regarding manual data transcription.

E.1 Field Datasheets

1. Transcribe data from the Core Sampling for Belowground Biomass Field Datasheets (RD[05]) to the 'perbout' ingest form.
 -) Consult the Belowground Biomass Soil Core ingest document (RD[06]) to determine appropriate values and formats for each field in the ingest table.
2. If a representative clip cell did not support belowground biomass core sampling, noted as 'coringPossible = N' in the **remarks** field of the Field Datasheet, enter in the 'perbout' ingest form:
 -) **coringPossible = 'N'**
3. Update permanent digital versions of the Clip Lists with **date** and **status = '5'** data recorded in the field.

E.2 Lab Datasheets

-) Transcribe data from the 'Lab Weighing' datasheet into the 'Root Mass' ingest form.
 - o Consult the Belowground Biomass Soil Core ingest document (RD[06]) to determine appropriate values and formats for each field in the ingest table.
 - o If a core sample contained no fine root biomass within a given **sizeCategory**, enter '0' in the **dryMass** field.
-) On the 'Chemistry Pooling' form, indicate which samples were pooled and ground for chemical analysis to enable generation of chemistry and archive sub-sample IDs.
-) Transcribe data from the 'Lab Dilution' datasheet into the 'dilution' ingest form.

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SOP F Sample Shipment

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

F.1 Timelines

Dried, root samples may be stored indefinitely before shipping.

F.2 Storage / Shipping Conditions

Dried root samples sealed in 20 mL plastic or glass vials may be shipped at ambient temperature without preservatives.

F.3 Grouping / Splitting Samples

Samples originating from the same clip cell should be grouped together for shipment, if possible.

F.4 Procedure

1. Take scintillation vial box containing processed samples out of temporary storage for shipment.
2. Wrap the box in bubble wrap and tape securely, then place in a FedEx box for shipment.
3. Navigate to the “Shipping Information for External Facilities” document on CLA’s NEON intranet site.
 -) Determine which additional documentation is required to accompany the shipment (e.g., USDA permits and/or cover letters).
 -) Check the intranet instructions frequently, *as shipping instructions are subject to change*.
4. Print out required documents (if needed), and include in the shipment box.
5. Prepare a shipping inventory detailing the contents of the shipment, using the protocol-specific template on CLA’s NEON intranet site. Include a printed copy in the shipment box.
6. Address shipping label appropriately, and ship ground.
7. Send an electronic copy of the shipping inventory to the email addresses listed in the “Shipping Information for External Facilities” document. Include the shipment Tracking Number in the email.

F.5 Laboratory Contact Information and Shipping / Receipt Days

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

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8 REFERENCES

- 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.

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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

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

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APPENDIX B QUICK REFERENCES

B.1 Soil Core Sampling in the Field

1. Select the first available clip cell from the Clip List, and assess for suitability. Be sure to check if Herbaceous Biomass sampling has already occurred in the current season, and if a cell has already been clipped, choose the clipped cell to co-locate sampling.
2. Take one core from the North sampling area, and another core from the South sampling area.
3. Measure and record the depth of the core hole.
4. Create a label for each core on waterproof paper, and be sure to record all required sampling metadata.
5. Record the date and time the core was placed in the cooler in the field.

QUALITY DEPENDS ON PROPER:

-) Labeling of core samples.
-) Measurement of core hole depth.
-) Maintaining samples in cold storage.

B.2 Processing Belowground Biomass Samples in the Laboratory

1. Figure out ahead of time which 20 cores will be randomly selected for Dilution Sampling.
2. Soak cores prior to sieving in a plastic bin or bucket.
3. Process one small aliquot of the core through the sieve stack at a time – **avoid overflowing the fine bottom sieve!**
4. Use a wire gauge to determine root **sizeCategory** – **always measure root diameter through the gap in side of the wire gauge**. Do NOT pass the root through the hole of the gauge.
5. Sort roots by **sizeCategory** and **rootStatus**.
6. Dry sorted roots for a minimum of 48 h at 65 °C.

QUALITY DEPENDS ON PROPER:

-) Passing the samples through the sieves – DO NOT OVERFLOW!
-) Removal of mineral soil and organic material from roots.
-) Use of the wire gauge for **sizeCategory** sorting.

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B.3 Dilution Sampling for Fine Root Fragments

1. Retain the residual fraction from randomly selected cores for Dilution Sampling.
2. Label all sample tins to ensure that samples can be tracked.
3. Work in pairs to quickly obtain representative subsamples of the suspended residual fraction.
4. Adjust the size of the beaker (**sampleVolume**) and the size of the sub-sample (**subSampleVolume**) to keep sorting time manageable.
 - a. Aim for approximately 10-15 minutes per tin pair.
5. Dry sorted root fragments and OM for a minimum of 48 h at 65 °C.

QUALITY DEPENDS ON:

-) Choosing an appropriately sized beaker for suspending the residual fraction. Too concentrated will take too long to sort, and too dilute will result in masses too light to accurately weigh.
-) Dispersing the residual fraction evenly throughout the sample volume in the beaker to generate representative subsamples.
-) Accurately distinguishing roots from organic material.

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APPENDIX C REMINDERS

TBD

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APPENDIX D ESTIMATED DATES FOR ONSET AND CESSATION OF SAMPLING

Belowground biomass soil core sampling is ideally timed to broadly 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 **Error! Reference source not found.**

Table 11. Estimated average dates after which greenness begins to decrease for each NEON site based on MODIS-EVI phenology data. Ideally, soil core sampling and aboveground biomass clip harvests should occur on or near these dates.

Domain	Site	Start Date (MM/DD)	Additional Information
01	BART	08/08	
	HARV	08/08	
02	BLAN	07/29	
	SCBI	08/08	
	SERC	08/08	
03	DSNY	07/09	
	JERC	08/08	
	OSBS	07/09	
04	GUAN	10/15	
	LAJA	10/01	
05	STEI	08/03	
	TREE	08/03	
	UNDE	08/03	
06	KONA	07/28	
	KONZ	07/29	
	UKFS	07/15	
07	GRSM	08/03	
	MLBS	08/08	
	ORNL	07/29	
08	DELA	07/24	
	LENO	07/19	
	TALL	07/14	
09	DCFS	07/24	
	NOGP	07/19	
	WOOD	07/29	

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Domain	Site	Start Date (MM/DD)	Additional Information
10	CPER	07/29	Soil may be too hard for coring at greenness decrease date; earlier start date timed to soil moisture may be advised (late spring).
	RMNP	07/29	
	STER	2-4 wks before crop harvest	
11	CLBJ	10/01	
	OAES	10/16	
12	YELL	07/09	
13	MOAB	08/12	
	NIWO	08/08	
14	JORN	09/02	
	SRER	08/28	
15	ONAQ	06/18	
16	ABBY	07/23	
	WREF	07/29	
17	SJER	04/05	
	SOAP	07/04	
	TEAK	07/24	
18	BARR	07/29	
	TOOL	07/24	
19	BONA	07/24	
	DEJU	07/29	
	HEAL	07/29	
20	PUUM	TBD	

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APPENDIX E SOIL CORE ASSEMBLY

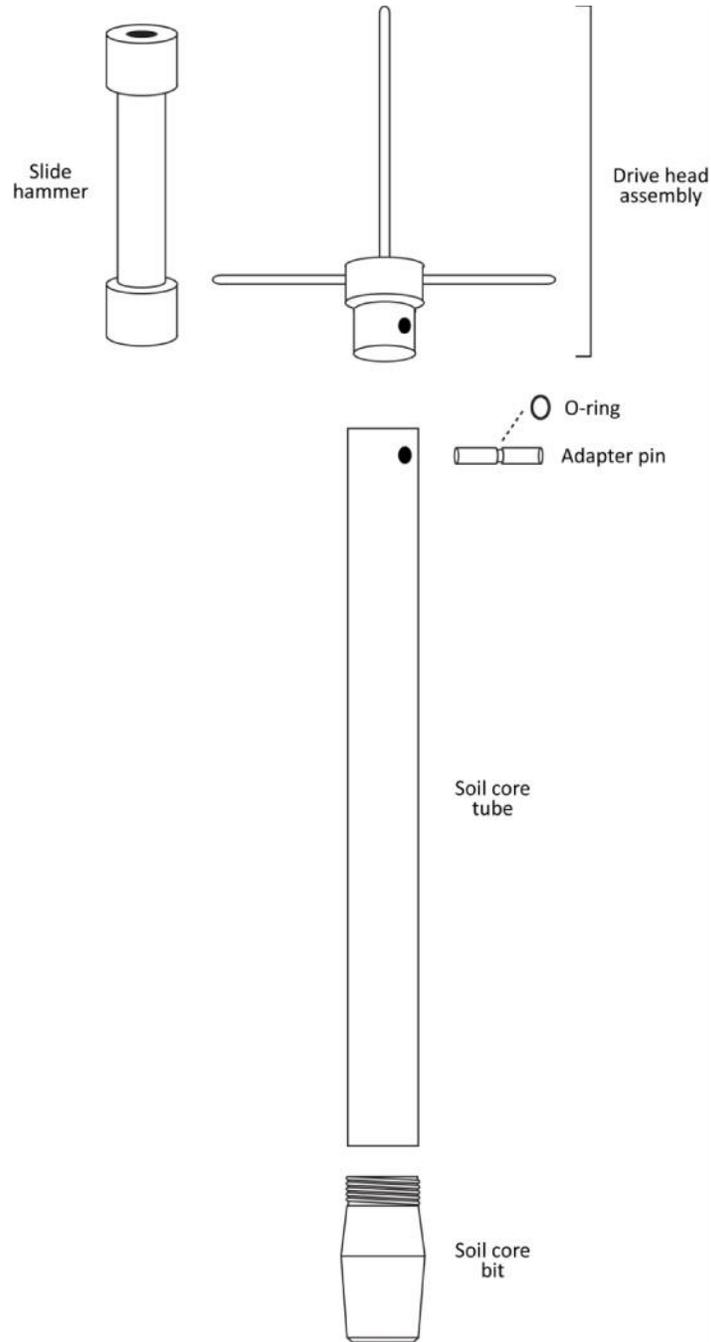


Figure 7. Component parts of the Giddings soil core assembly.

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APPENDIX F MANAGING EXPOSURE TO *TOXICODENDRON* SPECIES

General guidelines for preventing and mitigating exposure to toxic oils from *Toxicodendron* species can be found in RD[12].

The following are protocol-specific best-practice techniques for minimizing exposure to toxic oil during plant Belowground Biomass Coring.

Table 12. Equipment list – Minimizing exposure to toxic oils from roots of *Toxicodendron spp.* that may be encountered during plant Belowground Biomass Coring.

Item No.	R/S	Description	Purpose	Quantity
Durable Items				
	R	Labeled clippers, dedicated to clipping <i>Toxicodendron spp.</i> (see Table 5)	Prevent spread of toxic oils to multiple clippers	1
	R	Labeled sieve set(s), dedicated to sieving samples containing <i>Toxicodendron</i> . (Set contains 2mm sieve and 250 µm sieve. See Table 5 .)	Prevent spread of toxic oils to multiple sieves.	As needed
	R	Labeled forceps, blunt tip, stainless steel; dedicated to <i>Toxicodendron</i> samples	Prevent spread of toxic oils to multiple forceps.	As needed
Consumable Items				
	R	See RD[12]		

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:

-) 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.

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) Bring a clean, new plastic bag to the field for storing and transporting contaminated gloves, soil coring equipment, and clippers after use.

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 G CLIPCELLNUMBER COORDINATES AND MAPS

Belowground biomass soil core sampling and peak biomass clip harvest sampling ideally take place in the same clip cell in a given Tower plot. NEON Field Operations technicians must track the clip cell associated with coring and peak biomass clipping on the Clip Lists provided by Science Operations. When the Herbaceous Biomass clip harvest (RD[11]) precedes soil core sampling in the field, it is necessary to physically locate the clip cell in which the peak biomass clip occurred.

G.1 Maps of clipCellNumber by subplotID

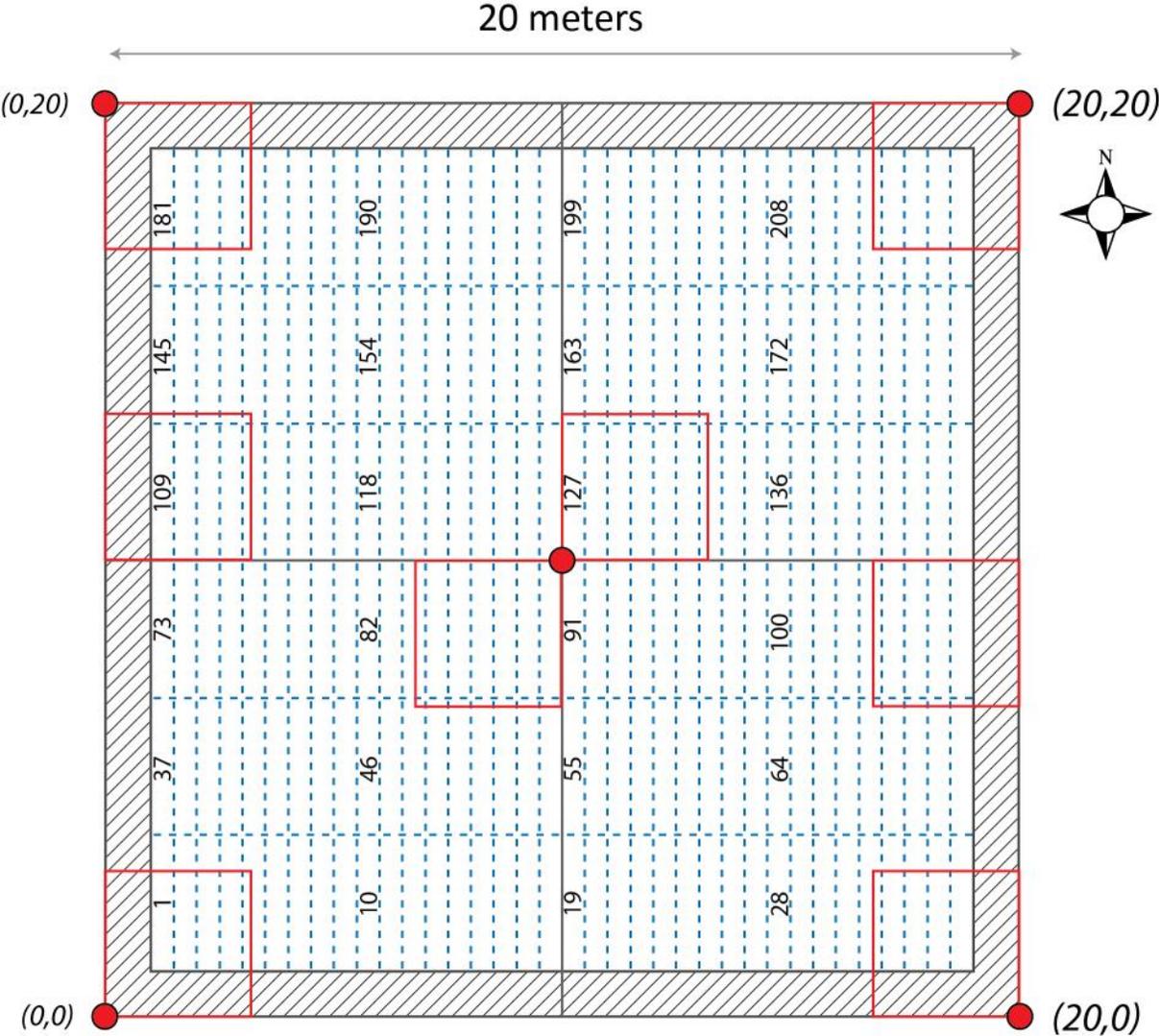


Figure 8. Map of clipCellNumbers in a 20m x 20m base plot (subplotID = 31 in provided Clip Lists). Red squares indicate nested subplots used for diversity sampling; clip cells that significantly overlap red squares are not used for fine root soil coring or clip sampling. However, cells with minimal overlap (e.g., 48-54, 68-72, 145-149) do support these sampling activities.

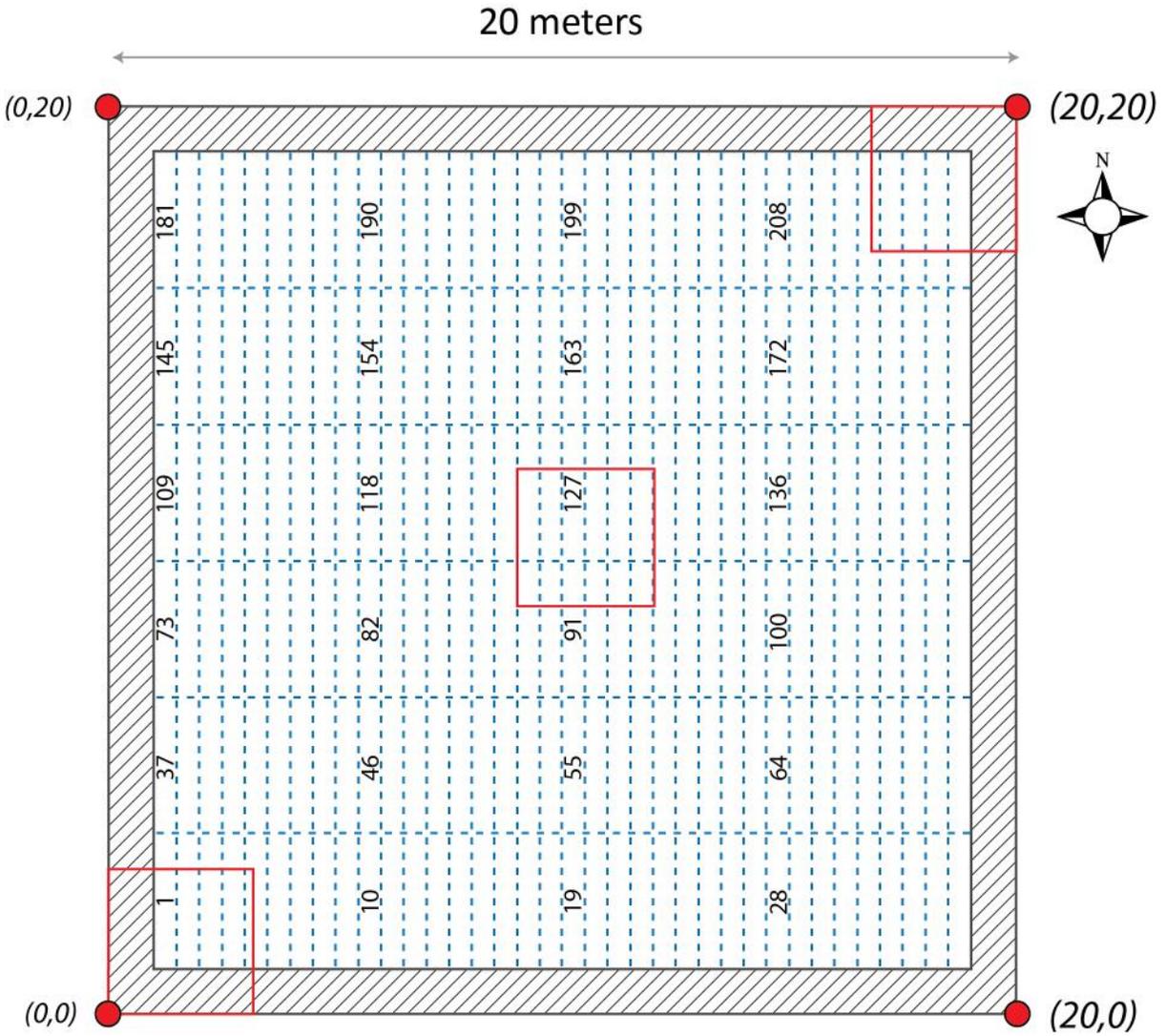


Figure 9. Map of clipCellNumbers for subplotID = 21 in a 40m x 40m Tower base plot. Cells that overlap nested subplots indicated by red squares are not used for fine root soil core or clip sampling.

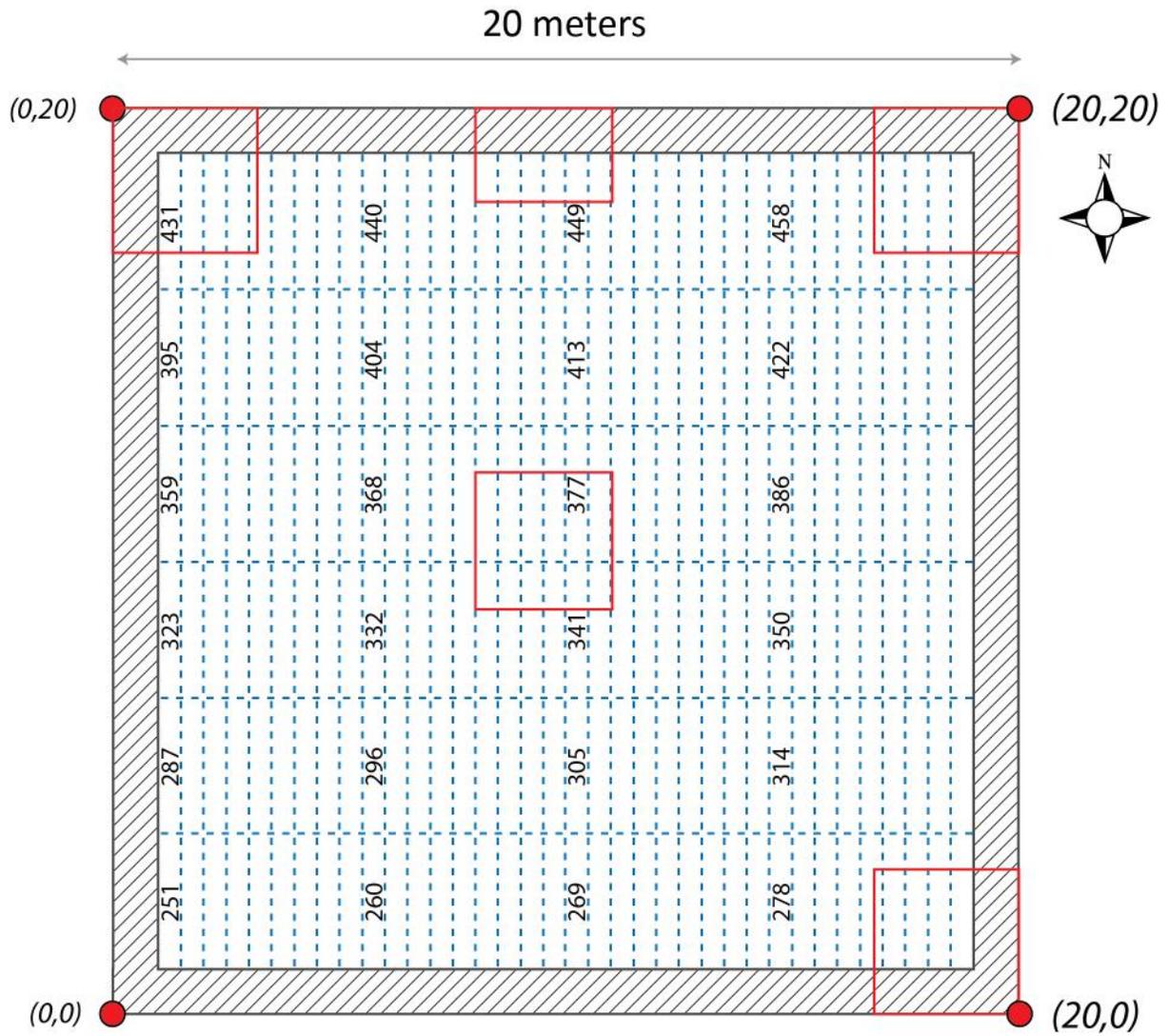


Figure 10. Map of clipCellNumbers for **subplotID = 23** in a 40m x 40m Tower base plot. Cells that overlap nested subplots indicated by red squares are not used for fine root soil core or clip sampling.

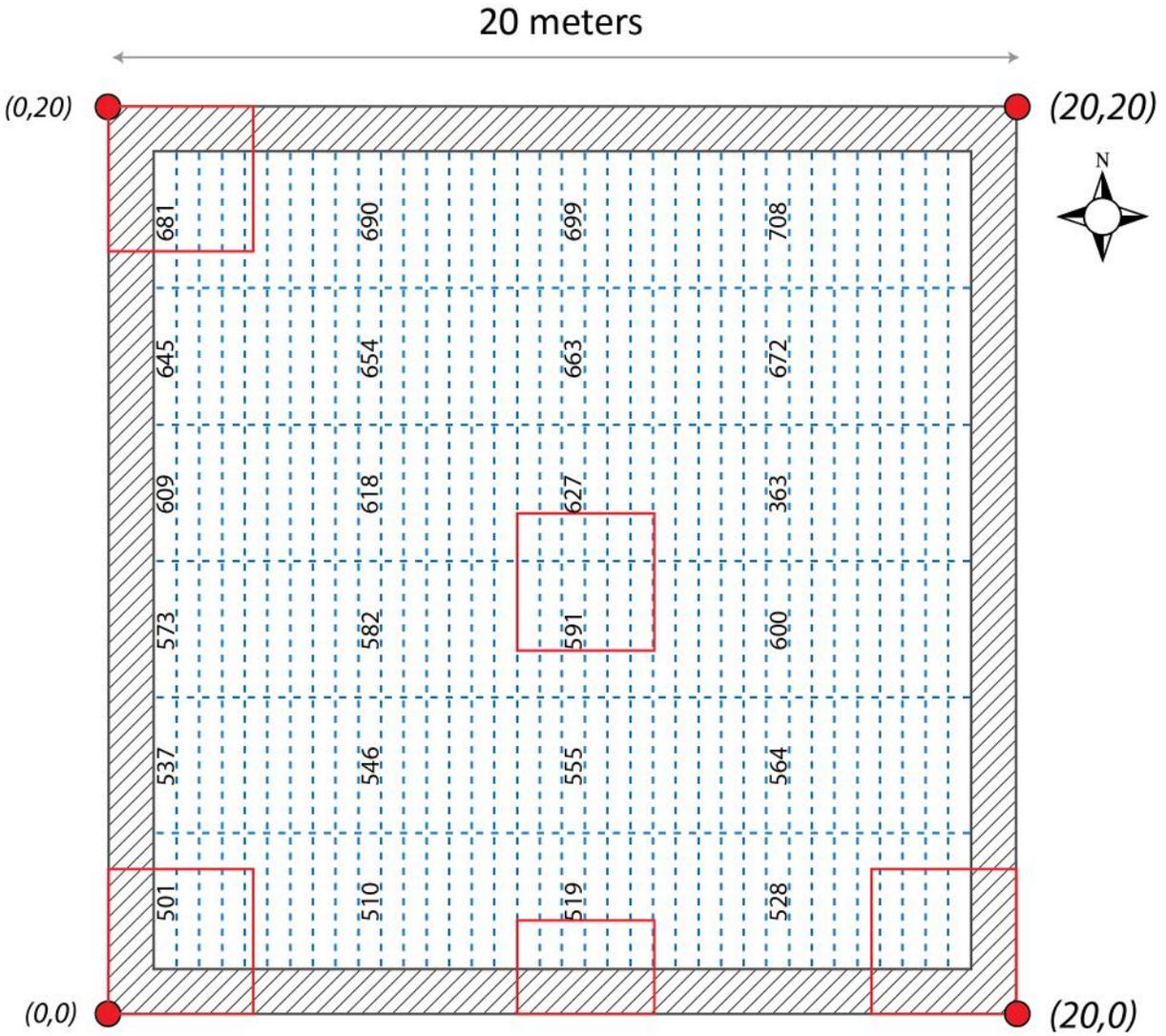


Figure 11. Map of clipCellNumbers for subplotID = 39 in a 40m x 40m Tower base plot. Cells that overlap nested subplots indicated by red squares are not used for fine root soil core or clip sampling.

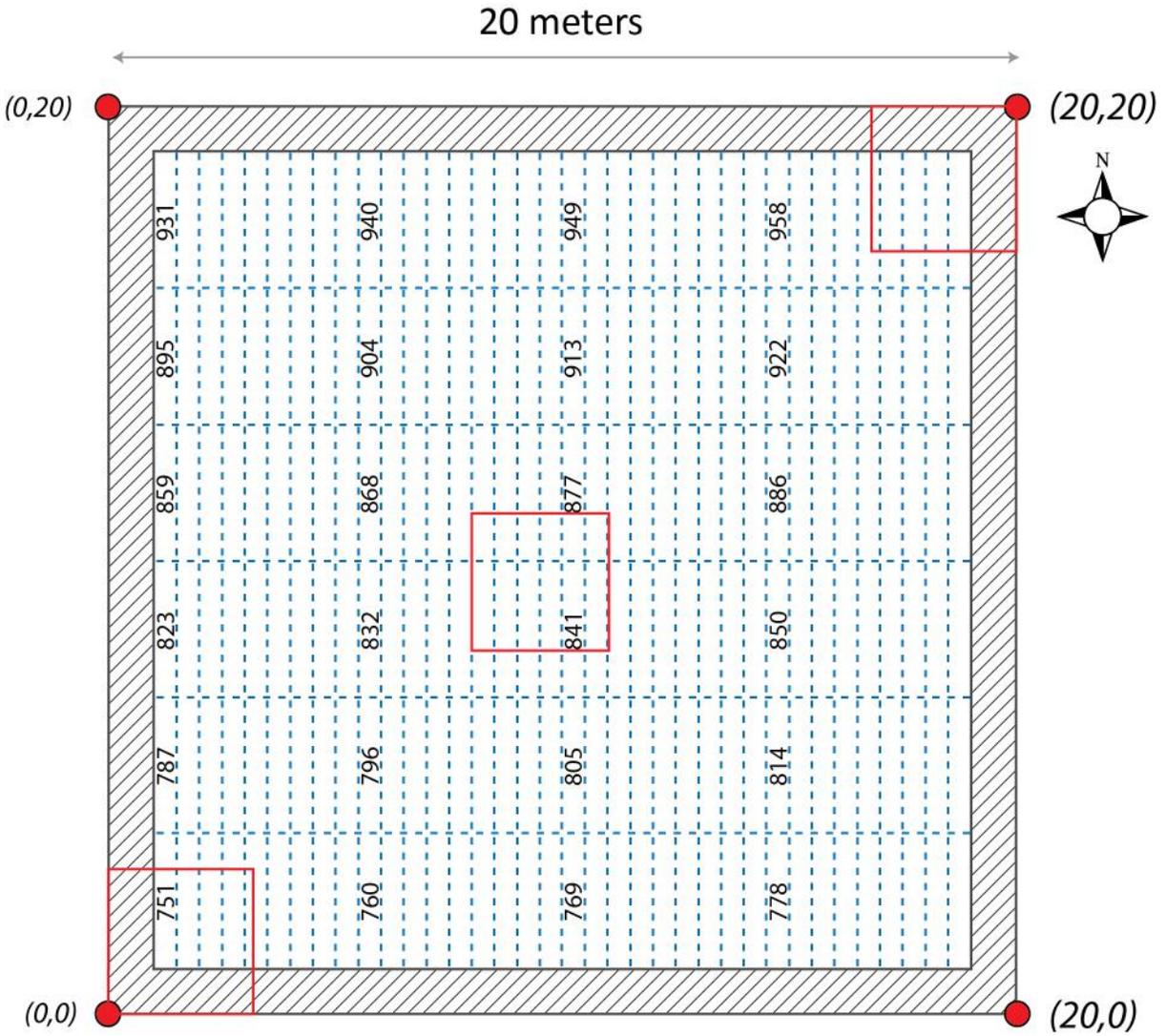


Figure 12. Map of clipCellNumbers for **subplotID = 41** in a 40m x 40m Tower base plot. Cells that overlap nested subplots indicated by red squares are not used for fine root soil core or clip sampling.

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G.2 Coordinates for clipCellNumbers by subplotID

Table 13. List of clipCellNumbers by subplotID and associated easting and northing coordinates. Coordinates correspond to the SW corner of a 0.1m x 2m Clip Strip, and indicate the distance in meters relative to the SW corner of the plot (subplotID = 31) or subplot (subplotID = 21, 23, 39, 41).

clipCellNumber subplotID = 31	clipCellNumber subplotID = 21	clipCellNumber subplotID = 23	clipCellNumber subplotID = 39	clipCellNumber subplotID = 41	easting offset	northing offset
1	1	251	501	751	1.2	1.5
2	2	252	502	752	1.7	1.5
3	3	253	503	753	2.2	1.5
4	4	254	504	754	2.7	1.5
5	5	255	505	755	3.2	1.5
6	6	256	506	756	3.7	1.5
7	7	257	507	757	4.2	1.5
8	8	258	508	758	4.7	1.5
9	9	259	509	759	5.2	1.5
10	10	260	510	760	5.7	1.5
11	11	261	511	761	6.2	1.5
12	12	262	512	762	6.7	1.5
13	13	263	513	763	7.2	1.5
14	14	264	514	764	7.7	1.5
15	15	265	515	765	8.2	1.5
16	16	266	516	766	8.7	1.5
17	17	267	517	767	9.2	1.5
18	18	268	518	768	9.7	1.5
19	19	269	519	769	10.2	1.5
20	20	270	520	770	10.7	1.5
21	21	271	521	771	11.2	1.5
22	22	272	522	772	11.7	1.5
23	23	273	523	773	12.2	1.5
24	24	274	524	774	12.7	1.5
25	25	275	525	775	13.2	1.5
26	26	276	526	776	13.7	1.5
27	27	277	527	777	14.2	1.5
28	28	278	528	778	14.7	1.5
29	29	279	529	779	15.2	1.5
30	30	280	530	780	15.7	1.5
31	31	281	531	781	16.2	1.5
32	32	282	532	782	16.7	1.5
33	33	283	533	783	17.2	1.5
34	34	284	534	784	17.7	1.5
35	35	285	535	785	18.2	1.5
36	36	286	536	786	18.7	1.5
37	37	287	537	787	1.2	4.5

clipCellNumber subplotID = 31	clipCellNumber subplotID = 21	clipCellNumber subplotID = 23	clipCellNumber subplotID = 39	clipCellNumber subplotID = 41	easting offset	northing offset
38	38	288	538	788	1.7	4.5
39	39	289	539	789	2.2	4.5
40	40	290	540	790	2.7	4.5
41	41	291	541	791	3.2	4.5
42	42	292	542	792	3.7	4.5
43	43	293	543	793	4.2	4.5
44	44	294	544	794	4.7	4.5
45	45	295	545	795	5.2	4.5
46	46	296	546	796	5.7	4.5
47	47	297	547	797	6.2	4.5
48	48	298	548	798	6.7	4.5
49	49	299	549	799	7.2	4.5
50	50	300	550	800	7.7	4.5
51	51	301	551	801	8.2	4.5
52	52	302	552	802	8.7	4.5
53	53	303	553	803	9.2	4.5
54	54	304	554	804	9.7	4.5
55	55	305	555	805	10.2	4.5
56	56	306	556	806	10.7	4.5
57	57	307	557	807	11.2	4.5
58	58	308	558	808	11.7	4.5
59	59	309	559	809	12.2	4.5
60	60	310	560	810	12.7	4.5
61	61	311	561	811	13.2	4.5
62	62	312	562	812	13.7	4.5
63	63	313	563	813	14.2	4.5
64	64	314	564	814	14.7	4.5
65	65	315	565	815	15.2	4.5
66	66	316	566	816	15.7	4.5
67	67	317	567	817	16.2	4.5
68	68	318	568	818	16.7	4.5
69	69	319	569	819	17.2	4.5
70	70	320	570	820	17.7	4.5
71	71	321	571	821	18.2	4.5
72	72	322	572	822	18.7	4.5
73	73	323	573	823	1.2	7.5
74	74	324	574	824	1.7	7.5
75	75	325	575	825	2.2	7.5
76	76	326	576	826	2.7	7.5
77	77	327	577	827	3.2	7.5
78	78	328	578	828	3.7	7.5
79	79	329	579	829	4.2	7.5

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clipCellNumber subplotID = 31	clipCellNumber subplotID = 21	clipCellNumber subplotID = 23	clipCellNumber subplotID = 39	clipCellNumber subplotID = 41	easting offset	northing offset
80	80	330	580	830	4.7	7.5
81	81	331	581	831	5.2	7.5
82	82	332	582	832	5.7	7.5
83	83	333	583	833	6.2	7.5
84	84	334	584	834	6.7	7.5
85	85	335	585	835	7.2	7.5
86	86	336	586	836	7.7	7.5
87	87	337	587	837	8.2	7.5
88	88	338	588	838	8.7	7.5
89	89	339	589	839	9.2	7.5
90	90	340	590	840	9.7	7.5
91	91	341	591	841	10.2	7.5
92	92	342	592	842	10.7	7.5
93	93	343	593	843	11.2	7.5
94	94	344	594	844	11.7	7.5
95	95	345	595	845	12.2	7.5
96	96	346	596	846	12.7	7.5
97	97	347	597	847	13.2	7.5
98	98	348	598	848	13.7	7.5
99	99	349	599	849	14.2	7.5
100	100	350	600	850	14.7	7.5
101	101	351	601	851	15.2	7.5
102	102	352	602	852	15.7	7.5
103	103	353	603	853	16.2	7.5
104	104	354	604	854	16.7	7.5
105	105	355	605	855	17.2	7.5
106	106	356	606	856	17.7	7.5
107	107	357	607	857	18.2	7.5
108	108	358	608	858	18.7	7.5
109	109	359	609	859	1.2	10.5
110	110	360	610	860	1.7	10.5
111	111	361	611	861	2.2	10.5
112	112	362	612	862	2.7	10.5
113	113	363	613	863	3.2	10.5
114	114	364	614	864	3.7	10.5
115	115	365	615	865	4.2	10.5
116	116	366	616	866	4.7	10.5
117	117	367	617	867	5.2	10.5
118	118	368	618	868	5.7	10.5
119	119	369	619	869	6.2	10.5
120	120	370	620	870	6.7	10.5
121	121	371	621	871	7.2	10.5

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clipCellNumber subplotID = 31	clipCellNumber subplotID = 21	clipCellNumber subplotID = 23	clipCellNumber subplotID = 39	clipCellNumber subplotID = 41	easting offset	northing offset
122	122	372	622	872	7.7	10.5
123	123	373	623	873	8.2	10.5
124	124	374	624	874	8.7	10.5
125	125	375	625	875	9.2	10.5
126	126	376	626	876	9.7	10.5
127	127	377	627	877	10.2	10.5
128	128	378	628	878	10.7	10.5
129	129	379	629	879	11.2	10.5
130	130	380	630	880	11.7	10.5
131	131	381	631	881	12.2	10.5
132	132	382	632	882	12.7	10.5
133	133	383	633	883	13.2	10.5
134	134	384	634	884	13.7	10.5
135	135	385	635	885	14.2	10.5
136	136	386	636	886	14.7	10.5
137	137	387	637	887	15.2	10.5
138	138	388	638	888	15.7	10.5
139	139	389	639	889	16.2	10.5
140	140	390	640	890	16.7	10.5
141	141	391	641	891	17.2	10.5
142	142	392	642	892	17.7	10.5
143	143	393	643	893	18.2	10.5
144	144	394	644	894	18.7	10.5
145	145	395	645	895	1.2	13.5
146	146	396	646	896	1.7	13.5
147	147	397	647	897	2.2	13.5
148	148	398	648	898	2.7	13.5
149	149	399	649	899	3.2	13.5
150	150	400	650	900	3.7	13.5
151	151	401	651	901	4.2	13.5
152	152	402	652	902	4.7	13.5
153	153	403	653	903	5.2	13.5
154	154	404	654	904	5.7	13.5
155	155	405	655	905	6.2	13.5
156	156	406	656	906	6.7	13.5
157	157	407	657	907	7.2	13.5
158	158	408	658	908	7.7	13.5
159	159	409	659	909	8.2	13.5
160	160	410	660	910	8.7	13.5
161	161	411	661	911	9.2	13.5
162	162	412	662	912	9.7	13.5
163	163	413	663	913	10.2	13.5

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clipCellNumber subplotID = 31	clipCellNumber subplotID = 21	clipCellNumber subplotID = 23	clipCellNumber subplotID = 39	clipCellNumber subplotID = 41	easting offset	northing offset
164	164	414	664	914	10.7	13.5
165	165	415	665	915	11.2	13.5
166	166	416	666	916	11.7	13.5
167	167	417	667	917	12.2	13.5
168	168	418	668	918	12.7	13.5
169	169	419	669	919	13.2	13.5
170	170	420	670	920	13.7	13.5
171	171	421	671	921	14.2	13.5
172	172	422	672	922	14.7	13.5
173	173	423	673	923	15.2	13.5
174	174	424	674	924	15.7	13.5
175	175	425	675	925	16.2	13.5
176	176	426	676	926	16.7	13.5
177	177	427	677	927	17.2	13.5
178	178	428	678	928	17.7	13.5
179	179	429	679	929	18.2	13.5
180	180	430	680	930	18.7	13.5
181	181	431	681	931	1.2	16.5
182	182	432	682	932	1.7	16.5
183	183	433	683	933	2.2	16.5
184	184	434	684	934	2.7	16.5
185	185	435	685	935	3.2	16.5
186	186	436	686	936	3.7	16.5
187	187	437	687	937	4.2	16.5
188	188	438	688	938	4.7	16.5
189	189	439	689	939	5.2	16.5
190	190	440	690	940	5.7	16.5
191	191	441	691	941	6.2	16.5
192	192	442	692	942	6.7	16.5
193	193	443	693	943	7.2	16.5
194	194	444	694	944	7.7	16.5
195	195	445	695	945	8.2	16.5
196	196	446	696	946	8.7	16.5
197	197	447	697	947	9.2	16.5
198	198	448	698	948	9.7	16.5
199	199	449	699	949	10.2	16.5
200	200	450	700	950	10.7	16.5
201	201	451	701	951	11.2	16.5
202	202	452	702	952	11.7	16.5
203	203	453	703	953	12.2	16.5
204	204	454	704	954	12.7	16.5
205	205	455	705	955	13.2	16.5

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clipCellNumber subplotID = 31	clipCellNumber subplotID = 21	clipCellNumber subplotID = 23	clipCellNumber subplotID = 39	clipCellNumber subplotID = 41	easting offset	northing offset
206	206	456	706	956	13.7	16.5
207	207	457	707	957	14.2	16.5
208	208	458	708	958	14.7	16.5
209	209	459	709	959	15.2	16.5
210	210	460	710	960	15.7	16.5
211	211	461	711	961	16.2	16.5
212	212	462	712	962	16.7	16.5
213	213	463	713	963	17.2	16.5
214	214	464	714	964	17.7	16.5
215	215	465	715	965	18.2	16.5
216	216	466	716	966	18.7	16.5